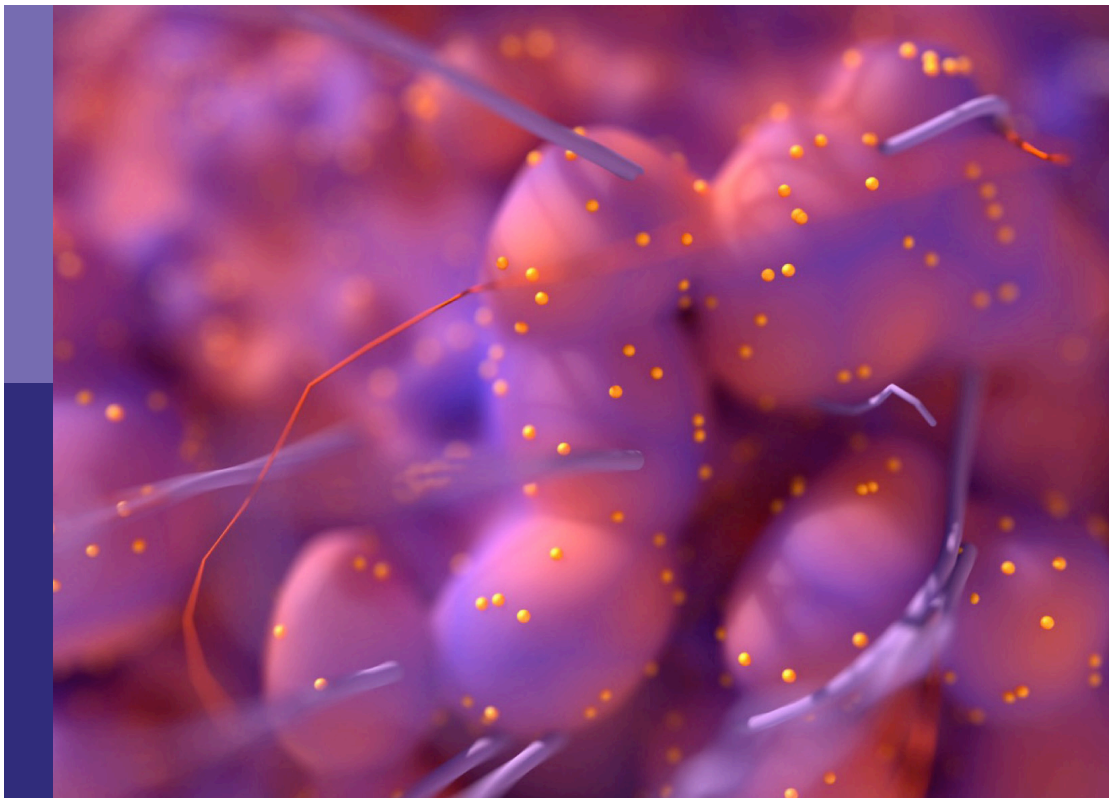
frontiers | Research Topics

Reviews in breast cancer

Edited by
Claudia Mello-Thoms and Maria Rosaria De Miglio

Published in
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-8325-2552-4
DOI 10.3389/978-2-8325-2552-4

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Reviews in breast cancer

Topic editors

Claudia Mello-Thoms — The University of Iowa, United States

Maria Rosaria De Miglio — University of Sassari, Italy

Citation

Mello-Thoms, C., De Miglio, M. R., eds. (2023). *Reviews in breast cancer*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2552-4

Table of contents

frontiers | Frontiers in Oncology

TYPE Editorial
PUBLISHED 11 May 2023
DOI 10.3389/fonc.2023.1161583

OPEN ACCESS

EDITED AND REVIEWED BY
Kara Britt,
Peter MacCallum Cancer Centre, Australia

*CORRESPONDENCE
Maria Rosaria De Miglio
demiglio@uniss.it
Claudia Mello-Thoms
claudia-mello-thoms@uiowa.edu

RECEIVED 08 February 2023
ACCEPTED 25 April 2023
PUBLISHED 11 May 2023

CITATION
De Miglio MR and Mello-Thoms C (2023) Editorial: Reviews in breast cancer.
Front. Oncol. 13:1161583.
doi: 10.3389/fonc.2023.1161583

Editorial: Reviews in breast cancer

Maria Rosaria De Miglio[1]* and Claudia Mello-Thoms[2]*

[1]Department of Medicine, Surgery and Pharmacy, University of Sassari, Sassari, Italy, [2]Department of Radiology, The University of Iowa, Iowa City, IA, United States

KEYWORDS

breast cancer, diagnosis, prognosis, education, anxiety, artificial intelligence

Editorial on the Research Topic
Reviews in breast cancer

According to the GLOBOCAN there were an estimated 19.3 million new cancer cases and 10 million cancer deaths worldwide in 2020 (1). Female breast cancer (BC) has surpassed lung cancer and today it is the most diagnosed type of cancer (2.3 million new cancer cases, representing 11.7% of all cancer cases). In terms of mortality, it ranks in 5th place, with 685,000 deaths in 2020. For women, BC represents 1 in 4 cancer cases and accounts for 1 in 6 cancer deaths (1). Moreover, the GLOBOCAN Cancer Tomorrow prediction tool estimates that incidence will increase by more than 46% by 2040 (2). However, incidence rates are not equal around the world. They are 88% higher in developed countries than in developing countries (55.9 vs. 29.7 per 100.000 women, respectively), but mortality rates are 17% higher in developing countries compared to developed countries (15.0 vs. 12.8 per 100.000 women, respectively). There are a number of reasons for the higher incidence rates in the developed countries, including early age at menarche, later age at menopause, advanced age at first birth, fewer number of children, in addition to lifestyle factors such as obesity, physical inactivity and alcohol intake.

Incidence of BC rapidly increased in the 1980s and 1990s, but by the 2000s incidence had dropped or stabilized. However, since 2007 there has been a slow increase of BC incidence of 0.5% per year in the United States, and moderate increases have been reported in several countries in Europe and in Oceania (2). Using cancer registry data, supplemented with tumor marker information to further understand these increases in incidence, it has been found that most breast cancers are estrogen-receptor positive (1). This particular type of cancer is associated with the obesity epidemic and with mammography screening, which tends to detect slow growing cancers like estrogen-receptor positive cancers. The analysis has also shown that incidence rates are falling for estrogen-receptor negative cancers (1).

Five-years survival rates range between 85-90% for developed countries, whereas for developing countries, particularly those located in Africa, it is 66%. This is primarily due to late-stage presentation of the disease, which reflects on the lack of screening programs and weak health infrastructure. As a result, mortality rates in Africa are among the world's highest (1).

In this Special Issue our focus was to bring some state-of-the-art research in breast cancer to light. In here the reader will find papers on prognostic and potential therapeutic factors, such as immune cells in the tumor microenvironment, inflammations, small extracellular vesicles, RNA binding proteins, dysbiosis, etc. Moreover, social factors will

also be discussed, such as the risk of anxiety, depression and sexual disfunction, as well as health-related quality of life in BC patients. The effects of tobacco smoking and breast cancer risk will also be explored. In terms of breast cancer diagnosis, we will examine the diagnostic value of multiple ultrasound techniques, as well as the role of Artificial Intelligence. We will also explore the use of educational tools to improve radiologists' performance when detecting this disease. Finally, we will discuss the recent progress of therapeutic vaccines for breast cancer.

A growing body of evidence demonstrated a relationship between inflammation and cancer. It increases the risk of cancer development influencing occurrence and progression (3). IL-6 triggers chronic inflammation and cancer, it was higher in many solid tumors including BC (4) which correlated with poor prognosis and metastasis (5). As summarized by Chen et al. several antibodies for IL-6/IL-6R have been used, either as single drug or combined with chemotherapy, demonstrating a marked outcome in both preclinical and clinical trials. IL-6/JAK/STAT3 pathway suppresses anti-tumor immune responses in BC tumor microenvironment. Therefore, treatments against this pathway have given benefit for patients with BC by reducing tumor cell growth and stimulating anti-tumor immunity. Combining IL-6 pathway inhibitor with other targets therapies may represent a new strategy to treat human cancers.

The most important cause of BC death is disease progression due to metastases. Because of this challenge, the identification of unambiguous molecular biomarkers to predict the disease response is needed. Wang et al. conducted a meta-analysis assessing that higher CD68+ and CD163+ tumor-associated macrophages (TAM) density, accounting for approximately 50% of tumor microenvironment cells, is associated with poor outcome in BC patients and also with higher tumors size, no vascular invasion, and positive ER expression, highlighting the significant prognostic value for TAMs in BC patients.

The triple negative breast cancer (TNBC) is the most aggressive and invasive BC subtype, with rapid progression, short response duration to available treatment and poor clinical outcomes. Therefore, there is an urgent need to develop new early diagnosis tools and therapies with good efficacy. Zhou et al. summarized the role of small extracellular vesicles (sEVs) in TNBC. sEVs are natural nano-sized extracellular vesicles with lipid membrane outside and bioactive contents inside, produced by nearly all cell types, play a significant role in intercellular communications. sEVs contribute to angiogenesis, immune escape, tumor proliferation, invasion and distant metastasis, and drug resistance in TNBC. sEVs can be simply detected in body fluids. So, they hold great promise as biomarkers for early diagnosis, prognosis and treatment approach of TNBC. Huertas-Caro et al. argued that higher levels of tumor infiltrating lymphocytes (TILs) in TNBC have been associated with better outcomes and a higher rate of pathological complete response to neoadjuvant chemotherapy. Similar results were observed for CD4 +, CD8+ TILs, independently to the human population analyzed. All together these results suggest that TILs subpopulations might have a prognostic role in TNBC, although the underlying mechanism demands to be elucidated.

Cancer stem cells are a small population of cancer cells with self-renewal and differentiation potential, responsible for tumor heterogeneity, recurrence, metastasis and drug resistance (6). Xu et al. reviewed that breast cancer stem cells (BCSCs) obtained from the same tumor exhibit heterogeneity in terms of mutations, transcriptional programs, immune characteristics and functional properties. Therefore, BCSC concept not only has extensive and great implications for cancer biology, but also has strongly clinical significance for the development of personalized therapies.

RNA binding proteins (RBPs) are key regulators of RNA metabolism. mRNAs as unstable and degradable biomacromolecules bind to specific RBPs and form complexes to maintain their stability in cells, within which RBPs control their localization, stability, translation, and degradation binding to specific mRNAs regions (7). Presently, functional inactivation or abnormal expression of RBPs may be closely associated with BC development, which means that RBPs may become good diagnostic and prognostic biomarkers for BC. Chen et al. described the role of several RBPs and their target genes in the BC development and progression, as well as Lu et al. summarized the function of RBPs in BC cells and their regulatory mechanisms. The RBPs role in drug resistance is still little know and can become a new research direction. Although, as described by Chen et al. therapeutic strategies are developing against RBPs, as the inhibition of HuR by KH-3 that blocks the invasion of BC cells by destroying the HuR-FOXQ1 mRNA interaction, the compound ZM-32 that prevent the formation of HuR-RRM1/2–VEGFA mRNA complex suppressing proliferation, migration, growth, and angiogenesis of BC cells.

Zhang et al. discussed about the emergent role of gastrointestinal microbiome as an important player in the risk and progression of BC. Supposing that the treatment of gut microbiota to stabilize the microenvironment may decrease the production and propagation of pro-tumorigenic factors and determining new approaches to stabilize these deleterious fluctuations is of interest in the treatment and prognosis of BC.

Zhang et al. provided a meta-analysis to evaluate the prognostic differences between multicentric/multifocal (MM) and unifocal BC, in order to illustrate a theoretical basis for the design of an applicable therapeutic strategy for treating MMBC patients. However, MMBC patients showed a higher death risk, but it may not be independently associated with poorer outcomes. MMBC and UFBC patients with appropriate surgery and adjuvant therapies showed the same prognosis, although the prognostic impact of every lesion in MMBC still needs further investigation.

Lei et al. summarized that germline BRCA1/2 mutations are common in Chinese patients with hereditary breast, ovarian, prostate and pancreatic cancers. Although Chinese consensuses recommend BRCA1/2 genetic testing for cancer patients only, depending on cost-effectiveness and social and political factors, public interest and patients' benefits. The Authors recommended that healthy individuals harboring pathogenic mutations should be identified to promote prevention, early diagnosis, and timely treatment of BRCA mutation-related cancers, which may increase 5-year survival for these patients.

Social factors that affect breast cancer patients were also discussed. For example, anxiety and depression risk in Taiwanese women with breast cancer and women with cervical cancer was explored by Yang et al. As they compared these two populations of patients, the authors found that they are both at an elevated

likelihood of developing anxiety and depression, but that the risk for developing depression was slightly higher in breast cancer patients.

In addition, sexual dysfunctions in breast cancer patients were examined by Hernandez-Blanquisett et al.. The authors report that up to 75% of women treated for breast cancer report sexual disorders, but oncologists are not trained to recognize which patients are at high risk for developing this disease. The authors suggest that the choice of less toxic treatments in the surgical, chemotherapy and radiation therapy domains could lead to a reduced risk of female sexual dysfunction without increasing the risk for breast cancer recurrence or the effectiveness of treatment.

In another meta-analysis and systematic review, Chen et al. studied the health-related quality of life in breast cancer patients in Asia. The authors reported that Asian breast cancer patients suffer from poor quality of life and were severely impacted by the effects of fatigue and hair loss, pain, insomnia, and anxiety.

Also in this Research Topic, He et al. carried out a systematic review and meta-analysis on the relationship between tobacco and breast cancer. They showed that active or passive smoking increased the risk of BC in women, and that the effect of smoking was influenced by factors such as duration, intensity, number of years since quitting, as well as population-related factors (such as fertility status) and breast cancer subtypes.

In terms of breast cancer diagnosis, Li et al. explored the diagnostic value of multiple ultrasound techniques for assessment of lymph node metastases in breast cancer patients. As the authors posit, early diagnosis of lymph node metastases is very important for prognosis of breast cancer development. Currently the most commonly used method is lymph node biopsy, however it is an invasive method that may bring complications to the patients (such as lymphedema). The authors found that the combination of ultrasound with contrast-enhanced ultrasound led to the best performance among all the ultrasound techniques tested.

Moreover, the use of Artificial Intelligence for the diagnosis and prognosis prediction of breast cancer was explored by Jones et al. In their review the authors focused on two tasks (1): better understanding the association between radiomics features and tumor microenvironment; and (2) the progress developing new computer-assisted aid schemes for predicting breast cancer risk, determining the likelihood of tumor malignancy, and determining tumor response to treatment.

Aiming to improve radiologists' performance when detecting early BC, Trieu et al. explored the use of an educational intervention, BREAST (Breastscreen REader Assessment STrategy), which helps radiologists' interpretation skills when reading both mammograms and Digital Breast Tomosynthesis cases. The authors described the use of the BREAST platform in countries with screening programs for breast cancer (such as Australia, Singapore) and countries without (such as China, Vietnam).

The recent progress on the development of therapeutic vaccines for BC has been explored by Zhang et al. in this issue. Although advanced BC is still considered to be a poorly immunogenic disease, the great success of cancer immunotherapy is paving the way for a new era in cancer treatment. Vaccine targets have included both tumor-associated antigens and tumor-specific antigens. However, as only a few women seem to benefit from neoantigens, more attention is being paid to overexpressed antigen-based treatments, such as HER-2-derived peptide vaccines.

Finally, Lyu et al. have determined the research trends and hot spots of breast cancer management during the COVID-19 pandemic. The authors suggest that during the epidemic the management of breast cancer patients changed considerably, including all aspects of management such as screening, treatment, follow-up and rehabilitation.

Conclusions

Breast cancer is currently the most diagnosed type of cancer for women worldwide. Moreover, the GLOBOCAN Cancer Tomorrow estimates that incidence of this disease will increase by more than 46% by 2040, making it critical that we device new ways to detect, diagnose and treat breast cancer.

In this Special Issue we presented reviews and meta-analyses that promoted knowledge of the mechanisms of breast cancer progression, as well as its prevention, diagnosis and treatment. We believe that this information will be useful for both scientists and clinicians.

Author contributions

Paper design: CMT. Writing: MRdM and CMT. Revisions: CMT. All authors read and agreed to the submitted version of the manuscript.

Funding

This work was partially supported by a grant from NIH/NCI (1 R01 CA259048) to CMT.

Acknowledgments

We are grateful to the Frontiers in Oncology Editorial Team members for their continuous, valuable and helpful support given to us for this Research Topic.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71:209–49. doi: 10.3322/CAAC.21660

2. Heer E, Harper A, Escandor N, Sung H, McCormack V, Fidler-Benaoudia MM. Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study. *Lancet Glob Heal* (2020) 8:e1027–37. doi: 10.1016/S2214-109X(20)30215-1

3. Solinas G, Marchesi F, Garlanda C, Mantovani A, Allavena P. Inflammation-mediated promotion of invasion and metastasis. *Cancer Metastasis Rev* (2010) 29:243–8. doi: 10.1007/S10555-010-9227-2

4. Guo Y, Xu F, Lu T, Duan Z, Zhang Z. Interleukin-6 signaling pathway in targeted therapy for cancer. *Cancer Treat Rev* (2012) 38:904–10. doi: 10.1016/J.CTRV.2012.04.007

5. Zhang GJ, Adachi I. Serum interleukin-6 levels correlate to tumor progression and prognosis in metastatic breast carcinoma. *Anticancer Res* (1999) 19(2B):1427–32.

6. Bai X, Ni J, Beretov J, Graham P, Li Y. Cancer stem cell in breast cancer therapeutic resistance. *Cancer Treat Rev* (2018) 69:152–63. doi: 10.1016/J.CTRV.2018.07.004

7. Ding Z, Yang HW, Xia TS, Wang B, Ding Q. Integrative genomic analyses of the RNA-binding protein, RNPC1, and its potential role in cancer prediction. *Int J Mol Med* (2015) 36:473–84. doi: 10.3892/IJMM.2015.2237/HTML

frontiers | Frontiers in Oncology

REVIEW
published: 06 June 2022
doi: 10.3389/fonc.2022.905832

Recent Progress on Therapeutic Vaccines for Breast Cancer

Lianru Zhang[1], *Xipeng Zhou*[2], *Huizi Sha*[1], *Li Xie*[1*] *and Baorui Liu*[1*]

[1] *The Comprehensive Cancer Centre of Drum Tower Hospital, Medical School of Nanjing University & Clinical Cancer Institute of Nanjing University, Nanjing, China,* [2] *Department of oncology, Yizheng People's Hospital, Yangzhou, China*

Breast cancer remains the most frequently diagnosed malignancy worldwide. Advanced breast cancer is still an incurable disease mainly because of its heterogeneity and limited immunogenicity. The great success of cancer immunotherapy is paving the way for a new era in cancer treatment, and therapeutic cancer vaccination is an area of interest. Vaccine targets include tumor-associated antigens and tumor-specific antigens. Immune responses differ in different vaccine delivery platforms. Next-generation sequencing technologies and computational analysis have recently made personalized vaccination possible. However, only a few cases benefiting from neoantigen-based treatment have been reported in breast cancer, and more attention has been given to overexpressed antigen-based treatment, especially human epidermal growth factor 2-derived peptide vaccines. Here, we discuss recent advancements in therapeutic vaccines for breast cancer and highlight near-term opportunities for moving forward.

Keywords: breast cancer, cancer vaccines, cancer immunotherapy, clinical trials, concurrent therapies

OPEN ACCESS

Edited by:
Vajihe Akbari,
Isfahan University of Medical Sciences, Iran

Reviewed by:
Rodney Macedo Gonzales,
Albert Einstein College of Medicine, United States
Chiara Focaccetti,
Università di Roma Tor Vergata, Italy

***Correspondence:**
Baorui Liu
baoruiliu@nju.edu.cn
Li Xie
sherry01130@163.com

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *27 March 2022*
Accepted: *11 May 2022*
Published: *06 June 2022*

Citation:
Zhang L, Zhou X, Sha H, Xie L and Liu B (2022) Recent Progress on Therapeutic Vaccines for Breast Cancer. Front. Oncol. 12:905832. doi: 10.3389/fonc.2022.905832

INTRODUCTION

Breast cancer (BC) is the leading cause of cancer worldwide (1). Although there has been an increase in the overall survival rate in BC because of improvements in early-stage diagnosis and targeted therapies, almost all metastatic tumors develop drug resistance and cannot be cured. It is still a difficult problem to reduce the recurrence rate of early breast cancer and to prolong the survival time of advanced breast cancer. Immune-based interventions could be a beacon of hope to decrease morbidity and mortality of cancer. Although immune checkpoint inhibitors (ICIs) have been proven to increase the survival rate in lung cancer, melanoma, gastric cancer and so on, the indications of ICIs for the treatment of BC are only focused on first-line and neoadjuvant therapy for triple-negative breast cancer (TNBC) (2) to date.

The tumor microenvironment (TME) plays a crucial role in the recognition and prevention of cancer and early eradication. The TME may also interact with tumor cells and promote the progression of cancer. The immunoediting hypothesis describes the dynamic interaction between the immune system and tumor cells in three phases: elimination phase, equilibrium phase and escape phase (3). Tumor cells that avoid immune recognition and elimination steps enter the escape phase and present a clinically detectable tumor. The advantage of active immunotherapy is to develop a protective effect against tumor tissue, modifying the immune microenvironment and resetting the immune system to an antitumor surveillance status. Therapeutic cancer vaccines led by neoantigens are hotspots of active immunotherapy. Combination strategies with ICIs have shown clinical benefits in multiple types of cancer (4, 5). To date, only one vaccine named sipuleucel-T has

been approved by the FDA and is used to treat metastatic castration-resistant prostate cancer in a limited group of nearly asymptomatic patients (6). No BC vaccine has been approved for clinical use. BC is a heterogeneous disease and can be classified into 4 common groups: luminal A, luminal B, human epidermal growth factor 2 (HER2)-positive, and TNBC (7). BC is traditionally considered a poorly immunogenic tumor. However, recently published data on TNBC have shown that a significant number of tumor infiltrating lymphocytes infiltrate TNBC tissues (8), indicating that an immunotherapeutic approach may be suitable for this hard-to-treat malignancy. A series of clinical trials for TNBC vaccines are underway. In addition, increasing numbers of clinical trials are being conducted demonstrating that vaccination is capable of inducing an antitumor-specific response in BC. In this review, we discuss recent progress on therapeutic vaccines from the perspective of tumor development and clinical data, and a blueprint for personalized vaccines is also presented.

SPECTRUM OF VACCINE TARGETS

Therapeutic tumor antigens are divided into two main categories: tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) (9) (**Figure 1**). TAAs include tumor germline antigens, tumor differentiation antigens and overexpressed antigens (10). Tumor germline antigens, or cancer testis antigens, are expressed at high levels in the germinal cells of the testis, ovaries, and placenta and are not expressed in somatic cells under normal conditions (11). They are expressed in malignant cells of various cancer types, including BC. In BC, the expression of a number of cancer testis antigens has been reported, such as MAGE-A1 (12), NY-ESO1 (13) and KK-LC-1 (14). Serum antibodies against cancer testis antigens can be detected as useful biomarkers for predicting the clinical benefits of immunotherapy (14–16). Tumor differentiation antigens are proteins expressed in tumor cells and in normal tissue from which the tumor originates, such as Melan-A/Mart-1 (17), gp100 (18), PSA (19), CEA (20) and NY-BR-1 (21, 22). Overexpressed antigens are proteins expressed at low levels in normal cells and at high levels in cancer cells. The most common overexpressed antigens targeted in BC are HER2 (23), MUC-1 (24), hTERT (25) and survivin (26). TAA-based vaccines must be sufficiently immunogenic to activate the remaining low-affinity TAA-reactive T cells because central and peripheral immune tolerance mechanisms have removed T cells with strong TAA affinity.

TSAs are expressed specifically in tumor cells, mainly including oncoviral antigens and neoantigens (27). Neoantigens are products of genomic alterations and consist of simple point mutations that change single amino acids, frameshift insertion or deletion mutations, splice-site alterations that lead to exon skipping, structural alterations that lead to the formation of fusion proteins and other forms of collateral damage (28). Although there are thousands of genomic alterations in the process of tumor formation, only a handful of neoantigens succeed in eliciting antitumor immune responses. BC shows an intermediate genomic mutational load, and only a few cases benefiting from neoantigen-

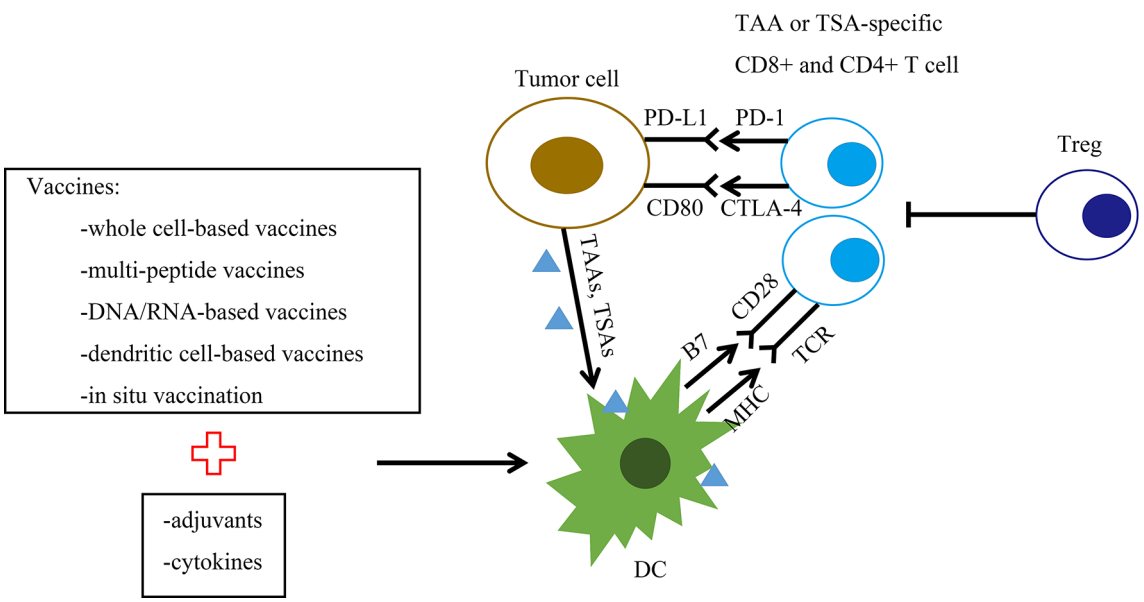


FIGURE 1 | Graphic representation of the therapeutic breast cancer vaccine platforms and their mechanism of action. The figure summarizes the spectrum of vaccine targets and vaccine platforms for breast cancer. Vaccine platforms are whole cell-based vaccines, multipeptide vaccines, DNA/RNA-based vaccines, dendritic cell (DC)-based vaccines and *in situ* vaccination. DCs present processed tumor-associated antigen (TAA) or tumor-specific antigen (TSA) to CD4+ and CD8+ T cells. This interaction generates TAA/TSA-specific effector T cells, leading to the killing of tumor cells. B7, B7 protein; CD28, T cell costimulatory molecule CD28; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; DC, dendritic cell; MHC, major histocompatibility complex; PD-1, programmed cell death protein 1; PDL-1, programmed death ligand 1; TAAs, tumor-associated antigens; TSAs, tumor-specific antigens; TCR, T cell receptor; Treg, regulatory T cell.

based treatment have been reported in BC (29). Since TNBC is recognized as a potential suitable subtype for immunotherapy, clinical trials of neoantigens are enrolling TNBC patients to evaluate the safety and induction of specific T cell responses. Clinical trials using autologous dendritic cells (DCs) pulsed with tumor-specific neoantigen (NCT04105582) or neoantigen DNA vaccine administered with durvalumab (NCT03199040) or personalized synthetic long peptide (SLP) neoantigen vaccine administered with durvalumab and nab-paclitaxel (NCT03606967) are currently enrolling TNBC patients. The neoantigen prediction process includes identifying tumor-specific somatic mutations and predicting major histocompatibility complex (MHC)-binding epitopes. Whole-exome sequencing is performed using tumor biopsy specimens and nonmalignant tissue samples to identify tumor-specific somatic mutations (30, 31). Tumor and germline DNA are compared to exclude germline mutations, while RNA sequencing provides additional information on mutated genes and confirms the mutation calls (32–34). Owing to human leukocyte antigen (HLA) restriction, various algorithm-based computational approaches have been developed to predict the binding of a tumor antigen to MHC molecules (35, 36). Peptides predicted with a moderate-to-strong HLA-binding affinity (IC50 < 150 nmol/l) are considered more likely to induce CD8+ T cell responses. Mass spectrometry-based immunopeptidomics can be used to identify neoantigens or to validate those predicted by in silico strategies. Recently, a new strategy based on using signaling and antigen-presenting bifunctional receptor (SABR) libraries was developed, enabling the identification of specific TCR-pMHC interactions (37).

In addition to TAAs and TSAs, multiple TME-targeting vaccine-based clinical trials are underway for patients with BC (38). Resident cells in the TME are likely more genomically stable than tumor cells. Pathological angiogenesis in the vascular TME can suppress effective immunotherapies. Multiple strategies targeting whole-cell endothelial cells (39), tumor blood vessel antigens (40), epidermal growth factor receptor (EGFR) (41), CD105 (42), platelet-derived growth factor receptor (PDGFR)-β (43) and vascular endothelial growth factor receptor (VEGFR) (44) have been tested in preclinical models of BC. A phase I study of pulsed DCs with tumor blood vessel antigens was completed recently (NCT02479230). Cancer-associated fibroblasts of the TME are vaccine targets as well. However, cancer-associated fibroblast vaccine strategies are all in the preclinical stage (45–47). Mads Hald Andersen et al. (48) designed an innovative investigational approach to target immune inhibitory pathways in the TME, modulating immune regulation. Therapeutic vaccination with long peptide epitopes is derived from proteins including indoleamine 2,3-dioxygenase (IDO), tryptophan 2,6-dioxygenase, arginase, and programmed death ligand 1 (PD-L1). Endogenous anti-regulatory T cells are activated because they recognize these peptides, and these pro-inflammatory cells are attracted to the TME, potentially altering tolerance to tumor antigens. Vaccinations against IDO or PD-L1 have been proven to be safe in clinical trials. Tryptophan 2,6-dioxygenase (TDO) is another enzyme involved in tryptophan degradation in the TME and is expressed in many cancers, including breast cancer, making it an interesting target for therapeutic vaccinations against the TME for BC. Vaccines are also currently being developed to target gene products associated with epithelial-mesenchymal transition (EMT) and cancer cells with stem-like characteristics (49, 50).

VACCINE DELIVERY PLATFORMS

Diverse vaccine platforms have now been evaluated in clinical trials, including whole cell-based vaccines, multipeptide vaccines, DNA/RNA-based vaccines, dendritic cell-based vaccines and *in situ* vaccination (**Table 1**).

Whole Cell-Based Vaccines

Whole cell-based vaccines are derived from autologous or allogenic tumor cells (56). Immunizing BC patients with tumor cells isolated from the patient can circumvent the problems associated with antigen selection and epitope prediction. In addition, whole cell-based vaccines present the patient's immune system with a wide variety of TAAs as immunogens. However, whole cell-based vaccines have shown relatively poor immunogenic potential (57). The immunogenicity can be increased by engineering tumor cell lines to secrete granulocyte-macrophage colony stimulating factor (GM-CSF), combined with strong adjuvants or cytokines (58, 59). In addition, whole cell-based vaccines in combination with chemotherapy may also exert synergistic antitumor effects. Autologous tumor cell vaccines (ATCVs) present a unique set of antigens, such as particular point mutations or fusion gene products, from a given patient's own tumor (60–62). These antigens could help to launch a polyclonal response against a variety of tumor cells. However, the generation of ATCVs is patient specific with high complexity and high cost. Allogenic tumor cell vaccines, which typically contain two or three established human tumor cell lines, can be used as an alternative for the development of cell-based vaccines (56). In a phase I clinical trial enrolling 28 patients with stable metastatic breast cancer (mBC), the efficacy of a combination therapy using an allogenic GM-CSF-secreting BC vaccine along with chemotherapy was investigated (63). The vaccine was formulated from two HER2/neu+ mammary adenocarcinoma BC cell lines, SKBR3 and T47D. This vaccine was administered either alone or in sequence with common chemotherapeutic agents, such as cyclophosphamide and doxorubicin. The results suggest that the vaccine alone or in sequence with low-dose chemotherapy could induce an effective immune response. In another phase I study, a human leukocyte antigen (HLA)-A2+, HER2/neu(+) allogeneic MDA-MB-231 BC cell line was modified to express the costimulatory molecule B7-1 (CD80) and used as a vaccine to treat stage IV BC patients (64). Although this immunization strategy proved to induce tumor-specific immune responses in a minority of patients, no significant tumor regression was observed. In a single-arm feasibility study, an allogeneic HER2+ GM-CSF-secreting BC vaccine was given with low-dose cyclophosphamide and weekly trastuzumab in 20 patients with HER2+ mBC (65). This vaccination regimen was safe and demonstrated clinical effects in terms of objective response rate (ORR), progression-free survival (PFS), and

TABLE 1 | Comparison of different vaccine platforms.

Vaccine platforms	Mechanisms	Advantages	Disadvantages	Ref
Whole cell-based vaccines	Whole tumor cell lysates can be prepared by hypochlorous acid, ultraviolet B ray-irradiation, repeat cycles of freezing and thawing or hyperthermia	All tumor cells express a wide range of tumor-associated antigens Gene sequencing and bioinformatics predictive screening are not required Diminishes the chance of tumor escape	Complex and expensive production The immunogenicity is relatively poor	(51)
Multipeptide vaccines	Peptide vaccines contain tumor-specific epitopes that can be taken up and processed by antigen-presenting cells to activate T cell immune responses	Stable Safe Can be inoculated repeatedly Long peptides can stimulate both CD4+ and CD8+ T cell responses	The immunogenicity of synthetic peptide-based vaccines can be significantly affected by the delivery process	(52)
DNA/RNA-Based Vaccines	*In vitro* transcribed RNA or plasmid DNA encoding cancer antigens is introduced into the body, and cancer antigens are expressed by the host to induce antitumor response	Rapid and inexpensive production Mimics viral infection DNA vaccines have flexible platform for molecule engineering RNA vaccines have intrinsic adjuvant properties	RNA vaccine is susceptible to extracellular degradation by RNAses DNA vaccine has theoretical risk of host genome integration, relatively low immunogenecity	(53)
Dendritic cell-based vaccines	DC cells are stimulated with cytokines *in vitro* to become mature DCs upregulating costimulatory molecules, and mature DCs loaded with antigens migrate to lymph nodes resulting in the subsequent specific immune responses	Bypass conventional antigen presentation pathways	Time-consuming personalized process Less practical Hard to preserve	(54)
In situ vaccination	Manipulation of intratumoral myeloid cells by injecting immunomodulators and local ablative therapies which are used to release tumor antigens from the therapy-killed tumor cells such as radiation or combination with vaccines	Simple and cost-effective Minimal side effects Minimizes immune escape Adjuvant delivery is feasible and flexible	Requirement for intratumoral injection	(55)

overall survival (OS), with a trend toward longer PFS and OS in HER2-specific T-cell responders.

Peptide Vaccines

The advantages of peptide vaccines include ease of synthesis and storage, safety, cost-effectiveness, and tolerable side effects. The great limitation for peptide-based vaccines is the possibility of insufficient immunogenicity, which makes a great need for a suitable adjuvant to produce an efficient response. The expression of antigen epitopes within the tumor bed can be heterogeneous, while the immune response may be limited to a few epitopes. Multipeptide vaccines formulated from MHC class I-restricted TAAs are being tested for their antigen-specific immune response in clinical trials (66–70). Peptides with epitopes can bind directly to MHC class I molecules on the surface of antigen-presenting cells without cross-presentation, but they often result in only low-level, short-lived responses without the help of CD4+ T cells. CD4+ T cells can enhance the tumoricidal activity of other antitumor effector cells, such as CD8+ T cells and macrophages. Some CD4+ subsets influence angiogenesis to facilitate the infiltration of CD8+ T cells, in addition to direct cytotoxic functions (71). There are attempts to activate both CD4+ and CD8+ T cells by using multivalent synthetic long peptides (SLPs) containing both MHC class I and class II epitopes (72). SLP vaccines offer several advantages. They are not able to bind directly to MHC class I so that they have to be processed by DCs (73). SLP vaccines increase the duration of *in vivo* epitope presentation in the antigen-draining lymph node (74), which is shown to be important for clonal expansion (75) and for interferon-γ production by CD8+ T cells (76), and harbor both CD4+ and CD8+ T cell epitopes, ensuring a balanced CD4/CD8 response. Some well-designed peptide vaccines will be discussed in the 4th part of this review. In addition, delivery systems have been applied to improve antitumor immunity. Among them, nanomaterials, such as liposomes, micelles, dendrimers, microneedles, proteins, polymer-based conjugates, the B-subunit of Shiga toxin (STxB), and polyactin A (PAA), are under investigation to convey and release antigens and immunostimulatory molecules (77).

DNA/RNA-Based Vaccines

DNA or RNA-based vaccines are easy to design and can encode multiple epitopes. DNA vaccines have good stability and can be rapidly and easily modified. Plasmid DNA vaccines can be integrated with additional immune modulators to elicit a maximal immune response (78). Most DNA-based cancer vaccine studies

have targeted TAAs, such as HER2/neu and mammaglobin-A (MAM-A), in BC. The first clinical trial of a HER2/neu DNA vaccine evaluated the efficacy and tolerability of the vaccine in humans. The HER2/neu DNA vaccine was administered with low doses of interleukin-2 (IL-2) and GM-CSF in mBC patients in a pilot clinical trial, even though no significant T cell response was elicited (79). Currently, two phase I clinical trials of HER2/neu DNA vaccines are active (NCT00393783 and NCT00436254). The MAM-A DNA vaccine was also investigated in mBC in a phase I clinical trial. This vaccine was safe and succeeded in eliciting MAM-A-specific CD8+ T cell responses. PFS was improved in vaccinated patients, although the sample size was low (n=14) (80). Additionally, a clinical trial using a neoantigen DNA vaccine to treat TNBC was launched (NCT03199040). RNA vaccines are designed to enter the cytosol and thus avoid safety concerns related to integration into the host cell genome. RNA-based vaccines have an inherent function through Toll-like receptor 3 (TLR3), TLR7 and TLR8 stimulation to provide an adjuvant effect. However, RNA is very unstable, so delivery systems such as nanoparticles and liposomes are challenging. Viral vectors can be used to deliver nucleic acid vaccines to enter the cytosol. However, the production of antibodies against viral vectors attenuates the efficiency. PANVAC (containing transgenes for CEA, MUC-1 and 3 T cell costimulatory molecules) is a well-studied poxviral vaccine. For the 12 mBC patients, 5 patients had stable disease (SD) by RESIST lasting ≥ 4 months, with one patient having a complete response (CR) and remaining on study for ≥ 37 months (81).

Dendritic Cell-Based Vaccines

DCs are professional antigen-presenting cells that can process exogenous and endogenous antigens and present them to stimulate naïve T lymphocytes through the MHC I and II pathways. Therefore, DCs play a crucial role in the initiation of the primary response and induction of the antitumor-specific immune response. Most cancer vaccines are greatly dependent on the activation of DCs. Peptide-pulsed DCs have superiority in inducing antitumor responses compared to peptide vaccines with adjuvants (82). In a pilot study, autologous DCs were pulsed with HER2/neu- or MUC1-derived peptides to generate a DC-based vaccine. Ten patients suffering from advanced BC and ovarian cancer showed a strong immunogenic response with no side effects (83). A HER2 intracellular domain (ICD) protein-containing DC vaccine was tested in disease-free BC patients. Six patients out of seven had circulating anti-HER2 ICD antibodies, and all patients were alive and disease free at 4.6-6.7 years of follow-up (84). Autologous DCs were also pulsed with patient-derived tumor cells or cell lysates to facilitate a strong immunogenic response (85–87). However, ex vivo generation of DCs is complicated, and it is costly and time-consuming to generate the large number of DCs required for vaccination. The demanding production process of DC vaccines and lack of improvement in clinical benefits limit their application in the clinic.

In Situ Vaccination

In situ vaccination (ISV) refers to inducing and stimulating an immune response specially at the tumor site (88). ISV uses the tumor itself as the antigen source and should be defined as a treatment process or strategy. There are several advantages of ISV. It is simple and cost-effective with minimal side effects, and it utilizes all tumor antigens in the tumor which minimizes immune escape. There is no need to identify antigens and adjuvant delivery is feasible and flexible. Besides, there is a great chance to obtain synergistic effect with other therapies (55). One limitation may be due to intratumoral injection, because some internal tumors will not be accessible to injection. As to breast cancer, the primary tumor is superficial, skin and regional lymph node recurrence is common. Therefore, breast cancer is quite accessible to injection, making it a good candidate for ISV.

Food and Drug Administraion (FDA) has approved a number of ISV-based cancer immunotherapies, such as Bacilus Calmette-Guerin (BCG) for *in situ* vaccination, toll-like receptor agonists for *in situ* vaccination, oncolytic virus for *in situ* vaccination, and *in situ* vaccination with cytokines and immune checkpoint blockade. ISV involves manipulation of intratumoral immune cells by injecting immunomodulators (89) and local ablative therapies which are used to release tumor antigens from the therapy-killed tumor cells (90). Besides, local treatment with vaccines and adjuvant is another option to provoke immune system in situ (91). The combination of ISV with other immnutherapies is likely to provide the optimal local control and systemic antitumor effect. Yokoi et al. treated mammary tumors with *in situ* immunomodulation consisting of intratumoral injections of Fms-like tyrosine kinase 3 receptor ligand to mobilize conventional type-1 dendritic cells (cDC1s), local irradiation to induce immunogenic tumor cell death, and TLR3/CD40 stimulation to activate cDC1s. Circulating effector T cells and CD8+ T cells infiltrated into metastatic brain lesions were increased and resistance to anti-PD-1 therapy was overcome, resulting in improved survival. Radiation can elicit systemic response which is known as abscopal effect, and the potential mechanism is to release tumor antigens in the process of ISV (92). Numerous clinical data supported the concept of radiation as an important part during *in situ* vaccine treatment (93–95), and clinical trials are underway investigating combination therapy of radiation with other immunotherapies (91). Combination therapy with noninvasive low intensity focused ultrasound and ablative radiation therapy was reported to generate an *in situ* tumor vaccine as well (96). Like radiation, heat (hyperthermia) has been used to damage targeted tumors and could be further combined with ISV (97). More approaches will be integrated into future multi-modality therapy.

Therapeutic Vaccines for Breast Cancer in Clinical Trials

The treatment for BC at different stages includes neoadjuvant therapy, adjuvant therapy for early BC, rescue therapy and maintenance therapy for advanced BC. Therapeutic vaccines for BC at different stages are summarized.

Neoadjuvant Setting

Disease at an early stage presents with a more intact immune system and a lower tumor burden, possibly affording vaccines the

potential to confer a more favorable outcome. Therapeutic vaccines in the neoadjuvant setting are the theoretically most likely method to optimize the immune microenvironment and improve prognosis. Cancer treatment starts with modulation of the microenvironment and promotion of antitumor immunity before any inhibition occurs to the immune system.

Mucin or MUC-1 is a transmembrane glycoprotein that is expressed in the lung, colon, breast, ovary, pancreas and other cancer tumor cells. MUC-1 is considered a promising candidate for vaccine development in BC. Tecemotide is a synthetic 27 amino acid lipopeptide used as an MUC-1 immunogen that is applied in clinical trials of prostate, NSCLC and colon cancer with promising effects. In a prospective, multicenter, randomized 2-arm academic phase II trial (ABCSG 34), tecemotide was added to neoadjuvant standard-of-care treatment in early BC patients. Approximately 400 patients with early BC were recruited into this trial. No significant difference was observed in residual cancer burden or overall pathological complete response (pCR) rates between the two groups. This trial demonstrated that MUC-1-based vaccination strategies are safe but did not show an improved treatment effect when added to standard treatment in the neoadjuvant setting (98). However, disease-free survival data are still premature and may provide further information. Interestingly, tumors which achieved a residual cancer burden (RCB) 0/I and a pCR had a higher concentration of intratumoural and stromal tumor-infiltrating lymphocytes in the pre-therapeutic biopsy than those which did not. Several ongoing studies address vaccines for BC in the neoadjuvant setting (NCT03387553, NCT02204098, NCT03564782, NCT03572361, NCT04144023). Further data are needed to determine whether neoadjuvant vaccine therapy can reduce the risk of recurrence and prolong relapse-free survival.

Adjuvant Setting

Further immune elimination of subclinical lesions is an important function of vaccines for BC after tumor resection. There have been a number of clinical studies of preventive vaccines in the field of adjuvant therapy.

In a pilot clinical trial of oxidized mannan–MUC-1 (M-FP) for the treatment of patients with stage II BC, the follow-up at 12-15 years showed that the recurrence rate was 12.5% (2/16) in the vaccine group compared with 60% (9/15) in the placebo group. M-FP also benefits the overall survival of stage II BC patients (99). In a phase II clinical trial (NCT02764333), a folate receptor alpha-based vaccine (TPIV200) was investigated in TNBC patients. In this trial, an immunologic response was elicited, and more data has not been exposed.

Peptide vaccines for HER2 have been explored in the adjuvant setting. The E75 peptide vaccine (nelipepimut-S), an HLA-A2/A3-restricted extracellular HER2-domain-derived peptide, is an MHC class I epitope (100). A series of trials in the adjuvant setting were conducted at approximately E75, demonstrating not only a good safety profile of the E75 peptide vaccine but also a superiority of immune response in BC patients with low HER2 expression than vaccinated patients with high levels of HER2 expression (101). Mittendorf et al. further examined schedule optimization according to lymph node (LN) status and risk of disease recurrence in a phase I/II clinical trial (69). Analysis of disease-free survival (DFS) revealed that patients who had tumors with low HER2 expression (immunohistochemistry score 1+ or 2+ with fluorescence *in situ* hybridization negativity) and had positive lymph nodes benefited the most from vaccination therapy. In a phase I/II trial, 187 LN-positive and high-risk LN-negative breast cancer (IHC score 1-3) patients were evaluated in the adjuvant setting. E75 patients with GM-CSF versus placebo were administered to 108 patients with HLA-A2/3- and 79 HLA-A2/3-negative patients, respectively. The results concluded that the 5-year DFS was improved for those who received E75 with respect to controls (89.7% vs 80.2%, P=0.08) (102). Given these promising data, in phase III clinical trials, the study assessed the effects of vaccination with E-75 plus subcutaneous GM-CSF relative to placebo in LN+ BC patients with low expression of HER2 in the adjuvant setting (103). However, no significant difference was found in DFS between the vaccine group and the control group, leading to the termination of the trial. Future clinical trials should be carried out to study the combination of vaccines with other medications. Several studies were conducted combining traustuzumab plus E75 in hope of a synergistic effect of active immunotherapy and passive immunotherapy (104). In phase IIb, multicenter, randomized, single-blinded, controlled trial (NCT01570036), the efficacy of the combination with E-75 plus traustuzumab was evaluated in patients with HER2 low-expressing BC in the adjuvant setting. No significant difference in DFS was seen in the HER2 low-expressing BC; however, significant clinical benefit was seen in patients with TNBC (105). These findings warrant further investigation in a phase III randomized trial. GP2 is a 9 amino acid-long peptide vaccine derived from the transmembrane domain of the HER/neu protein. It binds to the HLA-A2 molecule but has poor binding affinity compared to E75 (106). A phase II clinical trial was conducted to investigate GP2 vaccine efficacy in preventing recurrence in LN+ and high-risk LN- HER2 breast cancer patients (IHC 1+–3+) in the adjuvant setting. The results of the primary analysis did not show a significant difference in response to the vaccine compared to the control group in the rate of recurrence (70). However, patients who were vaccinated with GP2+GM-CSF had a significant increase in their delayed type hypersensitivity (DTH) reaction compared to pre-vaccination ($p<0.001$), the post-vaccination response was significantly greater in vaccinated patients than in control patients ($p<0.001$). In addition, ex vivo immune responses were assessed by phenotypic clonal expansion assays and by T cell functional assays. The GP2+GM-CSF vaccine induced significant increase in both clonal expansion as well as improved CTL function compared to pre-vaccine levels while GM-CSF alone had no such effect. A post for a prospective, randomized, single-blinded, placebo-controlled, multicenter phase IIb clinical trial was presented during the 2020 San Antonio Breast Cancer Symposium (SABCS) on December 09, 2020. This trial was completed in 2018, and Kaplan–Meier analysis of DFS for patients treated with GP2 immunotherapy showed 100% survival (0% breast cancer recurrence, p=0.0338) in the HER2/neu-positive adjuvant setting after a median of 5 years of follow-up. Greenwich LifeSciences

announced an update of the GP2 phase III clinical trial design at the 2021 American Association for Cancer Research (AACR) annual meeting.

Metastatic Setting

Most mBC cannot be cured by surgery and is highly dependent on systemic therapy. Therapeutic vaccines can be used in combination with other therapies as part of rescue therapy, and other studies are exploring their value as maintenance therapy for advanced breast cancer.

Therapeutic vaccines for rescue therapy for mBC have rarely been reported. Wilms tumor 1 (WT-1) is a protein with transcription factor activity involved in the maintenance of tissue homeostasis, possibly as an oncogene in BC. In a phase I clinical trial, WT-I vaccination activated WT-1-specific cytotoxic T lymphocytes (CTLs) and resulted in cancer regression with a good safety profile in 2 patients with BC with overexpression of the WT-1 gene and HLA-A*2402-possibility (107). Yang et al (108) enrolled 10 patients with advanced cancers, including mBC, and treated them with a DC-based WT-1 vaccination. Two patients had a partial response (PR), and three patients had stable disease (SD) with a disease control rate up to 50%. WT-1-specific CTL responses were enhanced in patients. CEA is overexpressed in BC and has attracted much attention as a target of vaccines. In a pilot study, the recombinant PANVAC poxviral vaccine (containing transgenes for CEA and MUC-1 and three T cell costimulatory molecules) was tested in 12 heavily pretreated metastatic BC patients. One patient demonstrated a CR lasting >37 months, and 4 patients had SD lasting >4 months. The median time to progression (TTP) was 2.5 months, and the median OS was 13.7 months (81). In another open-label phase II clinical trial, 48 patients with mBC were enrolled to receive treatment with either docetaxel with PANVAC or docetaxel alone. The median PFS was 7.9 months in the vaccination group vs 3.9 months in the docetaxel alone group, but the difference was not significant (p=0.09) (109). There was also no statistical correlation seen between the generation of TAA-specific immune responses in peripheral blood mononuclear cells and time to progression in either group. Takahashi et al. (110) developed a novel regimen of personalized peptide vaccination (PPV), in which vaccine antigens were selected and administered from a pool of 31 different peptide candidates based on the pre-existing immunoglobulin G (IgG) responses specific to peptides before vaccination. Based on previous results in cancer patients, they conducted a phase II study of PPV for metastatic recurrent breast cancer patients who had failed standard chemotherapies. Boosting of CTL and/or IgG responses was observed in most of the patients after vaccination. In addition, three CR cases and six PR cases were observed, irrespective of the BC subtypes. In a more recent early phase II study including 14 advanced metastatic triple-negative breast cancer (mTNBC) patients, the treatment protocol consisted of a weekly vaccination of mixed 19-peptide cancer vaccine monotherapy for 6 weeks. An increase in peptide-specific IgG was observed in all patients. The median OS was 11.5 months in all 14 patients and 24.4 months in the patients who completed the vaccination (111). Human telomerase reverse transcriptase (hTERT) is nearly universally overexpressed in human cancers and contributes critically to oncogenesis. A phase I clinical trial was performed to evaluate the HLA-A2-restricted hTERT I540 peptide presented with keyhole limpet hemocyanin (KLH) by ex vivo-generated autologous DCs. hTERT-specific T lymphocytes were induced in 4/7 patients after vaccination. PR was seen in 1 patient in association with the induction of CD8+ tumor infiltrating lymphocytes (112). In conclusion, although no prospective large-sample studies have confirmed the efficacy of therapeutic vaccines in the rescue therapy of advanced BC, some studies have preliminary results suggesting their effectiveness and possible prospects.

Immunosurveillance using therapeutic vaccines to trigger active immunity when remission is achieved through rescue therapy such as radiotherapy or chemotherapy suggests novel opportunities for both therapeutic and prophylactic vaccine strategies for cancer treatment. MAM-A is overexpressed in 40-80% of breast tumors. Tiriveedhi et al. (80) enrolled 14 mBC patients with stable disease and treated them randomly with the MAM-A vaccine or placebo in a phase I clinical trial. Although this trial was not powered to evaluate PFS, improved PFS was seen in vaccinated patients. A significant increase in the frequency of MAM-A-specific CD8+ T cells (0.9% + 0.5% vs 3.8% + 1.2%; $p<0.001$) and an increase in the number of MAM-A-specific IFNγ-secreting T cells (41 + 32 vs 215 + 67 spots per million cells (spm); $p<0.001$) were observed. Increased Siayl-TN (STn) expression, which is a carbohydrate epitope found on a variety of glycoproteins, including MUC-1, has been proven to be associated with the progression and poor prognosis of BC (113). Miled et al. (114) conducted the largest phase III clinical trial in 1028 mBC patients across 126 centers. Patients were administered a vaccine made of STn conjugated to the carrier protein KLH versus placebo. Although clinically significant antibody titers specific for STn were produced in patients, no significant improvement in TTP or OS was observed (115). Ibrahim et al. conducted a subgroup analysis in which patients who were also on endocrine therapy (ET) had longer TTP and OS than the control group. Moreover, vaccinated patients on ET with higher antibody responses had longer OS (41.3 vs 25.4 months; p=0.0147). In an open-label prospective study, 19 patients with mBC refractory to at least one conventional therapy were treated with the hTERT peptide vaccine, and hTERT-specific CD8+ T cells were detected after vaccination in the peripheral blood of patients and exhibited effector functions *in vitro*, including proliferation, IFN-gamma production, and tumor lysis. In this small sample study, the median OS was significantly longer in patients who achieved an immune response to hTERT peptide than in patients who did not (116). All the results above suggest that therapeutic vaccines are a potentially feasible option for maintenance therapy of advanced BC, but no mature vaccine has been proven to be beneficial in a large-sample clinical trial. Another important issue that should be considered is that the essential immune capability to recognize and activate antigens should be conserved before vaccination.

FUTURE PERSPECTIVES

Tumor Stage Specific Vaccine Strategy

During cancer clonal evolution, both selection and neutral growth may progress simultaneously within the same tumor,

but both styles of tumor progression may alter dynamically over time (117). Metastatic BC shows an increase in mutational burden and clonal diversity compared to early BC because genomic alterations are acquired during the evolution of cancers from their early stages (118). A multitude of epigenetic mechanisms, including DNA methylation, chromatin remodeling and posttranslational modification of histones, contribute to diversity within tumors, and the heterogeneity becomes extensive. Intratumor heterogeneity (ITH) is a key factor contributing to the lethal outcome of cancer, therapeutic failure, and drug resistance. Some claim that tumors with high heterogeneity may generate neoantigens that attract immune cells (119), but others argue that immune cells provide selection pressure that shapes tumor heterogeneity. High heterogeneity tumors are associated with higher subclonal diversity, less immune cell infiltration, less activation of the immune response, and worse survival in BC (120). Immune-infiltrated tumor regions exhibit either HLA loss of heterozygosity (LOH) or depletion of expressed neoantigens, which will eventually make it increasingly difficult to treat mBC with an immune strategy (120–122). Finding the right target antigen and intervening at the right time are the most important issues of active immunotherapy. The continuous evolution of the immune microenvironment during tumorigenesis also suggests that different modes of treatment should be considered at different stages.

Neoantigen profiles keep changing while tumor-specific mutations change during tumorigenesis and progression. Therefore, individual immune status, clonal heterogeneity and stage of disease should be fully considered, and time specificity should be realized.

Universal Vaccines

Optimal antigens should be developed from publicly mutated genes or high frequency overexpressed genes that are shared by a number of patients. A punch of such public antigens that are consumed to cover most patients with one type of cancer can be used to develop public vaccines, also named universal vaccines (123). Universal vaccines have the great advantage of convenient production and reduction in cost. In addition, preprepared vaccines that can be quickly inoculated into patients also save time and are more practical. The efficacy of universal vaccines should be ensured. One important problem should be considered except for the restriction of MHC molecules. That is, the proportion of tumor antigen expression in the population. Although more than 900,000 neoantigens have been identified through a wide examination of 20 tumor histotypes, only 24 neoantigens among a tiny fraction of patients have the potential to become public vaccines (124). Therefore, it is more feasible to develop public vaccines based on TAAs. Public vaccines have broad coverage and can improve the immune surveillance function of individuals to prevent tumor metastasis and recurrence. It is theoretically more suitable for the stage of neoadjuvant and adjuvant therapy.

From a single genome point of view, improving the antitumor effect of tumor-specific T cells and memory T cells is important for designing therapeutic vaccines. Personalized therapeutic vaccines targeting trunk or driver mutations are more effective and have a more comprehensive antitumor effect than those targeting companion or passenger mutations. In addition, the option of designing vaccines needs to be weighed between selecting a large number of target antigens to avoid immune escape and selecting antigens with good immunogenic potential.

Concurrent Therapies With Vaccination

Conventional therapies such as chemotherapy, radiotherapy and targeted therapy constantly promote the emergence of new subclones of tumor cells as a result of the pressure of clonal evolution, resulting in treatment failure. Immunotherapy, as a new therapeutic strategy, has a totally different effect on tumor heterogeneity from conventional therapy. However, patients who have received multiline conventional therapies can hardly benefit from immunotherapy. How to maximize the therapeutic effect of immunotherapy by rational arrangement of comprehensive therapy is an important direction in the future. In addition, how to exert antitumor effects of therapeutic vaccines synergistically with various therapeutic means is a hotspot. It was reported that sequential treatment with vaccine and PD-1 blockade was more effective than a simultaneous treatment regimen (125). In the PACIFIC trial, when durvalumab therapy was initiated within 14 days of completing chemoradiotherapy, better progression free survival was observed than when it was initiated after 14 days (126). Thus, timing is an important factor in obtaining abscopal effect and the optimal scheduling of vaccines, immunotherapy, radiation and chemotherapy needs to be clearly established, ideally through clinical trials. The TME is a major reason for the disappointing clinical results in addition to tumor-intrinsic resistance mechanisms, so an inflammatory TME is needed for sterile immunity (38). Except for what we have mentioned above about TME-targeting vaccines, *in situ* TME modulation strategies include stimulation of professional antigen presenting cells, combination with checkpoint inhibitors and depletion of regulatory T cells (Treg cells). PVX-410 (PVX) is a multipeptide vaccine targeting X-Box Binding Protein 1 (XBP1), and CD138 is overexpressed in TNBC. The synergistic effects of PVX-410 and ICI pembrolizumab will be evaluated in a clinical trial (NCT03362060) for TNBC. Another phase I clinical trial (NCT02826434) tested the synergistic effects of durvalumab and PVX-410 for TNBC. In this trial, the levels of CD8+ CTLs increased in patients 14 weeks after the first injection. The combination therapy strategy to work together with vaccines will include, but is not limited to, ICIs, antiangiogenic therapy, epigenetic regulation therapy, low intensity focused ultrasound (55) and conventional chemoradiotherapy. Cyclophosphamide to block Treg cells has been evaluated as a vaccine adjuvant in clinical trials (NCT03012100, NCT02938442). Several other ongoing trials are further assessing the application of various promising vaccination therapies in early and metastatic disease (**Table 2**).

TABLE 2 | Ongoing trials of tumor vaccine-based combination therapy for BCs (data from ClinicalTrials.gov).

Drug Regimen	NCT.gov Identifier	Sample Size	Phase; Status	Population
Neoantigen DNA Vaccine Durvalumab	NCT03199040	10	I; ANR	Clinical Stage T1c-T4c, Any N, M0 TNBC Prior to Neoadjuvant Chemotherapy, with Residual Invasive BC after Neoadjuvant Therapy
VRP-HER2 Pembrolizumab	NCT03632941	39	II; R	Advanced HER2-overexpressing BC
PVX-410 Pembrolizumab	NCT03362060	20	I; ANR	HLA-A2 + Metastatic TNBC
Galinpepimut-S Pembrolizumab	NCT03761914	90	I/II; R	Advanced Tumors including Advanced TNBC
RO7198457 Atezolizumab	NCT03289962	770	I; R	Advanced Tumors including Advanced TNBC
PVX-410 Durvalumab Hiltonol	NCT02826434	22	Ib; ANR	HLA-A2 + Subjects Following Standard Treatment of Stage II or III TNBC
Multiepitope Folate Receptor Alpha Peptide Vaccine Cyclophosphamide	NCT03012100	280	II; R	Stage I-III TNBC
NeuVax Vaccine Trastuzumab	NCT02297698	100	II; ANR	Stage I-III Noninflammatory, HER2+ High-risk BC
A Peptide Mimotope-based Vaccine P10s-PADRE with MONTANIDE™ ISA 51 VG Doxorubicin Cyclophosphamide Paclitaxel	NCT02938442	102	I/II; R	Stage I, II or III TNBC
AE37 Peptide vaccine Pembrolizumab	NCT04024800	29	II; ANR	Advanced TNBC
Dendritic Cell Vaccine Neoadjuvant Chemotherapy	NCT03387553	30	I; R	HER-2/neu Positive Invasive BC during Neoadjuvant Therapy
Anti-HER2/HER3 Dendritic Cell Vaccine Recombinant Interferon Alfa-2b Celecoxib Pembrolizumab	NCT04348747	23	IIa; NYR	Patients With Asymptomatic Brain Metastasis From TNBC or HER2+ BC
Personalized Synthetic Long Peptide Vaccine Carboplatin Durvalumab Gemcitabine Hydrochloride Nab-paclitaxel Tremelimumab	NCT03606967	70	II; R	Advanced TNBC
Multiepitope HER2 Peptide Vaccine TPIV100 Pertuzumab Trastuzumab	NCT04197687	480	II; R	HER2 Positive, Stage II-III BC in Patients With Residual Disease After Chemotherapy and Surgery
pUMVC3-IGFBP2-HER2-IGF1R Plasmid DNA Vaccine Paclitaxel Trastuzumab Pertuzumab	NCT04329065	16	II; R	BC during Neoadjuvant Therapy
Brachyury-TRICOM Entinostat M7824 Ado-trastuzumab emtansine	NCT04296942	65	I; R	Advacned BC
In Situ Vaccination With Flt3 L, Radiation, and Poly-ICLC Pembrolizumab	NCT03789097	56	I/II; R	Advanced, Measurable, Biopsy-accessible Cancers including BC
In Situ Vaccination Durvalumab Tremelimumab	NCT02643303	58	I/II; ANR	Advanced, Measurable, Biopsy-accessible Cancers including BC

ANR, active; not recruiting; NYR, not yet recruiting; R, recruiting.

CONCLUSION

In recent years, the application of therapeutic vaccines has been gradually accepted in the field of BC, but both the candidates and the efficacy need further study. Increasing attention has been given to the use of therapeutic vaccines to modulate the immune microenvironment and fully mobilize the body's own immune system for active immunotherapy. However, the exploration of therapeutic vaccines for BC is still in the early stage and is bound to be long based on considering the stage of disease, personal

immune status and clonal heterogeneity. Fully combining therapeutic vaccines with not only ICIs but also other multiple treatment methods may take great advantage in the future treatment of BC.

AUTHOR CONTRIBUTIONS

The corresponding authors are responsible for ensuring that the descriptions are accurate and agreed by all authors. Authors have contributed in multiple roles. LZ is responsible for writing original draft and literature search. XZ is responsible for literature search. HS is responsible for literature search. LX is responsible for conceptualization and supervision. BL is responsible for review and editing for original draft and supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by a grant from the National Natural Science Foundation of China (Grant No. 81803093). The funding sources had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2021) 71:209–49. doi: 10.3322/caac.21660
2. Kwapisz D. Pembrolizumab and Atezolizumab in Triple-Negative Breast Cancer. *Cancer Immunol Immunother* (2021) 70:607–17. doi: 10.1007/s00262-020-02736-z
3. Schreiber R, Old L, Smyth M. Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion. *Science (New York NY)* (2011) 331:1565–70. doi: 10.1126/science.1203486
4. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An Immunogenic Personal Neoantigen Vaccine for Patients With Melanoma. *Nature* (2017) 547:217–21. doi: 10.1038/nature22991
5. Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, et al. Neoantigen Vaccine Generates Intratumoral T Cell Responses in Phase Ib Glioblastoma Trial. *Nature* (2019) 565:234–9. doi: 10.1038/s41586-018-0792-9
6. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer. *N Engl J Med* (2010) 363:411–22. doi: 10.1056/NEJMoa1001294
7. Fragomeni SM, Sciallis A, Jeruss JS. Molecular Subtypes and Local-Regional Control of Breast Cancer. *Surg Oncol Clin N Am* (2018) 27:95–120. doi: 10.1016/j.soc.2017.08.005
8. Tolba MF, Omar HA. Immunotherapy, an Evolving Approach for the Management of Triple Negative Breast Cancer: Converting non-Responders to Responders. *Crit Rev Oncol Hematol* (2018) 122:202–7. doi: 10.1016/j.critrevonc.2018.01.005
9. Hollingsworth R, Jansen K. Turning the Corner on Therapeutic Cancer Vaccines. *NPJ Vaccines* (2019) 4:7. doi: 10.1038/s41541-019-0103-y
10. Buonaguro L, Petrizzo A, Tornesello M, Buonaguro F. Translating Tumor Antigens Into Cancer Vaccines. *Clin Vaccine Immunol CVI* (2011) 18:23–34. doi: 10.1128/cvi.00286-10
11. Lim S, Zhang Y, Zhang J. Cancer-Testis Antigens: The Current Status on Antigen Regulation and Potential Clinical Use. *Am J Blood Res* (2012) 2:29–35.
12. Otte M, Zafrakas M, Riethdorf L, Pichlmeier U, Löning T, Jänicke F, et al. MAGE-A Gene Expression Pattern in Primary Breast Cancer. *Cancer Res* (2001) 61:6682–7.
13. Vodolazhsky D, Kutilin D, Mogushkova K, Kit O. Specific Features of Transcription Activity of Cancer-Testis Antigens in Patients With Metastatic and Non-Metastatic Breast Cancer. *Bull Exp Biol Med* (2018) 165:382–5. doi: 10.1007/s10517-018-4175-x
14. Kondo Y, Fukuyama T, Yamamura R, Futawatari N, Ichiki Y, Tanaka Y, et al. Detection of KK-LC-1 Protein, a Cancer/Testis Antigen, in Patients With Breast Cancer. *Anticancer Res* (2018) 38:5923–8. doi: 10.21873/anticanres.12937
15. Egland KA, Kumar V, Duray P, Pastan I. Characterization of Overlapping XAGE-1 Transcripts Encoding a Cancer Testis Antigen Expressed in Lung, Breast, and Other Types of Cancers. *Mol Cancer Ther* (2002) 1:441–50.
16. Sakai Y, Kurose K, Sakaeda K, Abo H, Atarashi Y, Ide N, et al. A Novel Automated Immunoassay for Serum NY-ESO-1 and XAGE1 Antibodies in Combinatory Prediction of Response to Anti-Programmed Cell Death-1 Therapy in non-Small-Cell Lung Cancer. *Clinica chimica acta; Int J Clin Chem* (2021) 519:51–9. doi: 10.1016/j.cca.2021.04.008
17. Kawakami Y, Eliyahu S, Sakaguchi K, Robbins P, Rivoltini L, Yannelli J, et al. Identification of the Immunodominant Peptides of the MART-1 Human Melanoma Antigen Recognized by the Majority of HLA-A2-Restricted Tumor Infiltrating Lymphocytes. *J Exp Med* (1994) 180:347–52. doi: 10.1084/jem.180.1.347
18. Bakker A, Schreurs M, De Boer A, Kawakami Y, Rosenberg S, Adema G, et al. Melanocyte Lineage-Specific Antigen Gp100 is Recognized by Melanoma-Derived Tumor-Infiltrating Lymphocytes. *J Exp Med* (1994) 179:1005–9. doi: 10.1084/jem.179.3.1005
19. Corman J, Sercarz E, Nanda N. Recognition of Prostate-Specific Antigenic Peptide Determinants by Human CD4 and CD8 T Cells. *Clin Exp Immunol* (1998) 114:166–72. doi: 10.1046/j.1365-2249.1998.00678.x
20. Tsang K, Zaremba S, Nieroda C, Zhu M, Hamilton J, Schlom J. Generation of Human Cytotoxic T Cells Specific for Human Carcinoembryonic Antigen Epitopes From Patients Immunized With Recombinant Vaccinia-CEA Vaccine. *J Natl Cancer Institute* (1995) 87:982–90. doi: 10.1093/jnci/87.13.982
21. Seil I, Frei C, Sültmann H, Knauer S, Engels K, Jäger E, et al. The Differentiation Antigen NY-BR-1 is a Potential Target for Antibody-Based Therapies in Breast Cancer. *Int J Cancer* (2007) 120:2635–42. doi: 10.1002/ijc.22620
22. Balafoutas D, Zur Hausen A, Mayer S, Hirschfeld M, Jaeger M, Denschlag D, et al. Cancer Testis Antigens and NY-BR-1 Expression in Primary Breast Cancer: Prognostic and Therapeutic Implications. *BMC Cancer* (2013) 13:271. doi: 10.1186/1471-2407-13-271
23. Fisk B, Blevins T, Wharton J, Ioannides C. Identification of an Immunodominant Peptide of HER-2/Neu Protooncogene Recognized by Ovarian Tumor-Specific Cytotoxic T Lymphocyte Lines. *J Exp Med* (1995) 181:2109–17. doi: 10.1084/jem.181.6.2109
24. Segal-Eiras A, Croce M. Breast Cancer Associated Mucin: A Review. *Allergol Immunopathol* (1997) 25:176–81.
25. Vonderheide R, Hahn W, Schultze J, Nadler L. The Telomerase Catalytic Subunit is a Widely Expressed Tumor-Associated Antigen Recognized by Cytotoxic T Lymphocytes. *Immunity* (1999) 10:673–9. doi: 10.1016/s1074-7613(00)80066-7
26. Andersen M, Pedersen L, Becker J, Straten P. Identification of a Cytotoxic T Lymphocyte Response to the Apoptosis Inhibitor Protein Survivin in Cancer Patients. *Cancer Res* (2001) 61:869–72.
27. Peng M, Mo Y, Wang Y, Wu P, Zhang Y, Xiong F, et al. Neoantigen Vaccine: An Emerging Tumor Immunotherapy. *Mol Cancer* (2019) 18:128. doi: 10.1186/s12943-019-1055-6
28. Mardis ER. Neoantigens and Genome Instability: Impact on Immunogenomic Phenotypes and Immunotherapy Response. *Genome Med* (2019) 11:71. doi: 10.1186/s13073-019-0684-0

29. Zhang X, Kim S, Hundal J, Herndon JM, Li S, Petti AA, et al. Breast Cancer Neoantigens Can Induce CD8(+) T-Cell Responses and Antitumor Immunity. *Cancer Immunol Res* (2017) 5:516–23. doi: 10.1158/2326-6066.Cir-16-0264
30. Hwang S, Kim E, Lee I, Marcotte E. Systematic Comparison of Variant Calling Pipelines Using Gold Standard Personal Exome Variants. *Sci Rep* (2015) 5:17875. doi: 10.1038/srep17875
31. Xu C. A Review of Somatic Single Nucleotide Variant Calling Algorithms for Next-Generation Sequencing Data. *Comput Struct Biotechnol J* (2018) 16:15–24. doi: 10.1016/j.csbj.2018.01.003
32. Karasaki T, Nagayama K, Kuwano H, Nitadori J, Sato M, Anraku M, et al. Prediction and Prioritization of Neoantigens: Integration of RNA Sequencing Data With Whole-Exome Sequencing. *Cancer Sci* (2017) 108:170–7. doi: 10.1111/cas.13131
33. Smart A, Margolis C, Pimentel H, He M, Miao D, Adeegbe D, et al. Intron Retention is a Source of Neoepitopes in Cancer. *Nat Biotechnol* (2018) 36:1056–8. doi: 10.1038/nbt.4239
34. Rathe S, Popescu F, Johnson J, Watson A, Marko T, Moriarity B, et al. Identification of Candidate Neoantigens Produced by Fusion Transcripts in Human Osteosarcomas. *Sci Rep* (2019) 9:358. doi: 10.1038/s41598-018-36840-z
35. Hombrink P, Hassan C, Kester M, De Ru A, Van Bergen C, Nijveen H, et al. Discovery of T Cell Epitopes Implementing HLA-Peptidomics Into a Reverse Immunology Approach. *J Immunol (Baltimore Md. 1950)* (2013) 190:3869–77. doi: 10.4049/jimmunol.1202351
36. Rammensee HG, Singh-Jasuja H. HLA Ligandome Tumor Antigen Discovery for Personalized Vaccine Approach. *Expert Rev Vaccines* (2013) 12:1211–7. doi: 10.1586/14760584.2013.836911
37. Joglekar A, Leonard M, Jeppson J, Swift M, Li G, Wong S, et al. T Cell Antigen Discovery via Signaling and Antigen-Presenting Bifunctional Receptors. *Nat Methods* (2019) 16:191–8. doi: 10.1038/s41592-018-0304-8
38. Gordon B, Gadi V. The Role of the Tumor Microenvironment in Developing Successful Therapeutic and Secondary Prophylactic Breast Cancer Vaccines. *Vaccines* (2020) 8:529. doi: 10.3390/vaccines8030529
39. Yan HX, Cheng P, Wei HY, Shen GB, Fu LX, Ni J, et al. Active Immunotherapy for Mouse Breast Cancer With Irradiated Whole-Cell Vaccine Expressing VEGFR2. *Oncol Rep* (2013) 29:1510–6. doi: 10.3892/or.2013.2282
40. Zhao X, Bose A, Komita H, Taylor JL, Chi N, Lowe DB, et al. Vaccines Targeting Tumor Blood Vessel Antigens Promote CD8(+) T Cell-Dependent Tumor Eradication or Dormancy in HLA-A2 Transgenic Mice. *J Immunol* (2012) 188:1782–8. doi: 10.4049/jimmunol.1101644
41. Lu Y, Wei YQ, Tian L, Zhao X, Yang L, Hu B, et al. Immunogene Therapy of Tumors With Vaccine Based on Xenogeneic Epidermal Growth Factor Receptor. *J Immunol* (2003) 170:3162–70. doi: 10.4049/jimmunol.170.6.3162
42. Wood LM, Pan ZK, Guirnalda P, Tsai P, Seavey M, Paterson Y. Targeting Tumor Vasculature With Novel Listeria-Based Vaccines Directed Against CD105. *Cancer Immunol Immunother* (2011) 60:931–42. doi: 10.1007/s00262-011-1002-x
43. Kaplan CD, Kruger JA, Zhou H, Luo Y, Xiang R, Reisfeld RA. A Novel DNA Vaccine Encoding PDGFRbeta Suppresses Growth and Dissemination of Murine Colon, Lung and Breast Carcinoma. *Vaccine* (2006) 24:6994–7002. doi: 10.1016/j.vaccine.2006.04.071
44. Jin D, Yu X, Chen B, Li Z, Ding J, Zhao X, et al. Combined Immunotherapy of Breast Cancer With EGF and VEGF Vaccines From DNA Shuffling in a Mouse Model. *Immunotherapy* (2017) 9:537–53. doi: 10.2217/imt-2017-0004
45. Sung Kim T, Cohen EP. Immunity to Breast Cancer in Mice Immunized With Fibroblasts Transfected With a cDNA Expression Library Derived From Small Numbers of Breast Cancer Cells. *Cancer Gene Ther* (2005) 12:890–9. doi: 10.1038/sj.cgt.7700853
46. Kim TS, Jung MY, Cho D, Cohen EP. Prolongation of the Survival of Breast Cancer-Bearing Mice Immunized With GM-CSF-Secreting Syngeneic/Allogeneic Fibroblasts Transfected With a cDNA Expression Library From Breast Cancer Cells. *Vaccine* (2006) 24:6564–73. doi: 10.1016/j.vaccine.2006.06.012
47. Xia Q, Zhang FF, Geng F, Liu CL, Xu P, Lu ZZ, et al. Anti-Tumor Effects of DNA Vaccine Targeting Human Fibroblast Activation Protein Alpha by Producing Specific Immune Responses and Altering Tumor Microenvironment in the 4T1 Murine Breast Cancer Model. *Cancer Immunol Immunother* (2016) 65:613–24. doi: 10.1007/s00262-016-1827-4
48. Andersen M. The T-Win® Technology: Immune-Modulating Vaccines. *Semin Immunopathol* (2019) 41:87–95. doi: 10.1007/s00281-018-0695-8
49. Palena C, Polev D, Tsang K, Fernando R, Litzinger M, Krukovskaya L, et al. The Human T-Box Mesodermal Transcription Factor Brachyury is a Candidate Target for T-Cell-Mediated Cancer Immunotherapy. *Clin Cancer Res* (2007) 13:2471–8. doi: 10.1158/1078-0432.Ccr-06-2353
50. Quaglino E, Conti L, Cavallo F. Breast Cancer Stem Cell Antigens as Targets for Immunotherapy. *Semin Immunol* (2020) 47:101386. doi: 10.1016/j.smim.2020.101386
51. Chiang CL, Benencia F, Coukos G. Whole Tumor Antigen Vaccines. *Semin Immunol* (2010) 22:132–43. doi: 10.1016/j.smim.2010.02.004
52. Liu W, Tang H, Li L, Wang X, Yu Z, Li J. Peptide-Based Therapeutic Cancer Vaccine: Current Trends in Clinical Application. *Cell Prolif* (2021) 54:e13025. doi: 10.1111/cpr.13025
53. Supabphol S, Li L, Goedegebuure SP, Gillanders WE. Neoantigen Vaccine Platforms in Clinical Development: Understanding the Future of Personalized Immunotherapy. *Expert Opin Investig Drugs* (2021) 30:1–13. doi: 10.1080/13543784.2021.1896702
54. Perez CR, De Palma M. Engineering Dendritic Cell Vaccines to Improve Cancer Immunotherapy. *Nat Commun* (2019) 105408. doi: 10.1038/s41467-019-13368-y
55. Sheen MR, Fiering S. *In Situ* Vaccination: Harvesting Low Hanging Fruit on the Cancer Immunotherapy Tree. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* (2019) 11:e1524. doi: 10.1002/wnan.1524
56. González FE, Gleisner A, Falcón-Beas F, Osorio F, López MN, Salazar-Onfray F. Tumor Cell Lysates as Immunogenic Sources for Cancer Vaccine Design. *Hum Vaccin Immunother* (2014) 10:3261–9. doi: 10.4161/21645515.2014.982996
57. Sanjay S, Patel JM, Bozeman EN, Imasuen IE, Sara H, Danielle D, et al. Allogeneic Tumor Cell Vaccines: The Promise and Limitations in Clinical Trials. *Hum Vaccines Immunother* (2013) 10:52–63. doi: 10.4161/hv.26568
58. Emens LA. Breast Cancer Vaccines: Maximizing Cancer Treatment by Tapping Into Host Immunity. *Endocr Related Cancer* (2005) 12:1. doi: 10.1677/erc.1.00671
59. Simons JW, Sacks N. Granulocyte-Macrophage Colony-Stimulating Factortransduced Allogeneic Cancer Cellular Immunotherapy: The GVAX Vaccine for Prostate Cancer. *Urol Oncol Semin Orig Invest* (2006) 24:419–24. doi: 10.1016/j.urolonc.2005.08.021
60. Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, et al. Vaccination With Irradiated Tumor Cells Engineered to Secrete Murine Granulocyte-Macrophage Colony-Stimulating Factor Stimulates Potent, Specific, and Long-Lasting Anti-Tumor Immunity. *Proc Natl Acad Sci USA* (1993) 90:3539–43. doi: 10.1073/pnas.90.8.3539
61. Kurtz SL, Ravindranathan S, Zaharoff D. Current Status of Autologous Breast Tumor Cell-Based Vaccines. *Expert Rev Vaccines* (2014) 13:1439–45. doi: 10.1586/14760584.2014.969714
62. Anderson K, Erick T, Chen M, Daley H, Campbell M, Colson Y, et al. The Feasibility of Using an Autologous GM-CSF-Secreting Breast Cancer Vaccine to Induce Immunity in Patients With Stage II-III and Metastatic Breast Cancers. *Breast Cancer Res Treat* (2022). doi: 10.1007/s10549-022-06562-y
63. Emens LA, Asquith JM, Leatherman JM, Kobrin BJ, Petrik S, Laiko M, et al. Timed Sequential Treatment With Cyclophosphamide, Doxorubicin, and an Allogeneic Granulocyte-Macrophage Colony-Stimulating Factor–Secreting Breast Tumor Vaccine: A Chemotherapy Dose-Ranging Factorial Study of Safety and Immune Activation. *J Clin Oncol* (2009) 27:5911. doi: 10.1200/JCO.2009.23.3494
64. Dols A, Smith JW, Meijer SL, Fox BA, Hu HM, Walker E, et al. Vaccination of Women With Metastatic Breast Cancer, Using a Costimulatory Gene (CD80)-Modified, HLA-A2-Matched, Allogeneic, Breast Cancer Cell Line: Clinical and Immunological Results. *Hum Gene Ther* (2003) 14:1117–23. doi: 10.1089/104303403322124828
65. Chen G, Gupta R, Petrik S, Laiko M, Leatherman J, Asquith J, et al. A Feasibility Study of Cyclophosphamide, Trastuzumab, and an Allogeneic GM-CSF-Secreting Breast Tumor Vaccine for HER2+ Metastatic Breast

Cancer. *Cancer Immunol Res* (2014) 2:949–61. doi: 10.1158/2326-6066.Cir-14-0058
66. Zaks TZ, Rosenberg SA. Immunization With a Peptide Epitope (P369-377) From HER-2/Neu Leads to Peptide-Specific Cytotoxic T Lymphocytes That Fail to Recognize HER-2/Neu+ Tumors. *Cancer Res* (1998) 58:4902–8.
67. Knutson KL, Schiffman K, Cheever MA, Disis ML. Immunization of Cancer Patients With a HER-2/Neu, HLA-A2 Peptide, P369-377, Results in Short-Lived Peptide-Specific Immunity. *Clin Cancer Res* (2002) 8:1014–8.
68. Carmichael MG, Benavides LC, Holmes JP, Gates JD, Mittendorf EA, Ponniah S, et al. Results of the First Phase 1 Clinical Trial of the HER-2/Neu Peptide (GP2) Vaccine in Disease-Free Breast Cancer Patients: United States Military Cancer Institute Clinical Trials Group Study I-04. *Cancer* (2010) 116:292–301. doi: 10.1002/cncr.24756
69. Mittendorf EA, Clifton GT, Holmes JP, Clive KS, Patil R, Benavides LC, et al. Clinical Trial Results of the HER-2/Neu (E75) Vaccine to Prevent Breast Cancer Recurrence in High-Risk Patients: From US Military Cancer Institute Clinical Trials Group Study I-01 and I-02. *Cancer* (2012) 118:2594–602. doi: 10.1002/cncr.26574
70. Mittendorf EA, Ardavanis A, Litton JK, Shumway NM, Hale DF, Murray JL, et al. Primary Analysis of a Prospective, Randomized, Single-Blinded Phase II Trial Evaluating the HER2 Peptide GP2 Vaccine in Breast Cancer Patients to Prevent Recurrence. *Oncotarget* (2016) 7:66192–201. doi: 10.18632/oncotarget.11751
71. Miggelbrink A, Jackson J, Lorrey S, Srinivasan E, Waibl Polania J, Wilkinson D, et al. CD4 T-Cell Exhaustion: Does it Exist and What are its Roles in Cancer? *Clin Cancer Res* (2021) 27:5742–52. doi: 10.1158/1078-0432.Ccr-21-0206
72. Zhang H, Hong H, Li D, Ma S, Di Y, Stoten A, et al. Comparing Pooled Peptides With Intact Protein for Accessing Cross-Presentation Pathways for Protective CD8+ and CD4+ T Cells. *J Biol Chem* (2009) 284:9184–91. doi: 10.1074/jbc.M809456200
73. Melief C, van der Burg S. Immunotherapy of Established (Pre)Malignant Disease by Synthetic Long Peptide Vaccines. *Nat Rev Cancer* (2008) 8:351–60. doi: 10.1038/nrc2373
74. Bijker M, Van Den Eeden S, Franken K, Melief C, van der Burg S, Offringa R. Superior Induction of Anti-Tumor CTL Immunity by Extended Peptide Vaccines Involves Prolonged, DC-Focused Antigen Presentation. *Eur J Immunol* (2008) 38:1033–42. doi: 10.1002/eji.200737995
75. Lefrançois L, Marzo A, Williams K. Sustained Response Initiation is Required for T Cell Clonal Expansion But Not for Effector or Memory Development *In Vivo*. *J Immunol (Baltimore Md. 1950)* (2003) 171:2832–9. doi: 10.4049/jimmunol.171.6.2832
76. Obst R, Van Santen H, Melamed R, Kamphorst A, Benoist C, Mathis D. Sustained Antigen Presentation can Promote an Immunogenic T Cell Response, Like Dendritic Cell Activation. *Proc Natl Acad Sci USA* (2007) 104:15460–5. doi: 10.1073/pnas.0707331104
77. Li WH, Li YM. Chemical Strategies to Boost Cancer Vaccines. *Chem Rev* (2020) 120:11420–78. doi: 10.1021/acs.chemrev.9b00833
78. Lopes A, Bastiancich C, Bausart M, Ligot S, Lambricht L, Vanvarenberg K, et al. New Generation of DNA-Based Immunotherapy Induces a Potent Immune Response and Increases the Survival in Different Tumor Models. *J Immunother Cancer* (2021) 9. doi: 10.1136/jitc-2020-001243
79. Norell H, Poschke I, Charo J, Wei W, Erskine C, Piechocki M, et al. Vaccination With a Plasmid DNA Encoding HER-2/Neu Together With Low Doses of GM-CSF and IL-2 in Patients With Metastatic Breast Carcinoma: A Pilot Clinical Trial. *J Trans Med* (2010) 8:53. doi: 10.1186/1479-5876-8-53
80. Tiriveedhi V, Tucker N, Herndon J, Li L, Sturmoski M, Ellis M, et al. Safety and Preliminary Evidence of Biologic Efficacy of a Mammaglobin-a DNA Vaccine in Patients With Stable Metastatic Breast Cancer. *Clin Cancer Res* (2014) 20:5964–75. doi: 10.1158/1078-0432.Ccr-14-0059
81. Mohebtash M, Tsang K, Madan R, Huen N, Poole D, Jochems C, et al. A Pilot Study of MUC-1/CEA/TRICOM Poxviral-Based Vaccine in Patients With Metastatic Breast and Ovarian Cancer. *Clin Cancer Res* (2011) 17:7164–73. doi: 10.1158/1078-0432.Ccr-11-0649
82. Dissanayake D, Murakami K, Tran MD, Elford AR, Millar DG, Ohashi PS. Peptide-Pulsed Dendritic Cells Have Superior Ability to Induce Immune-Mediated Tissue Destruction Compared to Peptide With Adjuvant. *PLoS One* (2014) 9:e92380. doi: 10.1371/journal.pone.0092380
83. Kugler A, Stuhler G, Walden P, Zöller G, Zobywalski A, Brossart P, et al. Regression of Human Metastatic Renal Cell Carcinoma After Vaccination With Tumor Cell-Dendritic Cell Hybrids. *Nat Med* (2000) 6:332–6. doi: 10.1038/73193
84. Morse M, Hobeika A, Osada T, Niedzwiecki D, Marcom P, Blackwell K, et al. Long Term Disease-Free Survival and T Cell and Antibody Responses in Women With High-Risk Her2+ Breast Cancer Following Vaccination Against Her2. *J Trans Med* (2007) 5:42. doi: 10.1186/1479-5876-5-42
85. Avigan D, Vasir B, Gong J, Borges V, Wu Z, Uhl L, et al. Fusion Cell Vaccination of Patients With Metastatic Breast and Renal Cancer Induces Immunological and Clinical Responses. *Clin Cancer Res* (2004) 10:4699–708. doi: 10.1158/1078-0432.Ccr-04-0347
86. Gelao L, Criscitiello C, Esposito A, De Laurentiis M, Fumagalli L, Locatelli M, et al. Dendritic Cell-Based Vaccines: Clinical Applications in Breast Cancer. *Immunotherapy* (2014) 6:349–60. doi: 10.2217/imt.13.169
87. Zhang P, Yi S, Li X, Liu R, Jiang H, Huang Z, et al. Preparation of Triple-Negative Breast Cancer Vaccine Through Electrofusion With Day-3 Dendritic Cells. *PLoS One* (2014) 9:e102197. doi: 10.1371/journal.pone.0102197
88. Pierce R, Campbell J, Pai S, Brody J, Kohrt H. *In-Situ* Tumor Vaccination: Bringing the Fight to the Tumor. *Hum Vaccines Immunother* (2015) 11:1901–9. doi: 10.1080/21645515.2015.1049779
89. Yokoi T, Oba T, Kajihara R, Abrams S, Ito F. Local, Multimodal Intralesional Therapy Renders Distant Brain Metastases Susceptible to PD-L1 Blockade in a Preclinical Model of Triple-Negative Breast Cancer. *Sci Rep* (2021) 11:21992. doi: 10.1038/s41598-021-01455-4
90. Savage T, Pandey S, Guha C. Postablation Modulation After Single High-Dose Radiation Therapy Improves Tumor Control *via* Enhanced Immunomodulation. *Clin Cancer* (2020) 26:910–21. doi: 10.1158/1078-0432.Ccr-18-3518
91. Cadena A, Cushman T, Anderson C, Barsoumian H, Welsh J, Cortez M. Radiation and Anti-Cancer Vaccines: A Winning Combination. *Vaccines* (2018) 6:9. doi: 10.3390/vaccines6010009
92. Demaria S, Ng B, Devitt M, Babb J, Kawashima N, Liebes L, et al. Ionizing Radiation Inhibition of Distant Untreated Tumors (Abscopal Effect) is Immune Mediated. *Int J Radiat Oncol Biol Phys* (2004) 58:862–70. doi: 10.1016/j.ijrobp.2003.09.012
93. Hiniker S, Chen D, Reddy S, Chang D, Jones J, Mollick J, et al. A Systemic Complete Response of Metastatic Melanoma to Local Radiation and Immunotherapy. *Trans Oncol* (2012) 5:404–7. doi: 10.1593/tlo.12280
94. Grimaldi A, Simeone E, Giannarelli D, Muto P, Falivene S, Borzillo V, et al. Abscopal Effects of Radiotherapy on Advanced Melanoma Patients Who Progressed After Ipilimumab Immunotherapy. *Oncoimmunology* (2014) 3:e28780. doi: 10.4161/onci.28780
95. Theurich S, Rothschild S, Hoffmann M, Fabri M, Sommer A, Garcia-Marquez M, et al. Local Tumor Treatment in Combination With Systemic Ipilimumab Immunotherapy Prolongs Overall Survival in Patients With Advanced Malignant Melanoma. *Cancer Immunol Res* (2016) 4:744–54. doi: 10.1158/2326-6066.Cir-15-0156
96. Skalina K, Singh S, Chavez C, Macian F, Guha C. Low Intensity Focused Ultrasound (LOFU)-Mediated Acoustic Immune Priming and Ablative Radiation Therapy for *in Situ* Tumor Vaccines. *Sci Rep* (2019) 9:15516. doi: 10.1038/s41598-019-51332-4
97. Ito A, Honda H, Kobayashi T. Cancer Immunotherapy Based on Intracellular Hyperthermia Using Magnetite Nanoparticles: A Novel Concept of "Heat-Controlled Necrosis" With Heat Shock Protein Expression. *Cancer Immunol Immunother CII* (2006) 55:320–8. doi: 10.1007/s00262-005-0049-y
98. Singer CF, Pfeiler G, Hubalek M, Bartsch R, Stöger H, Pichler A, et al. Efficacy and Safety of the Therapeutic Cancer Vaccine Tecemotide (L-BLP25) in Early Breast Cancer: Results From a Prospective, Randomised, Neoadjuvant Phase II Study (ABCSG 34). *Eur J Cancer* (2020) 132:43–52. doi: 10.1016/j.ejca.2020.03.018
99. Vassilaros S, Tsibanis A, Tsikkinis A, Pietersz GA, Mckenzie IF, Apostolopoulos V. Up to 15-Year Clinical Follow-Up of a Pilot Phase III Immunotherapy Study in Stage II Breast Cancer Patients Using Oxidized Mannan-MUC1. *Immunotherapy* (2013) 5:1177–82. doi: 10.2217/imt.13.126

100. Kawashima I, Hudson SJ, Tsai V, Southwood S, Takesako K, Appella E, et al. The Multi-Epitope Approach for Immunotherapy for Cancer: Identification of Several CTL Epitopes From Various Tumor-Associated Antigens Expressed on Solid Epithelial Tumors. *Hum Immunol* (1998) 59:1–14. doi: 10.1016/s0198-8859(97)00255-3
101. Peoples G,E. Clinical Trial Results of a HER2/neu (E75) Vaccine to Prevent Recurrence in High-Risk Breast Cancer Patients. *J Clin Oncol* (2005) 23:7536–45. doi: 10.1200/JCO.2005.03.047
102. Mittendorf EA, Clifton GT, Holmes JP, Schneble E, Van Echo D, Ponniah S, et al. Final Report of the Phase I/II Clinical Trial of the E75 (Nelipepimut-S) Vaccine With Booster Inoculations to Prevent Disease Recurrence in High-Risk Breast Cancer Patients. *Ann Oncol* (2014) 25:1735–42. doi: 10.1093/annonc/mdu211
103. Mittendorf EA, Lu B, Melisko M, Hiller JP, Bondarenko I, Brunt AM, et al. Efficacy and Safety Analysis of Nelipepimut-S Vaccine to Prevent Breast Cancer Recurrence: A Randomized, Multicenter, Phase III Clinical Trial. *Clin Cancer Res* (2019) 25:4248–54. doi: 10.1158/1078-0432.CCR-18-2867
104. Gall VA, Philips AV, Na Q, Clise-Dwyer K, Mittendorf EA. Trastuzumab Increases HER2 Uptake and Cross-Presentation by Dendritic Cells. *Cancer Res* (2017) 77:5374–83. doi: 10.1158/0008-5472.CAN-16-2774
105. Clifton G, Hale D, Vreeland T, Hickerson A, Litton J, Alatrash G, et al. Results of a Randomized Phase IIb Trial of Nelipepimut-S + Trastuzumab Versus Trastuzumab to Prevent Recurrences in Patients With High-Risk HER2 Low-Expressing Breast Cancer. *Clin Cancer Res* (2020) 26:2515–23. doi: 10.1158/1078-0432.Ccr-19-2741
106. Ayoub NM, Al-Shami KM, Yaghan RJ. Immunotherapy for HER2-Positive Breast Cancer: Recent Advances and Combination Therapeutic Approaches. *Breast Cancer: Targets Ther Volume* (2019) 11:53–69. doi: 10.2147/BCTT.S175360
107. Oka Y, Tsuboi A, Taguchi T, Osaki T, Kyo T, Nakajima H, et al. Induction of WT1 (Wilms' Tumor Gene)-Specific Cytotoxic T Lymphocytes by WT1 Peptide Vaccine and the Resultant Cancer Regression. *Proc Natl Acad Sci USA* (2004) 101:13885–90. doi: 10.1073/pnas.0405884101
108. Zhang W, Lu X, Cui P, Piao C, Xiao M, Liu X, et al. Phase I/II Clinical Trial of a Wilms' Tumor 1-Targeted Dendritic Cell Vaccination-Based Immunotherapy in Patients With Advanced Cancer. *Cancer Immunol Immunother* (2019) 68:121–30. doi: 10.1007/s00262-018-2257-2
109. Heery CR, Ibrahim NK, Arlen PM, Mohebtash M, Murray JL, Koenig K, et al. Docetaxel Alone or in Combination With a Therapeutic Cancer Vaccine (PANVAC) in Patients With Metastatic Breast Cancer: A Randomized Clinical Trial. *JAMA Oncol* (2015) 11087–95. doi: 10.1001/jamaoncol.2015.2736
110. Takahashi R, Toh U, Iwakuma N, Takenaka M, Otsuka H, Furukawa M, et al. Feasibility Study of Personalized Peptide Vaccination for Metastatic Recurrent Triple-Negative Breast Cancer Patients. *Breast Cancer Res* (2014) 16:R70. doi: 10.1186/bcr3685
111. Toh U, Sakurai S, Saku S, Takao Y, Okabe M, Iwakuma N, et al. Early Phase II Study of Mixed 19-Peptide Vaccine Monotherapy for Refractory Triple-Negative Breast Cancer. *Cancer Sci* (2020) 111:2760–9. doi: 10.1111/cas.14510
112. Vonderheide R, Domchek S, Schultze J, George D, Hoar K, Chen D, et al. Vaccination of Cancer Patients Against Telomerase Induces Functional Antitumor CD8+ T Lymphocytes. *Clin Cancer* (2004) 10:828–39. doi: 10.1158/1078-0432.ccr-0620-3
113. Kinney A, Sahin A, Vernon S, Frankowski R, Annegers J, Hortobagyi G, et al. The Prognostic Significance of Sialyl-Tn Antigen in Women Treated With Breast Carcinoma Treated With Adjuvant Chemotherapy. *Cancer* (1997) 80:2240–9. doi: 10.1002/(sici)1097-0142(19971215)80:12<2240::aid-cncr4>3.0.co;2-y
114. Miles D, Roché H, Martin M, Perren TJ, Cameron DA, Glaspy J, et al. Phase III Multicenter Clinical Trial of the Sialyl-TN (STn)-Keyhole Limpet Hemocyanin (KLH) Vaccine for Metastatic Breast Cancer. *Oncologist* (2011) 16:1092–100. doi: 10.1634/theoncologist.2010-0307
115. Ibrahim N, Murray J, Zhou D, Mittendorf E, Sample D, Tautchin M, et al. Survival Advantage in Patients With Metastatic Breast Cancer Receiving Endocrine Therapy Plus Sialyl Tn-KLH Vaccine: *Post Hoc* Analysis of a Large Randomized Trial. *J Cancer* (2013) 4:577–84. doi: 10.7150/jca.7028
116. Domchek S, Recio A, Mick R, Clark C, Carpenter E, Fox K, et al. Telomerase-Specific T-Cell Immunity in Breast Cancer: Effect of Vaccination on Tumor Immunosurveillance. *Cancer Res* (2007) 67:10546–55. doi: 10.1158/0008-5472.Can-07-2765
117. Mcgranahan N, Swanton C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. *Cell* (2017) 168:613–28. doi: 10.1016/j.cell.2017.01.018
118. Bertucci F, Ng CKY, Patsouris A, Droin N, Piscuoglio S, Carbuccia N, et al. Genomic Characterization of Metastatic Breast Cancers. *Nature* (2019) 569:560–4. doi: 10.1038/s41586-019-1056-z
119. Heemskerk B, Kvistborg P, Schumacher T. The Cancer Antigenome. *EMBO J* (2013) 32:194–203. doi: 10.1038/emboj.2012.333
120. Mcdonald K, Kawaguchi T, Qi Q, Peng X, Asaoka M, Young J, et al. Tumor Heterogeneity Correlates With Less Immune Response and Worse Survival in Breast Cancer Patients. *Ann Surg Oncol* (2019) 26:2191–9. doi: 10.1245/s10434-019-07338-3
121. Jager E, Ringhoffer M, Altmannsberger M, Arand M, Karbach J, Jager D, et al. Immunoselection *In Vivo*: Independent Loss of MHC Class I and Melanocyte Differentiation Antigen Expression in Metastatic Melanoma. *Int J Cancer* (1997) 71:142–7. doi: 10.1002/(sici)1097-0215(19970410)71:2<142::aid-ijc3>3.0.co;2-0
122. Rosenthal R, Cadieux EL, Salgado R, Bakir MA, Moore DA, Hiley CT, et al. Neoantigen-Directed Immune Escape in Lung Cancer Evolution. *Nature* (2019) 567:479–85. doi: 10.1038/s41586-019-1032-7
123. Armistead PM. Cellular Therapy Against Public Neoantigens. *J Clin Invest* (2019) 129:506–8. doi: 10.1172/jci126116
124. Charoentong P, Finotello F, Angelova M, Mayer C, Efremova M, Rieder D, et al. Pan-Cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. *Cell Rep* (2017) 18:248–62. doi: 10.1016/j.celrep.2016.12.019
125. Kim Y, Kang S, Shin H, Kim T, Yu B, Kim J, et al. Sequential and Timely Combination of a Cancer Nanovaccine With Immune Checkpoint Blockade Effectively Inhibits Tumor Growth and Relapse. *Angewandte Chemie (International Ed English)* (2020) 59:14628–38. doi: 10.1002/anie.202006117
126. Antonia S, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab After Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. *N Engl J Med* (2017) 377:1919–29. doi: 10.1056/NEJMoa1709937

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

SYSTEMATIC REVIEW
published: 30 June 2022
doi: 10.3389/fonc.2022.905846

The Prognostic and Clinical Value of Tumor-Associated Macrophages in Patients With Breast Cancer: A Systematic Review and Meta-Analysis

Changjun Wang[1†], Yan Lin[1†], Hanjiang Zhu[2], Yidong Zhou[1], Feng Mao[1], Xin Huang[1], Qiang Sun[1] and Chenggang Li[3,4*]*

[1] Department of Breast Surgery, Peking Union Medical College Hospital, Beijing, China, [2] Department of Dermatology, 90 Medical Center Way, Surge 110, University of California, San Francisco, San Francisco, CA, United States, [3] State Key Laboratory of Medicinal Chemical biology, Nankai University, Tianjin, China, [4] College of Pharmacy, Nankai University, Tianjin, China

OPEN ACCESS

Edited by:
Ke-Da Yu,
Fudan University, China

Reviewed by:
Jingxian Ding,
The Third Hospital of Nanchang, China
Xianyu Zhang,
Harbin Medical University Cancer Hospital, China

***Correspondence:**
Qiang Sun
sunqiangpumch@sina.com
Chenggang Li
lichenggang@nankai.edu.cn

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: 28 March 2022
Accepted: 26 May 2022
Published: 30 June 2022

Citation:
Wang C, Lin Y, Zhu H, Zhou Y, Mao F, Huang X, Sun Q and Li C (2022) The Prognostic and Clinical Value of Tumor-Associated Macrophages in Patients With Breast Cancer: A Systematic Review and Meta-Analysis. Front. Oncol. 12:905846. doi: 10.3389/fonc.2022.905846

Background: The prognostic and clinical value of tumor-associated macrophages (TAMs) in patients with breast cancer (BCa) remains unclear. We conducted the current meta-analysis to systematically evaluate the association of CD68+ and CD163+ TAM density with the prognosis and clinicopathologic features of BCa patients.

Methods: Searches of Web of Science, PubMed, and EMBASE databases were performed up to January 31, 2022. The meta-analysis was conducted using hazard risks (HRs) and 95% confidence intervals (CIs) for survival data including overall survival (OS), disease-free survival (DFS), and BCa specific survival. Sensitivity and meta-regression analyses were also conducted to identify the robustness of the pooled estimates.

Results: Our literature search identified relevant articles involving a total of 8,496 patients from 32 included studies. Our analysis indicates that a high CD68+ TAM density in the tumor stoma was significantly linked with poor OS (HR 2.46, 95% CI, 1.83–3.31, P<0.001) and shorter DFS (HR 1.77, 95% CI, 1.08–2.89, P=0.02) compared to low CD68+ TAM density. A significant association was also found in the tumor nest. Analysis of CD163+ TAM density showed similar results (all P<0.001). Notably, the pooled analysis with multivariate-adjusted HRs for OS and DFS also found that a high TAM density was significantly related to poorer outcomes for BCa patients (all P<0.05). In addition, BCa patients with high TAM density were more likely to have larger tumors, no vascular invasion, and positive estrogen receptor expression (all P<0.05).

Conclusion: This meta-analysis indicates that a high CD68+ and CD163+ TAM density is associated with poor OS and shorter DFS in BCa patients. Further clinical studies and *in vivo* experiments are needed to elucidate the underlying mechanism of TAMs.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022304853, identifier CRD42022304853.

Keywords: tumor-associated macrophages, breast cancer, survival, systematic review, meta-analysis

INTRODUCTION

Breast cancer (BCa) is one of the most frequent cancers among malignant diseases in women and is the leading cause of cancer-related deaths worldwide (1). Recently, BCa has exhibited a trend of early age onset, further threatening women's health and global disease burden (2). Despite great achievements in the diagnosis and clinical treatment of BCa, overall survival (OS) has not significantly improved, especially for patients with advanced-stage or triple-negative BCa (3, 4). Traditional prognostic indicators, such as TNM classification scheme, histological grade, progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor-2 (HER2), can not fully represent tumor biological behavior and BCa prognosis (5–7). Therefore, there remains a large unmet demand for novel effective biomarkers with superior prognostic and predictive power to deliver personalized and precise treatment for BCa.

Recently, the tumor microenvironment (TME) has gained increased interest in BCa research. Both clinical and pre-clinical studies found a mixture of tumor cells and host-activated immune cells including B cells, natural killer cells, and tumor-associated macrophages (TAMs) that predominated on the BCa TME (8, 9). It was demonstrated that tumor-associated immune cells are associated with tumor progression, metastasis, and acquired resistance. TAMs are the main component of the TME, accounting for approximately 50% of TME cells, playing a crucial role in antigen presentation, angiogenesis, tissue repair, and tumor cell apoptosis (10). TAMs can be classified into two main functional subtypes including classically activated M1 and alternatively activated M2 macrophages (11). Generally, M1 macrophages exert cytotoxic effects on cancer cells *via* proinflammatory cytokine molecules such as lipopolysaccharide, interleukin-12, and interferon-γ. In contrast, M2 macrophages function as "tumor promotors", which facilitate tumor cell invasion and metastasis and restrain anti-tumor immune response (9, 12).

Several studies focused on the prognostic significance of TAMs among different cancers, such as lung (13), liver (14), gastric (15), pancreatic (16) cancer, and BCa (17). The prognostic value of TAMs remains controversial and the results highly depend on macrophage subtypes and TAMs locations (18). This systematic review and meta-analysis was conducted to evaluate the impact of different TAMs markers and histologic locations on BCa prognosis. We also analyzed the association between TAMs infiltration and BCa clinicopathologic features. A clearer understanding of TAMs infiltration modes and prognostic value would be helpful to improve treatment efficacy in BCa.

Abbreviations: TAMs, Tumor-associated macrophages; BCa, Breast cancer; OS, Overall survival; PR, Progesterone receptor; ER, Estrogen receptor; HER2, Human epidermal growth factor receptor-2; TME, Tumor microenvironment; IHC, Immunohistochemistry; BCSS, Breast cancer specific survival; DFS, Disease-free survival; NOS, Newcastle–Ottawa Scale; TN, Tumor nest; TS, Tumor stroma; HRs, Hazard ratios; CIs, Confidence interval; KM, Kaplan–Meier; OR, Odds risk.

METHODS

This meta-analysis was performed in accordance with the Meta-Analyses and Systematic Reviews of Observational Studies (MOOSE) (19) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (20). The meta-analysis is registered with PROSPERO (CRD42022304853).

Literature Search

Two investigators (WCJ and LY) independently searched the Web of Science, PubMed (MEDLINE), and EMBASE databases for potential studies published in journals until January 31, 2022, without any language limitation. The main key words were "tumor-associated macrophages" + "breast cancer", and a detailed search strategy is shown in **Supplementary Table 1**. We also conducted forward and backward citation tracking to avoid missing any relevant literature. Unpublished literature and conference papers were not included. All studies reporting TAMs and BCa were included and screened by two authors independently based on the inclusion criteria.

Inclusion Criteria

We included studies reporting TAMs associated with BCa that met the following inclusion criteria: (i) patients with pathologically diagnosed BCa; (ii) BCa patients without any previous cancer history; (iii) TAMs were measured at the primary tumor site using immunohistochemistry (IHC) staining for CD68 and CD163; and (iv) the study design was a cohort study or case-control study, evaluating the association of TAMs with survival data [OS, breast cancer specific survival (BCSS), disease-free survival (DFS)] and other clinical outcomes.

Exclusion Criteria

We excluded studies measuring TAMs at metastases or local relapse sites. Comments, reviews, conference abstracts, and case reports were also excluded from our meta-analysis.

Quality Assessment and Data Extraction

The quality of each selected study was independently evaluated by two experienced researchers using the modified Newcastle–Ottawa Scale (NOS) based on the current PRISMA guidelines (21). The researchers focused on measurement and selection bias because most studies included in this review were cross-sectionally designed. Studies obtained a NOS score based on three evaluation indicators including study comparability,

patient selection, and outcome assessment. Eligible studies were graded as high quality with a NOS score ≥6. A third researcher resolved any disagreements and made the final decision for candidate articles.

Two authors independently extracted the data from the studies using a standardized data extraction form. The following data were extracted: name of the first author, publication year, country, study design, study period, sample size, age, treatment received, tumor size, histologic type, histological grade, the status of ER, PR, HER-2, and Ki-67 (positive or negative), macrophage markers, macrophage location site [tumor nest (TN) or tumor stroma (TS)], follow-up time, OS, DFS, and BCSS with adjusted or unadjusted hazard ratios (HRs) and 95% confidence interval (CIs). TAMs in the TN was defined as intraepithelial tumor-infiltrating macrophages, and TS was defined as the stromal tissue surrounding the tumor nest. We also collected prognostic information from studies that only reported a Kaplan–Meier (KM) plot and a *P*-value derived from log-rank analysis. HRs and 95% CIs were extracted from KM plots using Engauge Digitizer version 4.1 (free software downloaded from http://sourceforge.net) and calculated as previously described (22) . The low TAM group was used as a reference to calculate HRs. If the high TAM group was considered as a reference in the included study, then the relevant measures were inverted to ensure data uniformity. The corresponding author of the included study was contacted if there were any unclear or missing data.

Statistical Analysis

The statistical analysis was performed according to the recommendations from The Cochrane Collaboration. The HR with 95% CI was used to evaluate the association between TAM density and survival. The odds risk (OR) with 95% CI for the difference in clinicopathological features was used to measure dichotomous data. Heterogeneity across studies was assessed using the Cochran Q test and the I^2 statistics. For I^2 statistics, we considered I^2<25% as low heterogeneity and I^2> 5% as high heterogeneity. Data were also analyzed with a fixed-effects model for $P > 0.10$ and I^2<50%; otherwise, the random-effects model was applied. We performed meta-regression analysis to analyze the role of potential contributors to heterogeneity using the "metafor" package in R software (Version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria). Subgroup analysis and sensitivity analysis were also conducted to identify the source of heterogeneity. Potential publication bias was evaluated using funnel plots. All statistical analyses were conducted using Review Manager Version 5.3 software (The Nordic Cochrane Center, The Cochrane Collaboration, 2014, Copenhagen). A two-tailed *P*-value <0.05 was considered statistically significant.

RESULTS

A total of 14,781 articles were found in our initial search, and 3,145 duplicated articles and irrelevant studies were removed. After reviewing the title and abstract, 11,368 studies were excluded; after reviewing the full text 38 articles were excluded. Finally, 32 unique studies were included in the meta-analysis (**Supplementary Table 2**). The detailed screening method and results are presented in **Figure 1**.

Basic Characteristics and Quality Assessment

The main characteristics of the enrolled studies are summarized in **Table 1**. We included 32 studies in our meta-analysis that were published between 1996 and 2021 and conducted in 10 countries from 1985 to 2018 (England, Japan, America, UK, Sweden, China, Finland, Republic of Korea, Singapore, Germany). A total of 8,496 patients were included in the eligible studies, with the reported age from 23 to 97 years.

For TAM identification, 28 studies used CD68 and 12 studies used CD163, among which three studies used a combination of CD68 and PCNA. Five studies explored the role of TAMs in both TN and TS, 18 studies only detected TAMs in TN, and nine studies only included TAMs in TS. The majority of studies used the median number of macrophages per high-power field as the cut-off value to divide TAMs into the high and low TAM groups. Moreover, most studies assessed the association between TAMs and the prognosis of BCa patients, including OS (25 studies), DFS (24 studies), and BCSS (seven studies). The reported follow-up time ranged from 0.1 to 20.4 years. The NOS scores of all included studies ranged from 6 to 8 (**Table 1**).

Prognostic Significance of CD68+ TAMs

A total of 15 studies were included in the analysis of CD68+ TAMs on survival data in patients with BCa using the fixed-effect model for the absence of heterogeneity (all I^2<50% or P>0.10). Our meta-analysis indicated that a high CD68+ TAM density was significantly associated with poor OS compared to a low CD68+ TAM density in the TN with a pooled HR of 1.72 (95% CI 1.44–2.06, P<0.001) and in the TS with a pooled HR of 2.46 (95% CI, 1.83–3.31, P<0.001) (**Figures 2A, B**). For adjusted measurements of OS from five studies, the results also supported a poor OS in patients with a high CD68+ TAM density in the TN (HR 2.37, 95% CI 1.69–3.31, P<0.001) (**Figures 2C, D**). The results were similar for the association between CD68+ TAMs and BCSS in the TN (HR 1.25, 95% CI 1.03–1.52, P=0.03) and TS (HR 2.23, 95% CI 1.68–2.96, P<0.001) (**Supplementary Figure 1A**). However, there was no significant association between CD68+ TAMs and BCSS in the TN (HR 0.83, 95% CI 0.33–2.08, P=0.70) after excluding the study of Mahmoud et al. for high weight (84.9% of total weight), and the study of Murri et al. for high weight (69.3% of total weight in remaining four studies) (**Supplementary Figure 1B**).

A total of 14 studies were eligible to assess the correlation between CD68+ TAMs and DFS. The results showed that a high CD68+ TAM density in the TS was significantly correlated with shorter DFS compared to a low CD68+ TAMs density (HR 1.77, 95% CI 1.08–2.89, P=0.02) in a random-effects model with significant heterogeneity (I^2 =90%, P<0.001). No significant difference was found in the TN (HR 1.04, 95% CI 1.01–1.07,

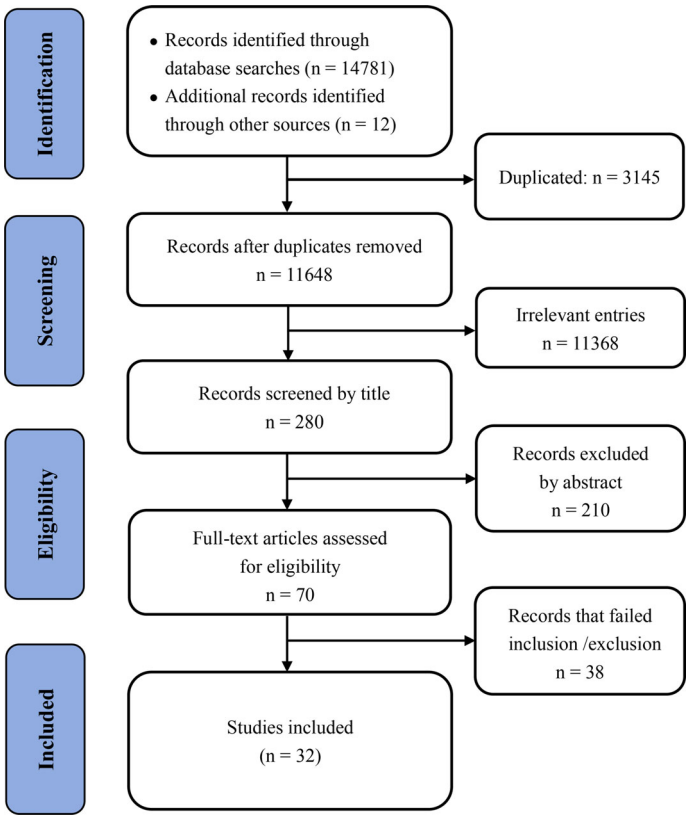


FIGURE 1 | Flow diagram of article selection.

P=0.02) (**Figures 3A, B**). However, the results showed that a high CD68+ TAM density in the TN was significantly correlated with shorter DFS (HR 1.50, 95% CI 1.19–1.89, *P*<0.001) after excluding the study of Leek et al. accounting for 98.4% of total weight (**Supplementary Figure 1C**). For adjusted measurements of DFS from 12 studies, the results support a poor DFS in patients with a high CD68+ TAM density (TN: HR 1.24, 95% CI 1.06–1.46, *P*=0.008; TS: HR 2.10, 95% CI 1.59–2.77, *P*<0.001) (**Figures 3C, D**), and the results still support a poor DFS in patients with a high CD68+ TAM density (TN: HR 1.52, 95% CI 1.16–2.01, *P*=0.003; TS: HR 1.96, 95% CI 1.27–3.02, *P*=0.003) even after excluding the studies of Mahmoud et al. and Yuan et al. accounting for 66.2% and 59.0% of the total weight, respectively (**Supplementary Figure 1D, E**).

Prognostic Significance of CD163+ TAMs

The following meta-analysis was conducted using the fixed-effect model for the absence of heterogeneity (all I^2<50% or *P*>0.10), except for adjusted measurements of OS in the TN (I^2 =79%, *P*=0.009). A total of nine studies were eligible to assess the association of CD163+ TAMs and survival data in patients with BCa. The results showed that a high CD163+ TAM density in the TN was significantly associated with poor OS (HR 1.50, 95% CI, 1.22–1.86, *P*<0.001), especially in the TS with a pooled HR of 2.17 (95% CI, 1.67–2.82, *P*<0.001) (**Figures 4A, B**). For adjusted measurements of OS from seven studies, the results also support a poor OS in patients with a high CD68+ TAM density (TN: HR 3.08, 95% CI 1.18–8.02, *P*=0.02; TS: HR 2.71, 95% CI 1.35–5.46, *P*=0.005) (**Figures 4C, D**). There was no significant association between CD163+ TAMs and BCSS in the TN (HR 1.17, 95% CI 0.45–3.05, *P*=0.74), but only two studies were included in this analysis (**Supplementary Figure 1F**).

For the correlation between CD163+ TAMs and DFS, the results indicated that a high CD163+ TAM density was significantly associated with shorter DFS both in the TN (HR 1.45, 95% CI 1.19–1.77, *P*<0.001) and TS (HR 2.48, 95% CI 1.87–3.27, *P*<0.001) (**Figures 5A, B**). For adjusted measurements of DFS from eight studies, the random-effects model was used to obtain HRs and the corresponding 95% CIs because the pooled data exhibited high heterogeneity (TN: I^2 =61%, *P*=0.05; TS: I^2 = 62%, *P*=0.03). The results also supported a poor DFS in patients with a high CD163+ TAM density (TN: HR 2.52, 95% CI 1.56–4.07, *P*<0.001; TS: HR 2.84, 95% CI 1.35–5.97, *P*=0.006) (**Figures 5C, D**).

Association Between TAMs (CD68+ or CD163+) and Clinicopathological Characteristics

We also analyzed the association between TAMs (CD68+ or CD163+) and clinicopathological characteristics in patients with

TABLE 1 | Characteristics of studies included in the meta-analysis.

Author	Country	Sample size	Markers	Cut-off value	Tissue distribution	Analysis	Follow-up	Outcome assessment	Selection	Comparability	Outcome	NOS
Leek et al., 1996 (23)	England	91	CD68+	Median 12	Tumor nest	Unavailable	60 months	OS, DFS	★★★	★★	★	6
Tsutsui et al., 2005 (24)	Japan	249	CD68+	55th percentile	Tumor nest	Unavailable	Unavailable	DFS	★★★★	★★	★	7
Murri et al., 2008 (25)	UK	168	CD68+	Tertiles	Tumor nest	Blind	Median 72 months	OS, BCSS	★★★★	★	★★	7
Campbell et al., 2010 (26)	American	216	CD68+/ PCNA+	5	Tumor nest	Blind	108 months	OS, DFS	★★★	★★	★★★	8
Mukhtar et al., 2011 (27)	American	70	CD68+/ PCNA+	Median 5	Tumor nest	Blind	Median 10.34 years	OS, DFS	★★★	★★	★★★	8
Mohammed et al., 2012 (28)	UK	468	CD68+	Tertiles	Tumor nest	Blind	10 years	OS, BCSS	★★★★	★	★★★	8
Medrek et al. 2012 (29)	Sweden	144	CD68+ CD163+	Median 50%	Tumor nest and stroma	Unavailable	Median 6.55 years (0.33-7.55)	OS, BCSS, DFS	★★★★	★	★★★	8
Mahmoud et al. 2012 (30)	UK	1902	CD68+	TN, 6 TS,17	Tumor nest and stroma	Blind	Unavailable	OS, BCSS, DFS	★★★	★	★★	6
Carrio et al., 2012 (31)	American	29	CD68+	Positive	Tumor nest	Unavailable	Unavailable	OS	★★★	★	★★★	7
Zhang et al., 2013 (32)	China	172	CD68+	Median 26	Tumor nest	Blind	Unavailable	OS, DFS	★★★	★★	★★	7
Campbell et al., 2013 (33)	American	102	CD68 +/PCNA+	Mean 24	Tumor nest	Unavailable	Unavailable	OS, DFS	★★★	★★	★★	7
Yuan et al., 2014 (34)	China	287	CD68+	16	Tumor stroma	Unavailable	Median 89 months (4-181)	OS, DFS	★★★	★	★★★	7
Gujam et al., 2014 (35)	UK	361	CD68+	Tertiles	Tumor stroma	Blind	Median 168 months	OS, BCSS	★★★★	★	★★★	8
Yang et al., 2015 (36)	China	100	CD68+	Median 61.14	Tumor nest	Unavailable	Mean 56.68 months	OS	★★★	★	★★	6
Sousa et al., 2015 (37)	Finland	562	CD68+ CD163+	Median CD68: 369 CD163: 167.5	Tumor nest	Double-blinded	Unavailable	DFS	★★★★	★	★★★	8
Gwak et al., 2015 (38)	Korea	276	CD68+	Median 24.2	Tumor nest	Unavailable	Median 7.7 years (0.1-10.6)	DFS	★★★	★★	★★	7
Tiainen et al. 2015 (17)	Finland	270	CD68+ CD163+	Median CD68: 34 CD163: 26	Tumor stroma	Blind	Median 6.3 years (0.4-11.1)	OS	★★★	★★	★★★	8
Ward et al., 2015 (39)	UK	129	CD68+	Mean value	Tumor nest	Unavailable	Median 78 months	DFS	★★★	★	★★	6
Koru-Sengul et al., 2016 (40)	American	150	CD163+	150	Tumor stroma	Blind	Unavailable	OS, DFS	★★★★	★	★★★	8
Tian et al., 2016 (41)	China	278	CD163+	Median 50%	Tumor stroma	Unavailable	Median 76 months (4-116)	OS	★★★	★	★★	6
Shiota et al., 2016 (42)	Japan	167	CD68+	Median 50%	Tumor nest	Blind	Median 86 months (1-159)	OS, BCSS, DFS	★★★★	★	★★★	8
Xu et al., 2017 (43)	China	102	CD68+	Mean number	Tumor stroma	Blind	Unavailable	OS, DFS	★★★★	★	★★★	8
Miyasato et al., 2017 (44)	Japan	149	CD68+ CD163+	190	Tumor nest	Blind	Unavailable	OS, BCSS, DFS	★★★★	★	★★★	8
Liu et al. 2017 (45)	China	203	CD163+	10%	Tumor stroma	Unavailable	Median 51 months (13-88)	OS, DFS	★★★	★★	★★	7
Yang et al. 2018 (46)	China	200	CD68+ CD163+	TN: 11; TS: 36	Tumor nest and stroma	Blind	Median 66 months (12-86)	OS, DFS	★★★	★★	★★★	8
Zhang et al., 2018 (47)	China	278	CD163+	Mean	Tumor nest	Blind	Median 87 months (8-130)	DFS	★★★	★★	★★	7

(Continued)

TABLE 1 | Continued

Author	Country	Sample size	Markers	Cut-off value	Tissue distribution	Analysis	Follow-up	Outcome assessment	Selection	Comparability	Outcome	NOS
Yuan et al., 2019 (48)	China	217	CD68+	Immunoreactivity scoring > 6	Tumor nest	Blind	5 years	DFS	★★★	★	★★★	7
Jeong et al., 2019 (49)	Korea	367	CD68+ CD163+	CD68+ TN:33 TS:17.8 CD163+ TN: 1.67 TS: 21	Tumor nest and stroma	Blind	Unavailable	OS, DFS	★★★	★	★★★	7
Jamiyan et al. 2020 (50)	Japan	107	CD68+ CD163+	Median value CD68+ TS: 26.2 TN: 11.2 CD163+ TS: 26.6 TN: 8.6	Tumor nest and stroma	Unavailable	Unavailable	OS, DFS	★★★	★	★★	6
Chen et al., 2020 (51)	Singapore	198	CD68+ CD163+	≥ 10%	Tumor stroma	Unavailable	Median 7.2 years (0-20.4)	DFS	★★★	★	★★★	7
Gunnarsdottir et al., 2020 (52)	Sweden	286	CD68+	10%	Tumor nest	Blind	Unavailable	OS	★★★	★★	★★	7
Lin et al., 2021 (53)	Germany	298	CD68+	≤ 4.5	Tumor stroma	Unavailable	12 years	OS, DFS	★★★	★★	★	6

TN, tumor nest; TS, tumor stroma; OS, overall survival; DFS, disease-free survival; BCSS, breast cancer specific survival; NOS: Newcastle-Ottawa Scale checklist

★*: A star means that the study obtain one score in NOS.*

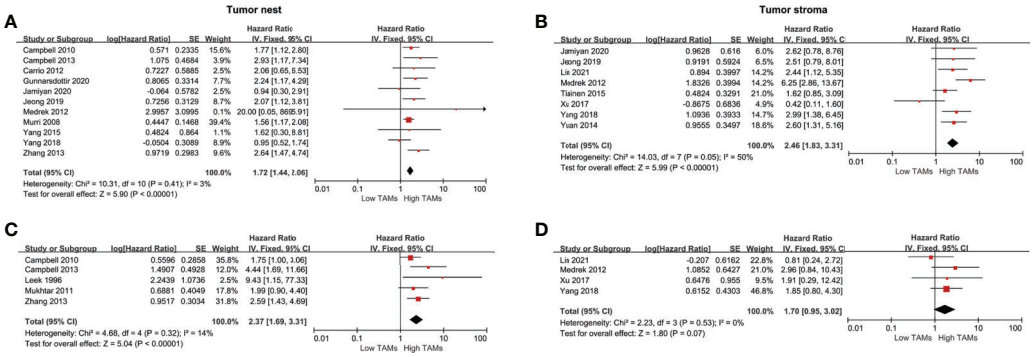


FIGURE 2 | Forest plots of HRs for OS between high and low CD68+ TAM density in BCa patients. **(A)** HRs of OS in raw data for CD68+ TAMs in the TN of BCa; **(B)** HRs of OS in raw data for CD68+ TAMs in the TS of BCa; **(C)** HRs of OS with adjusted measures for CD68+ TAMs in the TN of BCa; **(C)** HRs of OS with adjusted measures for CD68+ TAMs in the TS of BCa.

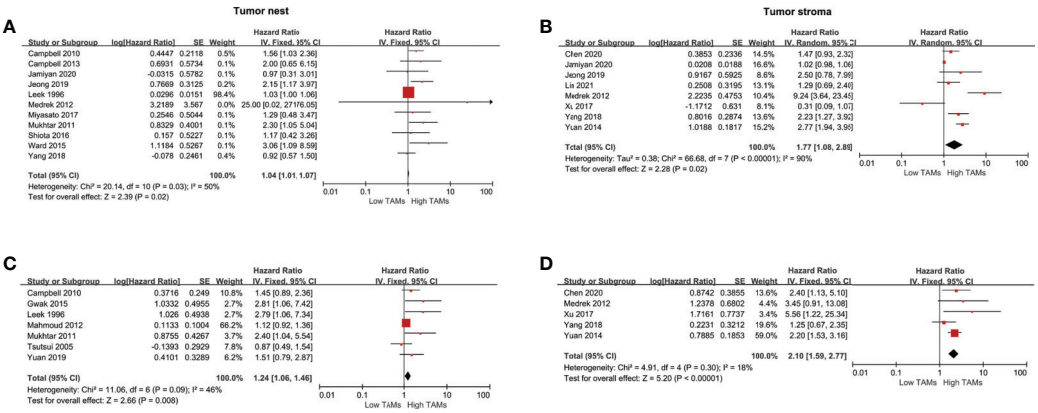


FIGURE 3 | Forest plots of HRs for DFS between high and low CD68+ TAM density in BCa patients. **(A)** HRs of DFS in raw data for CD68+ TAMs in the TN of BCa; **(B)** HRs of DFS in raw data for CD68+ TAMs in the TS of BCa; **(C)** HRs of DFS with adjusted measures for CD68+ TAMs in the TN of BCa; **(D)** HRs of DFS with adjusted measures for CD68+ TAMs in the TS of BCa.

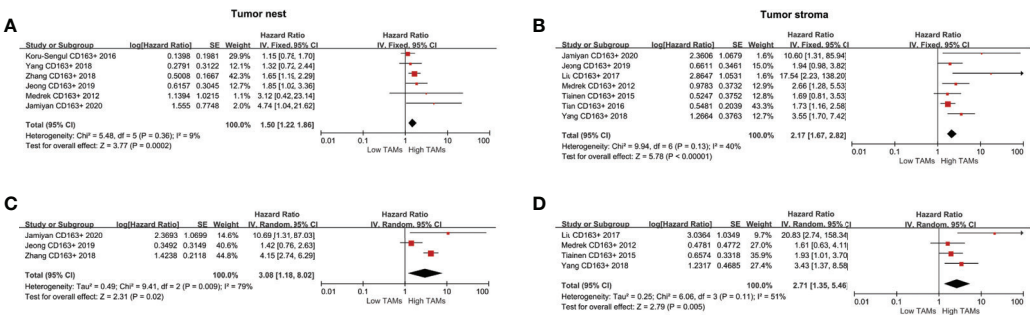


FIGURE 4 | Forest plots of HRs for OS between high and low CD163+ TAM density in BCa patients. **(A)** HRs of OS in raw data for CD163+ TAMs in the TN of BCa; **(B)** HRs of OS in raw data for CD163+ TAMs in the TS of BCa; **(C)** HRs of OS with adjusted measures for CD163+ TAMs in the TN of BCa; **(D)** HRs of OS with adjusted measures for CD163+ TAMs in the TS of BCa.

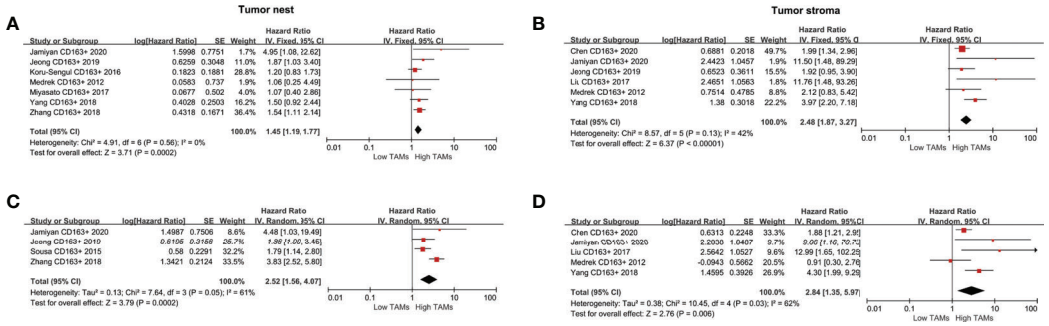


FIGURE 5 | Forest plots of HRs for DFS between high and low CD163+ TAM density in BCa patients. **(A)** HRs of DFS in raw data for CD163+ TAMs in the TN of BCa; **(B)** HRs of DFS in raw data for CD163+ TAMs in the TS of BCa; **(C)** HRs of DFS with adjusted measures for CD163+ TAMs in the TN of BCa; **(D)** HRs of DFS with adjusted measures for CD163+ TAMs in the TS of BCa.

BCa. The pooled results indicated that a high CD68+ TAM density was not significantly associated with age, lymph node status, histology classification, and PR in the TN or TS (all $P>0.05$) (**Table 2**). However, our meta-analysis using a random-effects model also revealed that a high CD68+ TAM density in the TN was significantly associated with larger tumor size (OR 0.36, 95% CI 0.15–0.85, $P=0.02$), no vascular invasion (OR 0.40, 95% CI 0.28–0.58, $P<0.001$), positive Ki-67 (OR 4.23, 95% CI 1.33–13.48, $P<0.001$), positive ER (OR 2.23, 95% CI 1.19–4.18, $P=0.01$), and negative HER-2 (OR 0.08, 95% CI 0.05–0.14, $P<0.001$), with significant heterogeneity (all $I^2 > 50\%$).

For the association between high CD163+ TAM density and clinicopathological characteristics, pooled analysis showed a significant correlation between high CD163+ TAMs in the TN and age ≥ 50 years (OR 0.21, 95% CI 0.13–0.34, $P<0.001$, random-effects model), large tumor size (OR 0.34, 95% CI 0.12–1.00, $P=0.05$, random-effects model), no vascular invasion (OR 0.56, 95% CI 0.38–0.82, $P=0.003$, fixed-effects model), and positive ER (OR 3.55, 95% CI 2.58–4.88, $P<0.001$, fixed-effects model) (**Table 3**). However, the results of the TS showed no significant association between high CD163+ TAM density and any clinicopathological characteristics, which could be due to insufficient CD163+ TAM data.

Heterogeneity

We used meta-regression analysis to quantitatively analyze the source of heterogeneity found in **Figure 4B**. A P-value <0.1 could be considered the main source of heterogeneity. The results of univariate analysis showed that region, year, sample size, and cut-off value for high or low TAM density may not be the main sources of heterogeneity between studies (**Table 4**). Multivariate analysis also showed that region, year, sample size, and cut-off value may not be a major source of between-study heterogeneity. Subgroup analysis was also conducted for CD68+ TAM density in the TS associated with DFS. The quantitative data for these subgroups are summarized in **Supplementary Table 3**. Subgroup analysis also showed that region, year, sample size, and cut-off value were not the potential sources of heterogeneity (all $P>0.05$).

Sensitivity Analysis

Due to the significant heterogeneity of CD68+ TAMs and DFS data, sensitivity analysis was conducted to evaluate the stability of the pooled HRs. After excluding individual studies one by one, the pooled HRs did not substantially change. Similarly, we performed sensitivity analysis for the association between CD163+ TAMs and OS data in the TN. When we removed the article by Jeong et al., we found that high CD163+ TAM density in the TN was associated with better OS with no significant heterogeneity (HR 4.30, 95% CI 2.86–6.47, $P<0.001$, $I^2 = 0\%$, $P=0.39$).

Publication Bias

We examined potential publication bias using funnel plots when the meta-analysis was conducted with more than five studies. The results showed no significant publication bias for TAMs (CD68+ or CD163+) with OS and DFS (**Supplementary Figures 2, 3**).

DISCUSSION

As the leading cause of death among women, BCa remains a significant global health threat, and new therapeutic strategies are required. TAMs are regarded as a potentially promising target for cancer treatment, and increasing studies have explored the possibility to suppress their tumor-promoting activity (54). Recent ongoing pre-clinical TAM-targeted studies indicated that TAMs are closely associated with poor prognosis and BCa progression (55, 56). Given the discordent conclusions among previous studies, the present meta-analysis was conducted to assess the association between TAMs and BCa prognosis.

This meta-analysis included 32 studies analyzing the prognostic value of TAMs in BCa. A total of 15 studies detected TAMs using a CD68+ biomarker, and 11 and eight of these studies identified TAMs in the TN and TS, respectively. CD163 was used in nine studies to identify TAMs, of which six and seven studies evaluated TAMs in the TN and TS,

TABLE 2 | Meta-analysis of high CD68+ TAMs density and clinicopathological features of breast cancer patients.

Clinicopathological features	References	No. of studies	Model	Pooled OR (95% CI)	*P* value	Heterogeneity	
						I^2 (%)	*P* value
Tumor nest							
Age (< 50 y vs ≥ 50 y)	≥ 50 years	9	Random	0.59 (0.33-1.04)	0.07	93	< 0.001
Tumor size (< 2cm vs ≥ 2cm)	≥ 2cm	9	Random	0.36 (0.15-0.85)	0.02	96	< 0.001
Lymph node status (N0 vs. N1-3)	N1-3	7	Random	0.74 (0.13-1.29)	0.28	90	< 0.001
Histological grade (I, II vs III)	III	13	Random	0.85 (0.46-1.56)	0.60	95	< 0.001
Vascular invasion (yes vs no)	No	3	Random	0.40 (0.28-0.58)	< 0.001	55	0.11
Ki-67 status (positive vs negative)	Negative	4	Random	4.23 (1.33-13.48)	0.01	94	< 0.001
ER status (positive vs negative)	Negative	9	Random	2.23 (1.19-4.18)	0.01	94	< 0.001
PR status (positive vs negative)	Negative	7	Random	1.34 (0.88-2.04)	0.17	78	< 0.001
HER-2 status (positive vs negative)	Negative	8	Random	0.08 (0.05-0.14)	< 0.001	88	< 0.001
Tumor stroma							
Age (< 50 y vs ≥ 50 y)	≥ 50 years	5	Random	0.48 (0.13-1.85)	0.29	96	< 0.001
Tumor size (< 2cm vs ≥ 2cm)	≥ 2cm	5	Random	0.59 (0.12-2.94)	0.52	97	< 0.001
Lymph node status (N0 vs. N1-3)	N1-3	3	Random	0.71 (0.21-2.42)	0.59	91	< 0.001
Histological grade (I, II vs III)	III	5	Random	0.32 (0.08-1.35)	0.12	97	< 0.001
Vascular invasion (yes vs no)	No	2	Random	0.08 (0.01-2.16)	0.13	94	< 0.001
Ki-67 status (positive vs negative)	Negative	1	–	0.32 (0.21-0.49)	–	–	–
ER status (positive vs negative)	Negative	3	Random	5.00 (3.68-6.80)	< 0.001	94	< 0.001
PR status (positive vs negative)	Negative	3	Random	1.23 (0.60-2.55)	0.57	80	0.006
HER-2 status (positive vs negative)	Negative	3	Random	0.21 (0.01-6.81)	0.38	99	< 0.001

TAMs, tumor-associated macrophages; OR, odds ratio; CI, confidence interval; ER, oestrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2.

TABLE 3 | Meta-analysis of high CD163+ TAMs density and clinicopathological features of breast cancer patients.

Clinicopathological features	References	No. of studies	Model	Pooled OR(95% CI)	*P* value	Heterogeneity	
						I^2 (%)	*P* value
Tumor nest							
Age (< 50 y vs ≥ 50 y)	≥ 50 years	4	Random	0.21 (0.13-0.34)	< 0.001	65	0.04
Tumor size (< 2cm vs ≥ 2cm)	≥ 2cm	5	Random	0.34 (0.12-1.00)	0.05	95	< 0.001
Lymph node status (N0 vs. N1-3)	N1-3	3	Random	0.94 (0.21-4.13)	0.93	95	< 0.001
Histological grade (I, II vs III)	III	5	Random	0.41 (0.13-1.31)	0.13	95	< 0.001
Vascular invasion (yes vs no)	No	2	Fixed	0.56 (0.38-0.82)	0.003	17	0.27
Ki-67 status (positive vs negative)	Negative	2	Random	4.70 (0.88-25.00)	0.07	93	< 0.001

(Continued)

TABLE 3 | Continued

Clinicopathological features	References	No. of studies	Model	Pooled OR(95% CI)	*P* value	Heterogeneity	
						I^2 (%)	*P* value
ER status (positive vs negative)	Negative	2	Fixed	3.55 (2.58-4.88)	< 0.001	51	0.15
PR status (positive vs negative)	Negative	1	–	1.81 (0.92-3.57)	0.09	–	–
HER-2 status (positive vs negative)	Negative	2	Random	0.11 (0.01-0.79)	0.03	94	< 0.001
Tumor stroma							
Age (< 50 y vs ≥ 50 y)	≥ 50 years	4	Random	1.71 (0.57-5.08)	0.34	90	< 0.001
Tumor size (< 2cm vs ≥ 2cm)	≥ 2cm	5	Random	0.31 (0.06-1.54)	0.15	96	< 0.001
Lymph node status (N0 vs. N1-3)	N1-3	4	Random	1.98 (0.44-8.96)	0.38	95	< 0.001
Histological grade (I, II vs III)	III	5	Random	0.36 (0.06-2.19)	0.27	97	< 0.001
Vascular invasion (yes vs no)	No	1	–	0.03 (0.01-0.09)	–	–	–
Ki-67 status (positive vs negative)	Negative	1	–	2.52 (1.30-4.85)	–	–	–
ER status (positive vs negative)	Negative	2	Random	2.96 (0.61-14.35)	0.18	91	0.001
PR status (positive vs negative)	Negative	3	Fixed	1.22 (0.87-1.71)	0.26	46	0.16
HER-2 status (positive vs negative)	Negative	3	Random	0.25 (0.02-2.53)	0.24	97	< 0.001

TAMs, tumor-associated macrophages; OR, odds ratio; CI, confidence interval; ER, oestrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2.

TABLE 4 | Univariable and multivariable meta-regressions for variables.

Variable	Univariable Meta-Regressions			Multivariable Meta-Regression		
	Standard deviation	*P* value	95%CI	Standard deviation	*P* value	95%CI
Region (Europe/Asian)	0.689	0.269	0.56-8.29	0.960	0.660	0.23-10.02
Year (after 2018/before 2018)	0.624	0.527	0.20-2.29	0.813	0.672	0.14-3.49
Sample size (<200/≥200)	0.620	0.571	0.21-2.37	0.990	0.324	0.05-2.62
Cut-off value (not median/median)	0.724	0.465	0.14-2.44	1.164	0.345	0.03-3.26

respectively. We systemically analyzed the association between TAMs (CD68+ or CD163+) and OS and DFS in BCa patients. The present study concluded that a high TAM density in the TME was significantly associated with poor prognostic (OS, and DFS) compared to a low TAM density, irrespective of TAM marker (CD68+ or CD163+, all $P<0.001$). Notably, the pooled results were further strengthened by OS and DFS multivariate analyses showing that a high TAM density was significantly related to poorer outcomes (all $P<0.05$). Compared to TAMs detected in the TN, a high TAMs density detected in the TS seems to show relatively higher prognostic value for BCa patients, validated both for CD68+ and CD163+ TAMs. We also analyzed the association between TAMs and clinicopathological characteristics in BCa patients, which indicated that a high TAM density was closely associated with larger tumor size, no vascular invasion, and positive ER. However, the heterogeneity was very large, requiring further clinical studies with larger sample sizes to validate this conclusion.

The conclusion of the present study is in line with two previous meta-analyses, involving 16 studies (57) and 13 studies (58), respectively. The study by Zhao et al. also showed a worse OS in the TS group compared to the TN group (57). Our findings are consistent with these studies, highlighting the significant prognostic value for TAMs in BCa patients. However, there were contradictory conclusions regarding the prognostic value of CD68 and CD163. Zhao et al. reported that CD68 was a more sensitive prognostic indicator than CD163 in BCa patients, while Ni et al. reported the opposite result. Our results indicated that both CD68+ and CD163+ TAMs were significantly related to poor OS and shorter DFS in both raw and adjusted measures. Compared with previous studies, the present meta-analysis has the advantage of a much larger sample size and more included studies, thus providing more reliable conclusions. Our subgroup analysis for different TAM locations (TN and TS), as well as for raw or adjusted measures, provides more insight into the value of TAM location for BCa prognosis.

Our study also found that a high TAM density in the TS tended to have superior prognostic value for BCa than TAMs in the TN. This finding was not only presented for BCa (50, 59), but also for gastric cancer (15) and oral squamous cell carcinoma (60). TAMs are prone to localize in certain cancer tissues and exhibit different biological behaviors (61). A previous study suggested that different histological locations could induce TAMs to perform distinct functions (62). High TAM density in the TS tended to cause stroma activation and extracellular matrix (ECM) remodeling, *via* interacting with other stromal components including lysyl oxidase, matrix metalloproteinase-9, and type IV collagen (63, 64). Fibroblasts and microvessels are the main supporting components for promoting angiogenesis and tumor metastasis. Activation of ECM remodeling enzymes might limit the function of immune cells and keep them out of the tumor (65). The consequences of these factors can result in tumor enlargement and potentially metastasis. However, these niches may be reshaped by anti-cancer therapy. For instance, immunotherapy increased the number of tertiary lymphoid structures, and anti-angiogenic therapy remodeled perivascular system and stroma niches (66). Moreover, several cytotoxic and targeted therapies have been shown to alter the comprehensive phenotype of tumor macrophages (67; 66) .

Although the present meta-analysis indicated that a high TAM density (both in CD68+ and CD163+) is associated with poor prognosis in patients with Bca, the results still need to be treated with caution. CD68 is a universal macrophage marker, as it stains both M1-like and M2-like TAMs, which exerts opposing effects on carcinogenesis. This may be the reason why CD68 was not an independent risk factor for prognosis in some multivariate analyses (29, 30, 46). CD68 can also be detected on some other non-monocyte cells (e.g. fibroblasts) (68, 69). Therefore, CD68 alone may not be a good marker of TAMs to predict OS. CD163 is a highly specific marker for M2-like macrophages. A previous study suggested that the presence of CD163+ TAMs was significantly associated with less favourable clinicopathological features than CD68+ TAMs (29). It has been found that TAMs tend to polarize to M2 in the TME, and their surface receptors and cytokines secreted are similar to M2-like macrophages (70). As a specific and predominant marker of macrophages in BCa, CD163 could be used as a general marker with prognostic impact alone or immunohistochemical double-staining with CD68 to detect macrophage subpopulations and calculate the ratio of M1/M2.

Furthermore, the subgroup analysis indicated that high TMA density was closely related to BCa patients with larger tumor size, no vascular invasion, or positive ER status. This implies that TAMs density may have prognostic, even therapeutic, value for BCa. A study by Castellaro et al. also reported that TAMs could promote proliferation, migration, invasiveness, and breast tumor growth of ER+ cells *via* rendering these estrogen-dependent breast cancer cells resistant to estrogen withdrawal and tamoxifen treatment (71). Therefore, TAM-targeted therapy may help improve BCa prognosis. Currently, several clinical trials on TAM-targeted therapy have been carried out. Interventions targeting TAMs include macrophages depletion, inhibition of macrophage-derived cytokines, anti-TAMs activation, chimeric antigen receptor macrophage (CAR-M) therapy, TAMs-based immune vaccine, and TAMs nanobiotechnology (70). CCL2, CSF-1, and CSF-1R inhibitors have been shown to effectively lower TAM density in both an animal model and clinical trials. (72–74). Given that M1 macrophages exert cytotoxic effects on cancer cells, another novel strategy could focus on inducing pro-tumor TAMs to an anti-tumor phenotype or M1 phenotype using typical agents such as CD40 agonists, CD47 inhibitors, STAT3 inhibitors, Bruton's tyrosine kinase (BTK) inhibitors, IL-1Ra inhibitors, and TLR agonists (72, 75, 76), However, despite numerous ongoing clinical and pre-clinical trials on TAM-targeting therapies, a further in-depth understanding of the underlying mechanism of TAMs-related carcinogenesis and the complexity of TAM subsets would be essential to fully realize their therapeutic potential.

There are several important strengths of this meta-analysis. First, the present study was the meta-analysis with the largest sample size, including several recently published papers, and thus the pooled results would be more reliable than previous studies. Second, our meta-analysis included different TAMs locations (TN and TS), which adds new information for the impact of TAM location on BCa survival. Third, our results indicated that a high TAM density is significantly related to poorer outcomes, especially for TAMs in the TS, as a useful prognostic marker. Fourth, given that preoperative adjuvant therapy might disturb TAM density, especially for large tumors, ER positive, and Ki-67 positive patients, the reliability of the results may be compromised. Most included studies excluded patients receiving preoperative neoadjuvant chemotherapy or anti-HER2 therapy, increasing the homogeneity of the study population and strengthening the conclusions.

Several limitations of our meta-analysis should be acknowledged. First, there is currently no consensus on the cut-off values of TAMs in BCa, as previous studies did not set a unified criterion. Most included studies adopted a median value as the cut-off for high/low TAMs. Although there is a concern that the inconsistent cut-off values used in the included studies may potentially introduce bias, the univariate and multivariate meta-regression analysis in the present study both demonstrated that the cut-off value was not the potential sources of heterogeneity, indicating studies using different cut-off value were homogeneous, further strengthening the final conclusions. Future large-scale randomized controlled trials and meta-analyses base on individual patient data are warranted to further elucidate the correlation between TAMs and BCa prognosis. Second, there was significant heterogeneity among the analysis of TAMs and clinicopathological features, even when making a distinction between TAM locations. The heterogeneity might be derived from the different antibodies and dilution applications to detect TAM density. Similarly, the cut-off value of Ki-67 expression (14% or 20%) varied in the included studies, which might have introduced heterogeneity. Third, all included articles were retrospective studies, which may have led to

selection bias in the pooled results. Fourth, excessive differences in the range of sample sizes may have increased the weight of the studies with big sample sizes in the pooled results and increased systematical biases. Therefore, future studies with larger sample sizes are required to validate the conclusions of our study.

CONCLUSION

In summary, the present systemic review and meta-analysis indicates that an elevated density of CD68+ and CD163+ TAMs is associated with poor OS and shorter DFS in BCa patients. Due to the limitations in our study, further well-designed studies with larger sample sizes are needed to validate our conclusion.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

CW, YZ, QS, and CL designed the project; CW, YL, QS, and CL performed the literature search and data acquisition; CW and YL performed data extraction; FM, HZ, and XH performed the statistical analyses for heterogeneity investigation; CW, HZ, and YZ supported the writing of the paper. All authors read and approved the final manuscript.

FUNDING

This study was funded by Key Projects in the National Science and Technology Pillar Program during the Twelfth Five-year Plan Period (No.2014BAI08B00), Beijing Municipal Science and Technology Project (No. D161100000816005), State Key Laboratory of Medicinal Chemical Biology (NanKai University) (No. 2019014) and LAM China Non-profit Organization Special Fund for LAM of Zhejiang Women and Children's Foundation (No. LAM001-202205). The funding agencies had no role in the design or conduct of the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.905846/full#supplementary-material

Supplementary Table 1 | The search strategy of databases.

Supplementary Table 2 | Reference of included studies.

Supplementary Table 3 | Subgroup analyses to explore the potential sources of heterogeneity for the impact of CD68+ TAMs density in tumor stroma on DFS.

Supplementary Figure 1 | Forest plots of HRs for BCa specific survival or DFS between high and low TAM density in BCa patients. **(A)** HRs of BCSS for CD68+ TAMs in the TN of BCa; **(B)** HRs of BCSS for CD68+ TAMs in the TN of BCa after excluding two studies with high weight; **(C)** HRs of DFS in raw data for CD68+ TAMs in the TN of BCa; **(D)** HRs of DFS with adjusted measures for CD68+ TAMs in the TN of BCa; **(E)** HRs of DFS with adjusted measures for CD68+ TAMs in the TS of BCa. **(F)** HRs of BCSS for CD163+ TAMs in the TN of BCa.

Supplementary Figure 2 | Funnel plot of studies with CD68+ TAM density for potential publication bias assessment. **(A)** OS and CD68+ TAMs in the TN; **(B)** DFS and CD68+ TAMs in the TN; **(C)** OS in adjusted measurements and CD68+ TAMs in the TN; **(D)** DFS in adjusted measurements and CD68+ TAMs in the TN; **(E)** BCSS and CD68+ TAMs in the TN; **(F)** OS and CD68+ TAMs in the TS; **(G)** OS in adjusted measurements and CD68+ TAMs in the TS; **(H)** DFS in adjusted measurements and CD68+ TAMs in the TS.

Supplementary Figure 3 | Funnel plot of studies with CD163+ TAM density for potential publication bias assessment. **(A)** OS and CD163+ TAMs in the TN; **(B)** DFS and CD163+ TAMs in the TN; **(C)** OS and CD163+ TAMs in the TS; **(D)** OS and CD163+ TAMs in the TS.

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics 2021. *CA Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654
2. Li N, Deng Y, Zhou L, Tian T, Yang S, Wu Y, et al. Global Burden of Breast Cancer and Attributable Risk Factors in 195 Countries and Territories, From 1990 to 2017: Results From the Global Burden of Disease Study 2017. *J Hematol Oncol* (2019) 12(1):140. doi: 10.1186/s13045-019-0828-0
3. Rossi L, Mazzara C, Pagani O. Diagnosis and Treatment of Breast Cancer in Young Women. *Curr Treat Options Oncol* (2019) 20(12):86. doi: 10.1007/s11864-019-0685-7
4. McDonald ES, Clark AS, Tchou J, Zhang P, Freedman GM. Clinical Diagnosis and Management of Breast Cancer. *J Nucl Med* (2016) 57 (Suppl 1):9S–16S. doi: 10.2967/jnumed.115.157834
5. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, et al. Breast Cancer Prognostic Classification in the Molecular Era: The Role of Histological Grade. *Breast Cancer Res* (2010) 12(4):207. doi: 10.1186/bcr2607
6. Barzaman K, Karami J, Zarei Z, Hosseinzadeh A, Kazemi MH, Moradi-Kalbolandi S, et al. Breast Cancer: Biology, Biomarkers, and Treatments. *Int Immunopharmacol.* (2020) 84:106535. doi: 10.1016/j.intimp.2020.106535
7. Yip CH, Rhodes A. Estrogen and Progesterone Receptors in Breast Cancer. *Future Oncol* (2014) 10(14):2293–301. doi: 10.2217/fon.14.110
8. Wagner J, Rapsomaniki MA, Chevrier S, Anzeneder T, Langwieder C, Dykgers A, et al. A Single-Cell Atlas of the Tumor and Immune Ecosystem of Human Breast Cancer. *Cell* (2019) 177(5):1330–45. doi: 10.1016/j.cell.2019.03.005
9. Huang X, Cao J, Zu X. Tumor-Associated Macrophages: An Important Player in Breast Cancer Progression. *Thorac Cancer* (2022) 13(3):269–76. doi: 10.1111/1759-7714.14268
10. Petty AJ, Yang Y. Tumor-Associated Macrophages: Implications in Cancer Immunotherapy. *Immunotherapy-UK* (2017) 9(3):289–302. doi: 10.2217/imt-2016-0135
11. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage Activation and Polarization: Nomenclature and Experimental Guidelines. *Immunity* (2014) 41(1):14–20. doi: 10.1016/j.immuni.2014.06.008

12. Linde N, Casanova-Acebes M, Sosa MS, Mortha A, Rahman A, Farias E, et al. Macrophages Orchestrate Breast Cancer Early Dissemination and Metastasis. *Nat Commun* (2018) 9(1):21. doi: 10.1038/s41467-017-02481-5
13. Zheng X, Weigert A, Reu S, Guenther S, Mansouri S, Bassaly B, et al. Spatial Density and Distribution of Tumor-Associated Macrophages Predict Survival in Non-Small Cell Lung Carcinoma. *Cancer Res* (2020) 80(20):4414–25. doi: 10.1158/0008-5472.CAN-20-0069
14. Fan QM, Jing YY, Yu GF, Kou XR, Ye F, Gao L, et al. Tumor-associated macrophages promote cancer stem cell-like properties *via* Transforming Growth Factor-Beta1-Induced Epithelial-Mesenchymal Transition in Hepatocellular Carcinoma. *Cancer Lett* (2014) 352(2):160–8. doi: 10.1016/j.canlet.2014.05.008
15. Li W, Zhang X, Wu F, Zhou Y, Bao Z, Li H, et al. Gastric Cancer-Derived Mesenchymal Stromal Cells Trigger M2 Macrophage Polarization That Promotes Metastasis and EMT in Gastric Cancer. *Cell Death Dis* (2019) 10 (12):918. doi: 10.1038/s41419-019-2131-y
16. Hutcheson J, Balaji U, Porembka MR, Wachsmann MB, McCue PA, Knudsen ES, et al. Immunologic and Metabolic Features of Pancreatic Ductal Adenocarcinoma Define Prognostic Subtypes of Disease. *Clin Cancer Res* (2016) 22(14):3606–17. doi: 10.1158/1078-0432.CCR-15-1883
17. Tiainen S, Tumelius R, Rilla K, Hamalainen K, Tammi M, Tammi R, et al. High Numbers of Macrophages, Especially M2-Like (CD163-Positive), Correlate With Hyaluronan Accumulation and Poor Outcome in Breast Cancer. *Histopathology* (2015) 66(6):873–83. doi: 10.1111/his.12607
18. Mehraj U, Qayoom H, Mir MA. Prognostic Significance and Targeting Tumor-Associated Macrophages in Cancer: New Insights and Future Perspectives. *Breast Cancer-Tokyo*. (2021) 28(3):539–55. doi: 10.1007/s12282-021-01231-2
19. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-Analysis of Observational Studies in Epidemiology: A Proposal for Reporting. Meta-Analysis of Observational Studies in Epidemiology (MOOSE) Group. *JAMA* (2000) 283(15):2008–12. doi: 10.1001/jama.283.15.2008
20. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *BMJ* (2009) 339:b2535. doi: 10.1136/bmj.b2535
21. Stang A. Critical Evaluation of the Newcastle-Ottawa Scale for the Assessment of the Quality of Nonrandomized Studies in Meta-Analyses. *Eur J Epidemiol*. (2010) 25(9):603–5. doi: 10.1007/s10654-010-9491-z
22. Altman DG, Bland JM. How to Obtain the Confidence Interval From a P Value. *BMJ* (2011) 343:d2090. doi: 10.1136/bmj.d2090
23. Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL. Association of Macrophage Infiltration With Angiogenesis and Prognosis in Invasive Breast Carcinoma. *Cancer Res* (1996) 56(20):4625–9.
24. Tsutsui S, Yasuda K, Suzuki K, Tahara K, Higashi H, Era S. Macrophage Infiltration and Its Prognostic Implications in Breast Cancer: The Relationship With VEGF Expression and Microvessel Density. *Oncol Rep*. (2005) 14 (2):425–31.
25. Murri AM, Hilmy M, Bell J, Wilson C, McNicol AM, Lannigan A, Doughty JC, McMillan DC. The Relationship Between the Systemic Inflammatory Response, Tumour Proliferative Activity, T-Lymphocytic and Macrophage Infiltration, Microvessel Density and Survival in Patients With Primary Operable Breast Cancer. *Br J Cancer* (2008) 99(7):1013–9. doi: 10.1038/sj.bjc.6604667
26. Campbell MJ, Tonlaar NY, Garwood ER, Huo D, Moore DH, Khramtsov AI, et al. Proliferating Macrophages Associated With High Grade, Hormone Receptor Negative Breast Cancer and Poor Clinical Outcome. *Breast Cancer Res Treat* (2011) 128(3):703–711. doi: 10.1007/s10549-010-1154-y
27. Mukhtar RA, Moore AP, Nseyo O, Baehner FL, Au A, Moore DH, Twomey P, et al. Elevated PCNA+ Tumor-Associated Macrophages in Breast Cancer Are Associated With Early Recurrence and Non-Caucasian Ethnicity. *Breast Cancer Res Treat* (2011) 130(2):635–44. doi: 10.1007/s10549-011-1646-4
28. Mohammed ZM, Going JJ, Edwards J, Elsberger B, Doughty JC, McMillan DC. The Relationship Between Components of Tumour Inflammatory Cell Infiltrate and Clinicopathological Factors and Survival in Patients With Primary Operable Invasive Ductal Breast Cancer. *Br J Cancer* (2012) 107 (5):864–73. doi: 10.1038/bjc.2012.347
29. Medrek C, Ponten F, Jirstrom K, Leandersson K. The Presence of Tumor Associated Macrophages in Tumor Stroma as a Prognostic Marker for Breast Cancer Patients. *BMC Cancer*. (2012) 12:306. doi: 10.1186/1471-2407-12-306
30. Mahmoud SM, Lee AH, Paish EC, Macmillan RD, Ellis IO, Green AR. Tumour-Infiltrating Macrophages and Clinical Outcome in Breast Cancer. *J Clin Pathol* (2012) 65(2):159–63. doi: 10.1136/jclinpath-2011-200355
31. Carrio R, Koru-Sengul T, Miao F, Glück S, Lopez O, Selman Y, et al. Macrophages as Independent Prognostic Factors in Small T1 Breast Cancers. *Oncol Rep* (2013) (1):141–8. doi: 10.3892/or.2012.2088
32. Zhang Y, Cheng S, Zhang M, Zhen L, Pang D, Zhang Q, Li Z. High-Infiltration of Tumor-Associated Macrophages Predicts Unfavorable Clinical Outcome for Node-Negative Breast Cancer. *PLoS One* (2013) 8(9):e76147. doi: 10.1371/journal.pone.0076147
33. Campbell MJ, Wolf D, Mukhtar RA, Tandon V, Yau C, Au A, et al. The Prognostic Implications of Macrophages Expressing Proliferating Cell Nuclear Antigen in Breast Cancer Depend on Immune Context. *PLoS One* (2013) 8(10):e79114. doi: 10.1371/journal.pone.0079114
34. Yuan ZY, Luo RZ, Peng RJ, Wang SS, Xue C. High Infiltration of Tumor-Associated Macrophages in Triple-Negative Breast Cancer Is Associated With a Higher Risk of Distant Metastasis. *Onco Targets Ther* (2014) 7:1475–80. doi: 10.2147/OTT.S61838
35. Gujam FJ, Edwards J, Mohammed ZM, Going JJ, McMillan DC. The Relationship Between the Tumour Stroma Percentage, Clinicopathological Characteristics and Outcome in Patients With Operable Ductal Breast Cancer. *Br J Cancer* (2014) 111(1):157–65. doi: 10.1038/bjc.2014.279
36. Yang J, Li X, Liu X, Liu Y. The Role of Tumor-Associated Macrophages in Breast Carcinoma Invasion and Metastasis. *Int J Clin Exp Pathol* (2015) 8 (6):6656–64. doi: 10.1111/imr.12719
37. Sousa S, Brion R, Lintunen M, Kronqvist P, Sandholm J, Mönkkönen J, et al. Human Breast Cancer Cells Educate Macrophages Toward the M2 Activation Status. *Breast Cancer Res* (2015) 17(1):101. doi: 10.1186/s13058-015-0621-0
38. Gwak JM, Jang MH, Kim DI, Seo AN, Park SY. Prognostic Value of Tumor-Associated Macrophages According to Histologic Locations and Hormone Receptor Status in Breast Cancer. *PLoS One* (2015) 10(4):e0125728. doi: 10.1371/journal.pone.0125728
39. Ward R, Sims AH, Lee A, Lo C, Wynne L, Yusuf H, et al. Monocytes and Macrophages, Implications for Breast Cancer Migration and Stem Cell-Like Activity and Treatment. *Oncotarget* (2015) 6(16):14687–99. doi: 10.18632/oncotarget.4189
40. Koru-Sengul T, Santander AM, Miao F, Sanchez LG, Jorda M, Glück S, et al. Breast Cancers From Black Women Exhibit Higher Numbers of Immunosuppressive Macrophages With Proliferative Activity and of Crown-Like Structures Associated With Lower Survival Compared to Non-Black Latinas and Caucasians. *Breast Cancer Res Treat* (2016) 158(1):113–126. doi: 10.1007/s10549-016-3847-3
41. Tian W, Wang L, Yuan L, Duan W, Zhao W, Wang S, et al. A Prognostic Risk Model for Patients With Triple Negative Breast Cancer Based on Stromal Natural Killer Cells, Tumor-Associated Macrophages and Growth-Arrest Specific Protein 6. *Cancer Sci* (2016) 107(7):882–9. doi: 10.1111/cas.12964
42. Shiota T, Miyasato Y, Ohnishi K, Yamamoto-Ibusuki M, Yamamoto Y, Iwase H, et al. The Clinical Significance of CD169-Positive Lymph Node Macrophage in Patients With Breast Cancer. *PLoS One* (2016) 11(11): e0166680. doi: 10.1371/journal.pone.0166680
43. Xu Y, Lan S, Zheng Q. Prognostic Significance of Infiltrating Immune Cell Subtypes in Invasive Ductal Carcinoma of the Breast. *Tumori* (2018) 104 (3):196–201. doi: 10.5301/tj.5000624
44. Miyasato Y, Shiota T, Ohnishi K, Pan C, Yano H, Horlad H, et al. High Density of CD204-Positive Macrophages Predicts Worse Clinical Prognosis in Patients With Breast Cancer. *Cancer Sci* (2017) 108(8):1693–700. doi: 10.1111/cas.13287
45. Liu H, Wang J, Zhang M, Xuan Q, Wang Z, Lian X, et al. Jagged1 Promotes Aromatase Inhibitor Resistance by Modulating Tumor-Associated Macrophage Differentiation in Breast Cancer Patients. *Breast Cancer Res Treat* (2017) 166(1):95–107. doi: 10.1007/s10549-017-4394-2
46. Yang M, Li Z, Ren M, Li S, Zhang L, Zhang X, et al. Stromal Infiltration of Tumor-Associated Macrophages Conferring Poor Prognosis of Patients With

Basal-Like Breast Carcinoma. *J Cancer.* (2018) 9(13):2308–16. doi: 10.7150/jca.25155
47. Zhang WJ, Wang XH, Gao ST, Chen C, Xu XY, Sun Q, et al. Tumor-Associated Macrophages Correlate With Phenomenon of Epithelial-Mesenchymal Transition and Contribute to Poor Prognosis in Triple-Negative Breast Cancer Patients. *J Surg Res* (2018) 222:93–101. doi: 10.1016/j.jss.2017.09.035
48. Yuan J, He H, Chen C, Wu J, Rao J, Yan H. Combined High Expression of CD47 and CD68 Is a Novel Prognostic Factor for Breast Cancer Patients. *Cancer Cell Int* (2019) 19:238. doi: 10.1186/s12935-019-0957-0
49. Jeong H, Hwang I, Kang SH, Shin HC, Kwon SY. Tumor-Associated Macrophages as Potential Prognostic Biomarkers of Invasive Breast Cancer. *J Breast Cancer* (2019) 22(1):38–51. doi: 10.4048/jbc.2019.22.e5
50. Jamiyan T, Kuroda H, Yamaguchi R, Abe A, Hayashi M. CD68- and CD163-Positive Tumor-Associated Macrophages in Triple Negative Cancer of the Breast. *Virchows Arch* (2020) 477(6):767–75. doi: 10.1007/s00428-020-02855-z
51. Chen XY, Thike AA, Md Nasir ND, Koh VCY, Bay BH, Tan PH. Higher Density of Stromal M2 Macrophages in Breast Ductal Carcinoma in Situ Predicts Recurrence. *Virchows Arch* (2020) 476(6):825–33. doi: 10.1007/s00428-019-02735-1
52. Björk Gunnarsdottir F, Auoja N, Bendahl PO, Rydén L, Fernö M, Leandersson K. Co-Localization of CD169+ Macrophages and Cancer Cells in Lymph Node Metastases of Breast Cancer Patients Is Linked to Improved Prognosis and PDL1 Expression. *Oncoimmunology* (2010) 9(1):1848067. doi: 10.1080/2162402X.2020.1848067
53. Lin L, Kuhn C, Ditsch N, Kolben T, Czogalla B, Beyer S, et al. Breast Adipose Tissue Macrophages (BATMs) Have a Stronger Correlation With Breast Cancer Survival Than Breast Tumor Stroma Macrophages (BTSMs). *Breast Cancer Res* (2021) 23(1):45. doi: 10.1186/s13058-021-01422-x
54. Tang X, Mo C, Wang Y, Wei D, Xiao H. Anti-Tumour Strategies Aiming to Target Tumour-Associated Macrophages. *Immunology* (2013) 138(2):93–104. doi: 10.1111/imm.12023
55. Turner NC, Neven P, Loibl S, Andre F. Advances in the Treatment of Advanced Oestrogen-Receptor-Positive Breast Cancer. *Lancet* (2017) 389 (10087):2403–14. doi: 10.1016/S0140-6736(16)32419-9
56. Oner G, Altintas S, Canturk Z, Tjalma W, Verhoeven Y, Van Berckelaer C, et al. Triple-Negative Breast Cancer-Role of Immunology: A Systemic Review. *Breast J* (2020) 26(5):995–9. doi: 10.1111/tbj.13696
57. Zhao X, Qu J, Sun Y, Wang J, Liu X, Wang F, et al. Prognostic Significance of Tumor-Associated Macrophages in Breast Cancer: A Meta-Analysis of the Literature. *Oncotarget* (2017) 8(18):30576–86. doi: 10.18632/oncotarget.15736
58. Ni C, Yang L, Xu Q, Yuan H, Wang W, Xia W, et al. CD68- and CD163-Positive Tumor Infiltrating Macrophages in Non-Metastatic Breast Cancer: A Retrospective Study and Meta-Analysis. *J Cancer.* (2019) 10(19):4463–72. doi: 10.7150/jca.33914
59. Ch'Ng ES, Tuan SS, Jaafar H. In Human Invasive Breast Ductal Carcinoma, Tumor Stromal Macrophages and Tumor Nest Macrophages Have Distinct Relationships With Clinicopathological Parameters and Tumor Angiogenesis. *Virchows Arch* (2013) 462(3):257–67. doi: 10.1007/s00428-012-1362-4
60. Ni YH, Ding L, Huang XF, Dong YC, Hu QG, Hou YY. Microlocalization of CD68+ Tumor-Associated Macrophages in Tumor Stroma Correlated With Poor Clinical Outcomes in Oral Squamous Cell Carcinoma Patients. *Tumour Biol* (2015) 36(7):5291–8. doi: 10.1007/s13277-015-3189-5
61. Komohara Y, Jinushi M, Takeya M. Clinical Significance of Macrophage Heterogeneity in Human Malignant Tumors. *Cancer Sci* (2014) 105(1):1–8. doi: 10.1111/cas.12314
62. Zhou D, Huang C, Lin Z, Zhan S, Kong L, Fang C, et al. Macrophage Polarization and Function With Emphasis on the Evolving Roles of Coordinated Regulation of Cellular Signaling Pathways. *Cell Signal* (2014) 26(2):192–7. doi: 10.1016/j.cellsig.2013.11.004
63. Peng C, Liu J, Yang G, Li Y. Lysyl Oxidase Activates Cancer Stromal Cells and Promotes Gastric Cancer Progression: Quantum Dot-Based Identification of Biomarkers in Cancer Stromal Cells. *Int J Nanomedicine.* (2018) 13:161–74. doi: 10.2147/IJN.S143871
64. Qian BZ, Pollard JW. Macrophage Diversity Enhances Tumor Progression and Metastasis. *Cell* (2010) 141(1):39–51. doi: 10.1016/j.cell.2010.03.014
65. Lu P, Weaver VM, Werb Z. The Extracellular Matrix: A Dynamic Niche in Cancer Progression. *J Cell Biol* (2012) 196(4):395–406. doi: 10.1083/jcb.201102147
66. DeNardo DG, Ruffell B. Macrophages as Regulators of Tumour Immunity and Immunotherapy. *Nat Rev Immunol* (2019) 19(6):369–82. doi: 10.1038/s41577-019-0127-6
67. Coffelt SB, de Visser KE. Immune-Mediated Mechanisms Influencing the Efficacy of Anticancer Therapies. *Trends Immunol* (2015) 36(4):198–216. doi: 10.1016/j.it.2015.02.006
68. Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES, Coussens LM. Leukocyte Composition of Human Breast Cancer. *Proc Natl Acad Sci U S A* (2012) 109 (8):2796–801. doi: 10.1073/pnas.1104303108
69. Gottfried E, Kunz-Schughart LA, Weber A, Rehli M, Peuker A, Muller A, et al. Expression of CD68 in Non-Myeloid Cell Types. *Scand J Immunol* (2008) 67 (5):453–63. doi: 10.1111/j.1365-3083.2008.02091.x
70. Xu T, Yu S, Zhang J, Wu S. Dysregulated Tumor-Associated Macrophages in Carcinogenesis, Progression and Targeted Therapy of Gynecological and Breast Cancers. *J Hematol Oncol* (2021) 14(1):181. doi: 10.1186/s13045-021-01198-9
71. Castellaro AM, Rodriguez-Baili MC, Di Tada CE, Gil GA. Tumor-Associated Macrophages Induce Endocrine Therapy Resistance in ER+ Breast Cancer Cells. *Cancers (Basel)* (2019) 11(2). doi: 10.3390/cancers11020189
72. Mehta AK, Kadel S, Townsend MG, Oliwa M, Guerriero JL. Macrophage Biology and Mechanisms of Immune Suppression in Breast Cancer. *Front Immunol* (2021) 12:643771. doi: 10.3389/fimmu.2021.643771
73. Guerriero JL. Macrophages: The Road Less Traveled, Changing Anticancer Therapy. *Trends Mol Med* (2018) 24(5):472–89. doi: 10.1016/j.molmed.2018.03.006
74. Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, et al. CCL2 Recruits Inflammatory Monocytes to Facilitate Breast-Tumour Metastasis. *Nature* (2011) 475(7355):222–5. doi: 10.1038/nature10138
75. Feng Y, Mu R, Wang Z, Xing P, Zhang J, Dong L, et al. A Toll-Like Receptor Agonist Mimicking Microbial Signal to Generate Tumor-Suppressive Macrophages. *Nat Commun* (2019) 10(1)2272. doi: 10.1038/s41467-019-10354-2
76. Wiehagen KR, Girgis NM, Yamada DH, Smith AA, Chan SR, Grewal IS, et al. Combination of CD40 Agonism and CSF-1R Blockade Reconditions Tumor-Associated Macrophages and Drives Potent Antitumor Immunity. *Cancer Immunol Res* (2017) 5(12):1109–21. doi: 10.1158/2326-6066.CIR-17-0258

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

REVIEW
published: 18 July 2022
doi: 10.3389/fonc.2022.903800

IL-6: The Link Between Inflammation, Immunity and Breast Cancer

Juan Chen[1†], Yanghui Wei[2†], Weiqin Yang[3], Qingnan Huang[2], Yong Chen[2], Kai Zeng[2] and Jiawei Chen[2*]*

[1] Department of Medicine and Rehabilitation, Tung Wah Eastern Hospital, Hong Kong, Hong Kong SAR, China, [2] Department of Surgery, The Eighth Affiliated Hospital, Sun Yat-Sen University, Shenzhen, China, [3] School of Biomedical Sciences, The Chinese, University of Hong Kong, Hong Kong, Hong Kong SAR, China

Breast cancer is one of the leading causes of mortality in females. Over the past decades, intensive efforts have been made to uncover the pathogenesis of breast cancer. Interleukin-6 (IL-6) is a pleiotropic factor which has a vital role in host defense immunity and acute stress. Moreover, a wide range of studies have identified the physiological and pathological roles of IL-6 in inflammation, immune and cancer. Recently, several IL-6 signaling pathway-targeted monoclonal antibodies have been developed for cancer and immune therapy. Combination of IL-6 inhibitory antibody with other pathways blockage drugs have demonstrated promising outcome in both preclinical and clinical trials. This review focuses on emerging studies on the strong linkages of IL-6/IL-6R mediated regulation of inflammation and immunity in cancer, especially in breast cancer.

Keywords: breast cancer, interleukin-6, inflammation, immune, target therapy

OPEN ACCESS

Edited by:
Monther Al-Alwan,
King Faisal Specialist Hospital and Research Centre, Saudi Arabia

Reviewed by:
Cheryl Lynn Jorcyk,
Boise State University, United States
Tomoki Nakamura,
Mie University Hospital, Japan

***Correspondence:**
Yanghui Wei
weiyh29@mail.sysu.edu.cn
Jiawei Chen
chenjw288@mail.sysu.edu.cn

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *24 March 2022*
Accepted: *20 June 2022*
Published: *18 July 2022*

Citation:
Chen J, Wei Y, Yang W, Huang Q, Chen Y, Zeng K and Chen J (2022) IL-6: The Link Between Inflammation, Immunity and Breast Cancer. Front. Oncol. 12:903800. doi: 10.3389/fonc.2022.903800

INTRODUCTION

Breast cancer is one of the leading diagnosed cancers in women with high mortality. According to International Agency for Research on Cancer (IARC), there were 2,261,419 women diagnosed with breast cancer in 2020 worldwide. It is a common cause of cancer-related death especially in less developed countries. Despite the recent advanced technique in breast cancer screening and early diagnosis, the high morbidity and mortality rates urge the need of investigation into the molecular mechanism of breast cancer.

Genome wide analyses have recently demonstrated thousands of mutations accumulated in breast cancer cells (1). In addition, as a multifactorial disease, the etiologies of breast cancer include not only distinct inherent factors such as genetic status, but also environmental factors such as obesity, lifestyle, and chronic inflammation (2).

Accumulating studies have been performed on the relationship between inflammation and cancer (3). It is well-accepted that inflammatory diseases could increase the risk of cancer development during tumor initiation, promotion, progression, and metastasis (3–6).

As one of the best-characterized pro-tumorigenic cytokines, IL-6 has been studied extensively for its central role in both physiological and pathological processes (7). Previous studies indicated that IL-6 regulate the pro-inflammatory and enhance monocyte infiltration at the inflammatory site during chronic inflammation (8). IL-6 responsive tissues would become resistant gradually during chronic inflammation, which correlated with high basal level of IL-6 (9, 10). IL-6 was also elevated in many solid tumors including breast cancer (11–13), which correlated with poor prognosis and

metastasis (14, 15). The current review will further discuss the intricate relationship between IL-6, inflammation, and breast cancer.

THE IL-6 SIGNALING PATHWAYS AND FUNCTIONS

The Il-6 Signaling Pathway

Human IL-6 is a 26 kDa glycoprotein known as a B-cell differentiation regulator (16) which is secreted by a number of cells (17). IL-6 is a multifunctional cytokine that plays both pro-inflammatory and anti-inflammatory roles in humans (18). IL-6 is a single chain phosphorylated glycoprotein consisting of four helix bundles (A-D), with A and B run in one direction while C and D run in the opposite direction. IL-6 transmits its signals through a cell-surface type-I receptor complex, which consists of the membrane-bound IL-6 receptor (IL-6R) and a signal-transducing component gp130 homodimer (19). IL-6R is expressed on a limited number of cell types, such as macrophages, B cells and subtypes of T cells (20, 21). IL-6R is 80 kDa α-chain and is also called as CD126 consisting of three domains namely D1, D2 and D3. Besides the membrane bound receptor (mIL-6R) as previously mentioned, soluble (sIL-6R) is the other form of IL-6R, which is expressed mainly in hepatocytes, neutrophils, monocytes, and T-cells (22). IL-6 selectively activates different signaling pathways, the classical signaling pathway through mIL-6R, and the trans-signaling pathway through sIL-6R. In both the cases, IL-6 binds to the receptor and then to gp130, but elicits different biological effects depending upon the receptor form (23). Cytokine IL-6 triggers the anti-inflammatory responses through classic signaling by binging to mIL-6R and gp130, while in contrast, trans-signaling can be manifested in all gp130-expressing cells, and leads to pro-inflammatory responses (24). The sIL-6R can be found at circulation with concentration from 25 to 35 ng/ml in human, which is generated by proteolytic cleavage of the membrane bound form IL-6R and by proteolytic cleavage of metalloproteinases gene family members, or by alternative splicing of IL-6R mRNA (25). There are three routes of the IL-6 signaling pathway. In route 1, Janus kinase (JAK) is phosphorylated and activated, subsequently activates dimerization of signal transducer and transcription-3 (STAT3) (26). In route 2, JAK activates Ras/Raf pathway, causing hyperphosphorylation of mitogen activated protein kinases (MAPK) and incudes its serine/threonine kinase activity (23). The third route involves the activation of phosphoinositol-3 kinase (PI3K)-protein kinase B (PKB)/Akt pathway (27).

IL-6 and Immunity

IL-6 is secreted by largely plasmacytoid dendritic cells (pDCs), which is critical for differentiation from B cells to plasma cells (28). This cytokine is also a vital modulator to maintain dynamic balance between Th1 and Th2 immune cells (29). For example, IL-6 is necessary during the differentiation from Th1 to Th2 cells (30). The process was proved to interfere with IFN-γ production *via* up-regulation of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in CD4+T cells (31). Meanwhile, together with transforming growth factor-β (TGF-β), IL-6 could promote the differentiation of Th17 cells *via* activating both retinoic acid-related orphan receptor γt (RORγt) and RORα (32). It was reported that STAT3 mediated the effectiveness of IL-6 on Th17 differentiation and this cytokine could inhibit the activity of Treg cells (33). Therefore, IL-6 is regarded as the main regulator of Treg/Th17 equilibrium (34).

IL-6 also plays a vital role in early differentiation of T follicular helper cells (Tfh), the main T helper cell subtype provides support for germinal center formation, affinity maturation, and immune cells' generation. Early BCl6 +/CXCR5+/Tfh differentiation would be mostly interfered in the case of IL-6 absence which was proved to mediate by STAT1 and STAT3 (35).

Novel agents against the IL-6/IL-6R signaling pathway have been proved to be effective for some inflammatory diseases. Preclinical studies have demonstrated that IL-6 has crucial functions in inflammatory cells recruitment (36). Tumor-associated macrophages (TAMs) secreted IL-6 and plays critical role in carcinogenesis and differentiation of myeloid-derived suppressor cells (MDSCs), which gives rise to intra-tumoral inflammatory processes (37, 38). A previous study demonstrated that inhibition of NF-κB decreased the stem cell compartment, which in turn reduced blood vessel formation in breast cancer (39). In addition, high expression of IL-6R on liver cells led to recruitment of acute phase proteins (40). High expression levels of acute phase proteins including CRP, fibrinogen and serum amyloid protein A were identified during both acute and chronic disease (41, 42). Interestingly, clinical observation found that CRP levels in patients with severe bacterial infections were not elevated when IL-6 was absent (43). Further studies demonstrated that blocking IL-6 signaling by neutralizing antibody may reverse low serum level of CRP (44). However, the application of IL-6/IL-6R blockers as anti-cancer agents has not been proved intensively in cancers including breast cancer.

IL-6 and Stem Cell

IL-6 family cytokines play an important role in generation and maintenance of stem/progenitor cells including cancer stem cells (CSCs) (45). As a member in IL-6 family, leukemia inhibitory factor (LIF) has an crucial role in both embryonic stem (ES) cells and cancer development (46), which is necessary to maintain mouse ES cells in an undifferentiated condition *via* STAT3 activation (47). Active LIF was detected in a wide range of malignancies including lung, breast, stomach, colon, liver, gallbladder, and pancreatic carcinoma (48). Once activated, STAT3 may induce gene expression including c-Myc, which contribute to the maintenance of undifferentiated state in mouse ES cells (49). It is also reported that IL-6 increased pluripotent stem (iPS) cell population by inducing c-Myc and Pim1 (50). The transcription factor C/EBPδ, was reported to be pro-tumorigenic in breast cancer cell lines by directly targeting IL-6R, leading to cancer progression with cancer stem cells activation (51). The IL-

6-JAK1-STAT3 pathway has a vital function in the transition from non-CSCs into CSCs by regulating OCT4 in human breast cancer cell lines (52). In lung cancer CSCs, IL-6Rα was detected in CSCs (53), whereas STAT3 was necessary for proliferation and survival in colon cancer-initiating cells (54, 55). It was reported that constitutive activation of STAT3 and NF-κB signaling in glioblastoma CSCs regulate Notch pathway, which played a key role in CSC maintenance and cell survival (56). STAT3 activation by IL-6 from adipose-derived stem cells could promote endometrial carcinoma proliferation and metastasis (57).

IL-6 is also crucial for epigenetic modification in stem cells (58, 59). NF-κB and STAT3 were identified as key regulators in epigenetic switch in inflammation (60, 61). Recently, a positive feedback loop involving microRNA let-7 has been demonstrated for maintaining chronic inflammatory status in malignant cells (60). Interestingly, this feedback loop regulated by IL-6 signaling could in turn activate NF-κB pathway and its downstream targets such as let-7 and Lin-28. Similarly, IL-6 was proved to be essential in keeping inflammatory loop in breast cancer CSCs (60, 61). In summary, IL-6 signaling plays a regulatory role in controlling cancer cell growth, CSC renewal and metastasis (62).

IL-6 and Tumor Microenvironment

Tumor microenvironment contributes significantly towards potentiating the stemness and metastasis properties of cancer cells. Solid tumors, including breast cancer cells were reported to have intense interaction with stromal cells such as mesenchymal stem cells (MSCs), adipocytes, cancer associated fibroblasts (CAFs), endothelial cells and immune cells in tumor microenvironment (63). Majority of these stromal cells within tumor microenvironment could secrete both IL-6 and IL-8 (63, 64). Mesenchymal cells could be either recruited from bone marrow (65) or normal breast stroma (66). In breast tumor cells, it has been identified that MSCs could be selectively recruited to the sites of growing carcinoma through cytokine such as IL-6 and CXCL7, where they interact with breast cancer CSCs (65, 66). In addition, MSCs are capable to differentiate into CAFs as well as adipocytes, which also interact with cancer cells (67).

CAFs have been demonstrated to have the ability to support tumorigenesis by stimulating angiogenesis, cell proliferation and invasion (68). CAFs in breast tumors expressed high levels of IL-6 (68, 69), which mediated epithelial-stromal interactions and promoted tumorigenesis (70). CAFs were reported to induce trastuzumab resistance in HER2 positive breast cancer cells (71). More importantly, IL-6 could in turn reactivate breast stromal fibroblasts through STAT3-dependent manner (72). CAFs could affect intra tumoral CD8+ and FoxP3+ T cells via IL-6 in tumor microenvironment (73). Recent findings also indicated miR-149's role in the crosstalk between tumor cells and CAFs, which highlighted the potential therapeutic strategy using interfering miRNAs (74). There was growing evidence support that CAFs promote stem cell-like properties of hepatocellular carcinoma via IL-6/STAT3/Notch signaling pathway (75).

In a recent study, a novel developed liposomal nanoparticle loaded with anti-IL6R antibody which deliver to tumor microenvironment achieved a significant effect in inhibiting the metastasis of breast cancer cells in mouse models (76).

Obesity has been recently identified as a negative prognostic factor in breast cancer (77, 78), which appears to be independent of menopausal status, tumor stage, and hormone-related factors (79). According to the reported literature, adipocytes produced inflammatory cytokines such as IL-6 in obesity individuals (80). IL-6 was reported to mediate crosstalk between preadipocytes and breast ductal carcinoma *in situ* cells which may lead to progression of early-stage breast cancer (81). In addition, adipose-derived stem cells (ADSCs) promoted tumor initiation and accelerated tumor growth through IL-6 production (82). Obesity was suggested to induce resistance to anti-VEGF therapy in breast cancer by up-regulating IL-6 (83).

IL-6'S FUNCTIONAL ROLE IN BREAST CANCER DEVELOPMENT

Experimental Studies

The predominant role of IL-6 in cancer is its key promotion of tumour growth. It has been demonstrated that deregulated IL-6 signaling pathway plays important roles in proliferation, migration, and adhesion among tumors (84–87). High level of IL-6 in breast cancer tissues stimulated Jagged-1 expression to promote cell growth and maintain the aggressive phenotype (88). High level of IL-6 secretion may facilitate tumor cell growth *via* suppressing apoptosis and promoting angiogenesis (89). High expression of IL-6Rα was also demonstrated to induce apoptosis resistance in breast cancer (90). In metastatic lesions of breast cancer patients, upregulated IL-6 was identified which may lead to chemotherapy resistance such as paclitaxel (91). The crosstalk between adipocytes and breast cancer cells in cancer progression has attracted much attention in recent years. The adipocyte-derived IL-6 was reported to promote breast cancer metastasis by inducing PLOD2 expression through activating the JAK/STAT3 and PI3K/AKT signaling pathways (92). In a recent study on triple-negative breast cancers (TNBCs), restraining of IL-6 and IL-8 expressions prominently suppressed both *in vitro* and *in vivo* cancer cell proliferation (93).

IL-12, which is produced by activated antigen presenting cells including dendritic cells and macrophages, was reported to inhibit tumor development (94). Some studies suggested that high expression level of IL-12 receptor were found to significantly increase breast cancer patients' survival, especially in the more aggressive subtypes (95). It is also critical to initiate the differentiation of naive CD4+ T cells to T helper type 1 (Th-1) cells (96). However, the correlation between IL-6 and IL-12 remains elusive in breast cancer. According to the reported literature, the Th-1/Th-2 imbalance plays important role in the development of breast cancer (97). And circulating Th-1 and Th-2 levels and their ratios are associated with ER-negative and TNBC, suggesting their contribution in breast cancers (98). IL-6 played dual functions on Th-1/Th-2 differentiation by promoting Th-2 differentiation and inhibiting Th-1 polarization simultaneously (29).

IL-6 is a vital player during acute inflammation, controlling not only the inflammatory response but also tissue metabolism

(99). Under chronic inflammation circumstance, IL-6 may induce cachexia through cytokines production and metabolism change in both lipids and proteins (100). Over-expression of IL-6 has been proved to be related with atrophy by promoting muscle protein metabolism (101). Cachexia and its related diseases account for approximately one third of all cancer-related deaths (102). Inflammatory breast cancer (IBC) describes a highly aggressive form of breast cancer of diverse molecular subtypes and clonal heterogeneity. The signature of IBC is recognized by its inflammation feature which is associated with IL-6 expression. A recent study published in May 2022 revealed that IL-6 signaling stimulate cell proliferation in IL-6R and HER2-expressing responsive sub-clones in IBC, and this effect was abrogated by the IL-6R neutralizing antibody Tocilizumab (103).

IL-6 is able to diffuse through cells structures and tissues in tumor microenvironment due to its low molecular weight (104). Tumor microenvironment-associated inflammation, mainly regulated by cytokines including IL-6, has been well-documented to contribute to every stage of cancer progression (105–108). Accumulating evidence has proved the significance of senescent cells in the microenvironment of cancer cells, of which pro-inflammatory IL-6 and IL-8 are consistently present. In this study, IL6 was reported to induce a self-reinforced senescence/inflammatory milieu responsible for the epithelial plasticity and stemness features which prone to a more aggressive phenotype in breast cancer (109).

Despite significant therapeutic achievements have been made in recent years, breast cancer is still one of the most common cancers with high mortality in women worldwide. Estrogen receptor (ER) α-positive breast cancers account for more than two thirds of all the category and endocrine therapies such as selective and aromatase inhibitors remain the standard adjuvant therapy for these tumors. However, majority of patients will develop drug resistance after treatment for several years and alternative hormone therapy is needed afterwards (110, 111). Interestingly, IL6/STAT3 signaling was suggested to drive metastasis in ER positive breast cancer independent of ER, decoupling IL6/STAT3 and ER oncogenic pathways could sensitize some hormonal resistant patients (112). In another study, similar conclusion was reported that Tocilizumab, an antibody that binds to IL-6R, could robustly reverse tamoxifen resistance (113). In compliance with this result, clinical breast cancer samples analysis confirmed that IL-6R expression was significantly associated with tamoxifen resistance in breast cancer tissues, with high IL-6R expression correlated with poor survival (113). Apart from the role in ER positive breast cancer, IL-6 was identified to trigger the migration and invasion of ER negative breast cancer cells *via* activation of YAP signals (114).

IL-6 could upregulate circulating VEGF in breast cancer patients, which was confirmed to promote angiogenesis and metastasis (115). Downregulation of IL-6 was related to the better response to breast cancer therapy (11, 116). Ligation of IL-6 with IL-6R activates Janus kinase (JAK) tyrosine kinases leading to phosphorylation of signal transducer and activator of transcription 3 (STAT3), which is a well-studied cancer signaling pathway. Moreover, the expression level of IL-6 was higher in aggressive tumors with multi-drug resistance and is negatively related to the expression of estrogen receptor in breast cancer patients (117, 118). Recently, the fact that IL-6-mediated Jagged1/Notch signaling pathway enhanced the ability for breast cancer cells metastasis has been demonstrated (119). All the evidence suggested that IL-6 and its receptor as attractive therapeutic targets.

Clinical Studies

In many preclinical models, IL-6 has been demonstrated to promote carcinogenicity, angiogenesis and metastasis (88, 118, 120, 121). IL-6 has been implicated in resistance to trastuzumab treatment in HER2 positive patients. The induction of IL-6 inflammatory feedback loop leads to the expanded population of CSCs, which lead to high levels of this cytokine secretion. The addition of tocilizumab, an anti-IL-6R antibody, was reported to be capable for the interruption against this feedback loop (122). Based on this finding, a Phase I clinical trial started from 2017 with combined treatment including trastuzumab and tocilizumab for patients with metastatic trastuzumab-resistant HER2+ breast cancer was carried out (NCT03135171). According to the reported literature, IL-6 signaling is a major determinant of TNBC cell proliferation and viability (123), and this chemotherapy-associated inflammatory cytokine may promote resistance mechanisms in TNBC cells as well (124). A Phase Ib/II, open-label, multicenter, randomized umbrella study is being carried out to evaluate the efficacy and safety of multiple immunotherapy-based treatment combinations including tocilizumab in patients with metastatic or inoperable locally advanced TNBC (NCT03424005).

The Prognostic Significance of IL-6 and Its Correlation With Survival

The prognostic impacts of preoperative IL-6 expression levels in patients with breast cancer remain controversial. In a meta-analysis extracted from thirteen articles containing 3,224 breast cancer patients showed that IL-6 expression was not associated with lymph node metastasis, tumor size, or histologic grade. Moreover, there was no correlation between IL-6 expression and disease-free survival. However, the combined hazard ratio for OS was 2.15 (125). Another study included 1,380 patients with early-stage invasive breast cancer revealed that high IL-6 expression is associated with better disease-free survival and breast cancer specific survival (126). However, anther investigation involving 55 female patients with invasive breast cancer demonstrated that the individuals with IL-6 ≥10.0 pg/ml had poorer overall survival compared with those with IL-6 <10.0 pg/ml (127). Similarly, it was reported that high level of serum IL-6 secreted by metastatic breast cancer cells were correlated with poor survival (15). Regarding the roles of IL-6 in ER positive breast cancers as previously described, we further summarized the prognostic value of IL-6 among different subtypes of breast cancer patients (**Table 1**). For example, in a prospective study included 240 patients who underwent surgery for management of newly diagnosed breast cancer, the associations between

TABLE 1 | Prognositc value of IL-6 in different types of breast cancers.

Tumor subtype[a]	Prognostic value of IL-6	Reference
Luminal A	•ER+ breast cancer cells express and/or secrete lower cytokine levels than ER- cells (128, 129) •High levels of gene expression of IL-6 receptor in luminal A and B (130)	(128–130)
Luminal B	•The luminal B HER2+ group was found to feature the highest spontaneous secretion of IL-6 among subgroups (131) •High levels of gene expression of IL-6 receptor in luminal A and B (130)	(130, 131)
HER2 (+/-)	•HER2- patients with recurrence had higher levels of circulating IL-6 (P=0.024) (132) •High IL-6 expression was significantly associated with DFS in HER2- (P = 0.026) (126) •High serum in HER2+ patients (P<0.05) (133) •IL6 as good indicator in both HER2- (P = 0.001) and HER2+ subgroups (P = 0.002) (134) •Association with HER2 or endocrine therapy resistance (122, 135)	(122, 126, 132–135)
TNBC	•Patients with recurrence had higher levels[b] of circulating IL-6 (P=0.024) (132) •High IL-6 expression was significantly associated with DFS in non-TNBC (P = 0.003) (126) •Induction of TNBC progression (123, 136, 137)	(123, 126, 132, 136, 137)
ER/PR status	•High IL-6 expression was significantly associated with DFS in ER+ (P = 0.025) (126) •High serum in ER+ patients (P<0.05) (133) •IL6 as the independent prognostic factor for good outcome (P=0.001) (134)	(126, 133, 134)
Metastasis	•Higher serum IL-6 level correlated with more metastatic sites (P<0.0001) (15)	(15)

[a]*Luminal A (ER+ and/or PR+, HER2-, and Ki-67 index<15%); luminal B ([ER+ and/or PR+, HER-, and Ki-67 index≥15%] or [ER+ and/or PR+,and HER2+]); HER2 only (ER-, PR-, and HER2 +); TNBC (ER-, PR-, and HER2-).*

[b]*High and low levels were determined based on the median value.*

plasma concentration of IL-6 and breast cancer recurrence during a six-year follow-up period were examined. The result showed that patients with recurrence had higher levels of circulating IL-6 only among those with HER2 negative tumors. Results of survival analyses revealed an association of high levels of IL-6 with poor recurrence-free survival in patients with HER2 negative and TNBC patients (132).

The approximate percentage of HER2 gene amplified in human breast cancer is 25%, which is characterized by a more aggressive phenotype (138). Trastuzumab, as one of the targeted therapeutic agents for HER2+ breast cancer patients, has totally changed the treatment course. Although many patients benefit from the HER2 targeted therapy, nearly half of them will develop drug resistance after one to two years of treatment (139). Evidence showed that overexpression of HER2 in breast CSCs increased IL-6 production, which could promote CSC self-renewal. The fact that HER2 targeted therapy could prominently activate the IL-6 inflammatory loop and expand the CSC population, signified the cause of IL-6 in Herceptin resistance (122). In ER-negative breast cancer, findings demonstrated that IL-66/Stat3/NF-κB inflammatory loop was activated (140). And it has been proved that leptin-induced STAT3 is partially cross activated through SK1-mediated IL-6 secretion and gp130 activation, suggesting the potential significance of this pathway (141).

A growing body of evidence indicated Bazedoxifene, which is a synthetic anti-gp130 compound, could effectively disrupt the IL-6R/gp130 interactions thus inhibit cell viability, and overall cell survive, proliferation as well as cell migration in TNBC (142). A novel in-house prepared IL-6 pathway inhibitor namely 6a, which is capable of selectively inhibiting STAT3 activation following IL-6 stimulation in MDA-MB-231 breast cancer (143). Sarilumab, an FDA-approved anti-IL-6R antibody for rheumatoid arthritis, which blocks both mIL-6R and sIL-6R, is currently under clinical studies for breast cancer (144). Siltuximab, which is a neutralizing anti-IL-6 antibody, delayed engraftment of MCF-7 humanized xenograft tumors and elicited tumor xenograft regression in tumors (145). The anti-IL-6 receptor antibody, Tocilizumab, is effective in the treatment of various autoimmune diseases such as rheumatoid arthritis (RA) (146). Experimental results demonstrates that IL-6 pathway targeted drugs may have additional benefit in HER2+ breast cancer (122). It has been proved that IL-6 receptor inhibitor suppressed bone metastases in a breast cancer cell line (147). Another study showed that IL-6R antagonist Tocilizumab significantly decreases breast cancer stem cell and inhibits tumor growth in Notch3-expressing breast cancers (148). The high level of IL-6R expression in spindle-shaped stromal cells such as CAF was not associated with the vasculature but could be used as prognostic determinant of early breast cancer (149). CAFs in tumor microenvironment played a vital role in developing trastuzumab resistance by magnifying CSCs bulge and activating multiple pathways (150). Regarding this, combination of anti-IL-6 antibody, or multiple pathway inhibitors with trastuzumab maybe novel strategy to reverse drug resistance in HER2+ breast cancer (71). Genotype of IL-6 was prominently related to early events among patients bearing with ER-negative tumors (151). The IL-6 signaling loop mediated drug resistance to PI3K inhibitors *via* inducing epithelial-mesenchymal transition (EMT) and CSCs expansion in human breast cancer cells (152). In summary, IL-6 signaling pathway may be potential treatment target for breast cancer patients in the future. The previously mentioned agents targeting the IL-6/IL-6R signaling for breast cancer therapy were listed in **Table 2**.

IL-6 could promote the response of acute phase inflammatory *via* increasing the production of acute inflammatory proteins. IL-6 was also correlated with elevated CRP in different kinds of cancers including breast cancer (154), renal cancer (155), lung cancer (156), and colorectal cancer (157). Although breast cancers rarely are characterized by inflammation, a growing body of evidence nevertheless suggests that inflammatory process also play an important role in breast cancer progression (158, 159). Based on the reported literature, the

TABLE 2 | Agents directly targeting the IL-6/IL-6R/gp130 complex for breast cancer therapy.

Agents	Antibody/Compound	Preclinical	Clinical trial	Mechanism
Bazedoxifene	Synthetic Anti-gp130 compound	Inhibit the growth of IL-6-induced SUM159 breast cancer cell line (153)	Breast tissue density change (NCT00774267) (NCT00418236)	1. Inhibition STAT3 phosphorylation by disrupting IL-6/gp130 interface (153) 2. Estrogen antagonist in breast tissue
6a	Anti-IL-6 synthetic pyrrolidinesulphonylaryl compound	Inhibition of STAT3 phosphorylation in IL-6 stimulated MDA-MB-231 breast cancer cell line (143)		Selective inhibition of STAT3 phosphorylation (143)
Sarilumab	IL-6R antagonist		To eliminate minimal residual disease in TNBC (NCT04333706)	Selective inhibition of STAT3 phosphorylation (143)
Siltuximab	CNTO-328, IL-6 mAb which received FDA-approval	Treatment in 6 orthotopically implanted PDX lines *in vivo* (145)		To prevent binding to soluble and membrane bound interleukin-6 receptors
Tocilizumab	IL-6R antagonist	Trastuzumab-resistant breast tumor xenograft mouse model	For metastatic HER2 positive breast cancer resistant to Trastuzumab (NCT03135171) Treatment combinations in patients with metastatic or inoperable locally advanced TNBC (NCT 03424005)	

results from epidemiologic studies in different centres are conflicting, with some showing significant association between elevated CRP levels and poor prognosis in breast cancers while others show no association (160–162). In a study consisted of 700 women with early-stage breast cancer found that elevated levels of CRP measured 2.5 years after diagnosis were associated with reduced DFS and OS (163). Similarly, another investigation included 2,910 women for up to seven years after invasive breast cancer diagnosis revealed elevated CRP levels were significantly associated with reduced DFS and OS (164). Preoperative CRP level was indicated as a more accurate prognostic factor compared with other factors, such as histological grade, tumor factor and node factor (127).

CONCLUSIONS

IL-6 is a pleiotropic cytokine in the regulations of various physiological and pathological processes. IL-6 causes uncontrolled inflammatory responses resulting in chronic inflammation and even carcinoma. IL-6 expression is associated with poor prognosis for breast cancer. The interaction network of IL-6 in breast cancer cells/stromal cells is listed as **Figure 1**. The IL-6 signal transduction pathway including IL-6, IL-6R, sIL-6R, gp130, JAK, and STAT3 has been suggested as promising therapeutic targets for breast cancer. Several antibodies for IL-6/IL-6R have been developed, either as single drug or combined with other traditional chemotherapy, have demonstrated dramatical

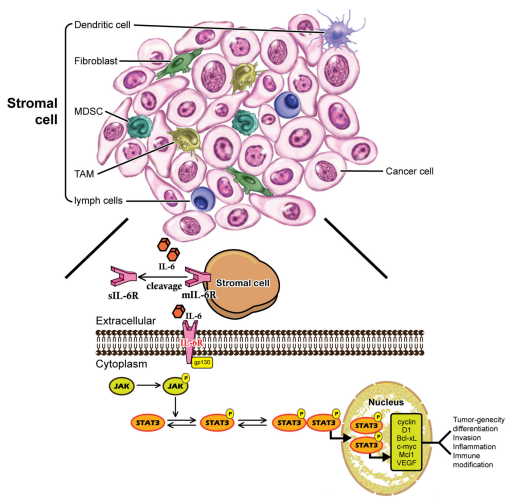


FIGURE 1 | The interaction network of IL-6 and breast cancer cells/stromal cells.

outcome in both preclinical and clinical trials. In addition to the critical roles of IL-6/JAK/STAT3 signaling in breast cancer, hyperactivation of this pathway has also been implicated in suppressing anti-tumor immune responses in tumor microenvironment. Treatments targeting the IL-6/JAK/STAT3 pathway have provided benefit for patients with breast cancer by directly inhibiting tumor cell growth and activating anti-tumor immunity. Taken together, strategy targeting the IL-6/JAK/STAT3 signaling pathway, which has already been shown to be beneficial in certain cancers including breast cancer, has proven to be effective. Combination of IL-6 signaling pathway inhibitor and other targets blockage drugs may serve as novel strategy to treat IL-6 mediated immune disease and human cancers.

AUTHOR CONTRIBUTIONS

Conception or design of the work, JC and YW. Data collection, JWC, WY, QH, YC, and KZ. Drafting the article, JC and JWC. Critical revision of the article, YW. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank Dr. Yao Huang for reviewing the article.

REFERENCES

1. Cancer Genome Atlas N. Comprehensive Molecular Portraits of Human Breast Tumours. *Nature* (2012) 490(7418):61–70. doi: 10.1038/nature11412
2. Pedersen BK. The Diseasome of Physical Inactivity–and the Role of Myokines in Muscle–Fat Cross Talk. *J Physiol* (2009) 587(Pt 23):5559–68. doi: 10.1113/jphysiol.2009.179515
3. Solinas G, Marchesi F, Garlanda C, Mantovani A, Allavena P. Inflammation-Mediated Promotion of Invasion and Metastasis. *Cancer Metastasis Rev* (2010) 29(2):243–8. doi: 10.1007/s10555-010-9227-2
4. Agarwal G, Pradeep PV, Aggarwal V, Yip CH, Cheung PS. Spectrum of Breast Cancer in Asian Women. *World J Surg* (2007) 31(5):1031–40. doi: 10.1007/s00268-005-0585-9
5. Agarwal G, Ramakant P. Breast Cancer Care in India: The Current Scenario and the Challenges for the Future. *Breast Care (Basel)* (2008) 3(1):21–7. doi: 10.1159/000115288
6. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. Helicobacter Pylori Infection and the Development of Gastric Cancer. *New Engl J Med* (2001) 345(11):784–9. doi: 10.1056/NEJMoa001999
7. Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. Il-6/Il-6 Receptor System and Its Role in Physiological and Pathological Conditions. *Clin Sci* (2011) 122(4):143–59. doi: 10.1042/cs20110340
8. Gabay C. Interleukin-6 and Chronic Inflammation. *Arthritis Res Ther* (2006) 8(2):S3. doi: 10.1186/ar1917
9. Scheele C, Nielsen S, Kelly M, Broholm C, Nielsen AR, Taudorf S, et al. Satellite Cells Derived From Obese Humans With Type 2 Diabetes and Differentiated Into Myocytes *in Vitro* Exhibit Abnormal Response to Il-6. *PloS One* (2012) 7(6):e39657. doi: 10.1371/journal.pone.0039657
10. Fischer CP, Berntsen A, Perstrup LB, Eskildsen P, Pedersen BK. Plasma Levels of Interleukin-6 and C-Reactive Protein Are Associated With Physical Inactivity Independent of Obesity. *Scandinavian J Med Sci sports* (2007) 17(5):580–7. doi: 10.1111/j.1600-0838.2006.00602.x
11. Guo Y, Xu F, Lu T, Duan Z, Zhang Z. Interleukin-6 Signaling Pathway in Targeted Therapy for Cancer. *Cancer Treat Rev* (2012) 38(7):904–10. doi: 10.1016/j.ctrv.2012.04.007
12. Liu X, Ma Y, Yang W, Wu X, Jiang L, Chen X. Identification of Therapeutic Targets for Breast Cancer Using Biological Informatics Methods. *Mol Med Rep* (2015) 12(2):1789–95. doi: 10.3892/mmr.2015.3565
13. Zubor P, Hatok J, Moricova P, Kapustova I, Kajo K, Mendelova A, et al. Gene Expression Profiling of Histologically Normal Breast Tissue in Females With Human Epidermal Growth Factor Receptor 2positive Breast Cancer. *Mol Med Rep* (2015) 11(2):1421–7. doi: 10.3892/mmr.2014.2863
14. Zhang GJ, Adachi I. Serum Interleukin-6 Levels Correlate to Tumor Progression and Prognosis in Metastatic Breast Carcinoma. *Anticancer Res* (1999) 19(2B):1427–32.
15. Salgado R, Junius S, Benoy I, Van Dam P, Vermeulen P, Van Marck E, et al. Circulating Interleukin-6 Predicts Survival in Patients With Metastatic Breast Cancer. *Int J Cancer* (2003) 103(5):642–6. doi: 10.1002/ijc.10833
16. Kishimoto T. Interleukin-6: Discovery of a Pleiotropic Cytokine. *Arthritis Res Ther* (2006) 8 Suppl 2:S2. doi: 10.1186/ar1916
17. Lotz M. Interleukin-6: A Comprehensive Review. *Cancer Treat Res* (1995) 80:209–33. doi: 10.1007/978-1-4613-1241-3_8
18. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The Pro- and Anti-Inflammatory Properties of the Cytokine Interleukin-6. *Biochim Biophys Acta* (2011) 1813(5):878–88. doi: 10.1016/j.bbamcr.2011.01.034
19. Varghese JN, Moritz RL, Lou M-Z, Donkelaar Av, Ji H, Ivancic N, et al. Structure of the Extracellular Domains of the Human Interleukin-6 Receptor α-Chain. *Proc Natl Acad Sci* (2002) 99(25):15959–64. doi: 10.1073/pnas.232432399
20. Scheller J, Rose-John S. Interleukin-6 and Its Receptor: From Bench to Bedside. *Med Microbiol Immunol* (2006) 195(4):173–83. doi: 10.1007/s00430-006-0019-9
21. Rose-John S, Scheller J, Elson G, Jones SA. Interleukin-6 Biology Is Coordinated by Membrane-Bound and Soluble Receptors: Role in Inflammation and Cancer. *J Leukoc Biol* (2006) 80(2):227–36. doi: 10.1189/jlb.1105674
22. Briso EM, Dienz O, Rincon M. Cutting Edge: Soluble Il-6r Is Produced by Il-6r Ectodomain Shedding in Activated Cd4 T Cells. *J Immunol* (2008) 180(11):7102–6. doi: 10.4049/jimmunol.180.11.7102
23. Bousoik E, Montazeri Aliabadi H. "Do We Know Jack" About Jak? A Closer Look at Jak/Stat Signaling Pathway. *Front Oncol* (2018) 8:287. doi: 10.3389/fonc.2018.00287
24. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The Complex Pattern of Cytokines in Serum From Patients With Meningococcal Septic Shock. Association Between Interleukin 6, Interleukin 1, and Fatal Outcome.+. *J Exp Med* (1989) 169(1):333–8. doi: 10.1084/jem.169.1.333
25. Honda M, Yamamoto S, Cheng M, Yasukawa K, Suzuki H, Saito T, et al. Human Soluble Il-6 Receptor: Its Detection and Enhanced Release by Hiv Infection. *J Immunol* (1992) 148(7):2175–80.
26. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of Interleukin (Il)-6-Type Cytokine Signalling and Its Regulation. *Biochem J* (2003) 374(Pt 1):1–20. doi: 10.1042/BJ20030407
27. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the Pi3k/Akt Pathway for Cancer Drug Discovery. *Nat Rev Drug Discovery* (2005) 4(12):988–1004. doi: 10.1038/nrd1902
28. Jego G, Palucka AK, Blanck JP, Chalouni C, Pascual V, Banchereau J. Plasmacytoid Dendritic Cells Induce Plasma Cell Differentiation Through Type I Interferon and Interleukin 6. *Immunity* (2003) 19(2):225–34. doi: 10.1016/S1074-7613(03)00208-5
29. Diehl S, Rincon M. The Two Faces of Il-6 on Th1/Th2 Differentiation. *Mol Immunol* (2002) 39(9):531–6. doi: 10.1016/s0161-5890(02)00210-9
30. Neveu WA, Allard JB, Dienz O, Wargo MJ, Ciliberto G, Whittaker LA, et al. Il-6 Is Required for Airway Mucus Production Induced by Inhaled Fungal Allergens. *J Immunol* (2009) 183(3):1732–8. doi: 10.4049/jimmunol.0802923

31. Diehl S, Anguita J, Hoffmeyer A, Zapton T, Ihle JN, Fikrig E, et al. Inhibition of Th1 Differentiation by Il-6 Is Mediated by Socs1. *Immunity* (2000) 13 (6):805–15. doi: 10.1016/S1074-7613(00)00078-9
32. Veldhoen M, Hocking RJ, Flavell RA, Stockinger B. Signals Mediated by Transforming Growth Factor-Beta Initiate Autoimmune Encephalomyelitis, But Chronic Inflammation Is Needed to Sustain Disease. *Nat Immunol* (2006) 7(11):1151–6. doi: 10.1038/ni1391
33. Ghoreschi K, Laurence A, Yang X-P, Tato CM, McGeachy MJ, Konkel JE, et al. Generation of Pathogenic Th17 Cells in the Absence of Tgf-B Signalling. *Nature* (2010) 467(7318):967–71. doi: 10.1038/nature09447
34. Kimura A, Kishimoto T. Il-6: Regulator of Treg/Th17 Balance. *Eur J Immunol* (2010) 40(7):1830–5. doi: 10.1002/eji.201040391
35. Choi HI, Chung KJ, Yang HY, Ren L, Sohn S, Kim PR, et al. Peroxiredoxin V Selectively Regulates Il-6 Production by Modulating the Jak2-Stat5 Pathway. *Free Radical Biol Med* (2013) 65:270–9. doi: 10.1016/j.freeradbiomed.2013.06.038
36. Fielding CA, McLoughlin RM, McLeod L, Colmont CS, Najdovska M, Grail D, et al. Il-6 Regulates Neutrophil Trafficking During Acute Inflammation *Via* Stat3. *J Immunol* (2008) 181(3):2189–95. doi: 10.4049/jimmunol.181.3.2189
37. Gabrilovich DI, Nagaraj S. Myeloid-Derived Suppressor Cells as Regulators of the Immune System. *Nat Rev Immunol* (2009) 9(3):162–74. doi: 10.1038/nri2506
38. Peranzoni E, Zilio S, Marigo I, Dolcetti L, Zanovello P, Mandruzzato S, et al. Myeloid-Derived Suppressor Cell Heterogeneity and Subset Definition. *Curr Opin Immunol* (2010) 22(2):238–44. doi: 10.1016/j.coi.2010.01.021
39. Liu M, Sakamaki T, Casimiro MC, Willmarth NE, Quong AA, Ju X, et al. The Canonical Nf-Kappab Pathway Governs Mammary Tumorigenesis in Transgenic Mice and Tumor Stem Cell Expansion. *Cancer Res* (2010) 70 (24):10464–73. doi: 10.1158/0008-5472.CAN-10-0732
40. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the Acute Phase Response. *Biochem J* (1990) 265(3):621–36. doi: 10.1042/bj2650621
41. Gulhar R, Ashraf MA, Jialal I. *Physiology, Acute Phase Reactants*. Treasure Island (FL: Statpearls (2020).
42. Sack GH. Serum Amyloid a – a Review. *Mol Med* (2018) 24(1):46. doi: 10.1186/s10020-018-0047-0
43. Slaats J, Ten Oever J, van de Veerdonk FL, Netea MG. Il-1beta/Il-6/Crp and Il-18/Ferritin: Distinct Inflammatory Programs in Infections. *PloS Pathog* (2016) 12(12):e1005973. doi: 10.1371/journal.ppat.1005973
44. Nanki T, Onoue I, Nagasaka K, Takayasu A, Ebisawa M, Hosoya T, et al. Suppression of Elevations in Serum C Reactive Protein Levels by Anti-Il-6 Autoantibodies in Two Patients With Severe Bacterial Infections. *Ann rheumatic Dis* (2013) 72(6):1100–2. doi: 10.1136/annrheumdis-2012-202768
45. Wei H. Interleukin 6 Signaling Maintains the Stem-Like Properties of Bladder Cancer Stem Cells. *Trans Cancer Res* (2019) 8(2):557–66. doi: 10.21037/tcr.2019.03.16
46. Kuphal S, Wallner S, Bosserhoff AK. Impact of Lif (Leukemia Inhibitory Factor) Expression in Malignant Melanoma. *Exp Mol Pathol* (2013) 95 (2):156–65. doi: 10.1016/j.yexmp.2013.06.012
47. Mathieu ME, Saucourt C, Mournetas V, Gauthereau X, Theze N, Praloran V, et al. Lif-Dependent Signaling: New Pieces in the Lego. *Stem Cell Rev* (2012) 8(1):1–15. doi: 10.1007/s12015-011-9261-7
48. Kamohara H, Sakamoto K, Ishiko T, Mita S, Masuda Y, Abe T, et al. Human Carcinoma Cell Lines Produce Biologically Active Leukemia Inhibitory Factor (Lif). *Res Commun Mol Pathol Pharmacol* (1994) 85(2):131–40. doi: 10.1002/stem.110
49. Bourillot PY, Aksoy I, Schreiber V, Wianny F, Schulz H, Hummel O, et al. Novel Stat3 Target Genes Exert Distinct Roles in the Inhibition of Mesoderm and Endoderm Differentiation in Cooperation With Nanog. *Stem Cells* (2009) 27(8):1760–71. doi: 10.1002/stem.110
50. Brady JJ, Li M, Suthram S, Jiang H, Wong WH, Blau HM. Early Role for Il-6 Signalling During Generation of Induced Pluripotent Stem Cells Revealed by Heterokaryon Rna-Seq. *Nat Cell Biol* (2013) 15(10):1244–52. doi: 10.1038/ncb2835
51. Balamurugan K, Mendoza-Villanueva D, Sharan S, Summers GH, Dobrolecki LE, Lewis MT, et al. C/Ebpdelta Links Il-6 and Hif-1 Signaling to Promote Breast Cancer Stem Cell-Associated Phenotypes. *Oncogene* (2019) 38(20):3765–80. doi: 10.1038/s41388-018-0516-5
52. Kim SY, Kang JW, Song X, Kim BK, Yoo YD, Kwon YT, et al. Role of the Il-6-Jak1-Stat3-Oct-4 Pathway in the Conversion of Non-Stem Cancer Cells Into Cancer Stem-Like Cells. *Cell signalling* (2013) 25(4):961–9. doi: 10.1016/j.cellsig.2013.01.007
53. Yi H, Cho HJ, Cho SM, Jo K, Park JA, Kim NH, et al. Blockade of Interleukin-6 Receptor Suppresses the Proliferation of H460 Lung Cancer Stem Cells. *Int J Oncol* (2012) 41(1):310–6. doi: 10.3892/ijo.2012.1447
54. Lin L, Liu A, Peng Z, Lin HJ, Li PK, Li C, et al. Stat3 Is Necessary for Proliferation and Survival in Colon Cancer-Initiating Cells. *Cancer Res* (2011) 71(23):7226–37. doi: 10.1158/0008-5472.CAN-10-4660
55. Lin S, Li S, Chen Z, He X, Zhang Y, Xu X, et al. Formation, Recognition and Bioactivities of a Novel G-Quadruplex in the Stat3 Gene. *Bioorg med Chem Lett* (2011) 21(19):5987–91. doi: 10.1016/j.bmcl.2011.07.121
56. Garner JM, Fan M, Yang CH, Du Z, Sims M, Davidoff AM, et al. Constitutive Activation of Signal Transducer and Activator of Transcription 3 (Stat3) and Nuclear Factor Kappab Signaling in Glioblastoma Cancer Stem Cells Regulates the Notch Pathway. *J Biol Chem* (2013) 288(36):26167–76. doi: 10.1074/jbc.M113.477950
57. Chu Y, Wang Y, Peng W, Xu L, Liu M, Li J, et al. Stat3 Activation by Il-6 From Adipose-Derived Stem Cells Promotes Endometrial Carcinoma Proliferation and Metastasis. *Biochem Biophys Res Commun* (2018) 500 (3):626–31. doi: 10.1016/j.bbrc.2018.04.121
58. D'Anello L, Sansone P, Storci G, Mitrugno V, D'Uva G, Chieco P, et al. Epigenetic Control of the Basal-Like Gene Expression Profile *Via* Interleukin-6 in Breast Cancer Cells. *Mol Cancer* (2010) 9:300. doi: 10.1186/1476-4598-9-300
59. Hodge DR, Hurt EM, Farrar WL. The Role of Il-6 and Stat3 in Inflammation and Cancer. *Eur J Cancer* (2005) 41(16):2502–12. doi: 10.1016/j.ejca.2005.08.016
60. Iliopoulos D, Hirsch HA, Struhl K. An Epigenetic Switch Involving Nf-Kappab, Lin28, Let-7 Microrna, and Il6 Links Inflammation to Cell Transformation. *Cell* (2009) 139(4):693–706. doi: 10.1016/j.cell.2009.10.014
61. Iliopoulos D, Jaeger SA, Hirsch HA, Bulyk ML, Struhl K. Stat3 Activation of Mir-21 and Mir-181b-1 *Via* Pten and Cyld Are Part of the Epigenetic Switch Linking Inflammation to Cancer. *Mol Cell* (2010) 39(4):493–506. doi: 10.1016/j.molcel.2010.07.023
62. Dethlefsen C, Hojfeldt G, Hojman P. The Role of Intratumoral and Systemic Il-6 in Breast Cancer. *Breast Cancer Res Treat* (2013) 138(3):657–64. doi: 10.1007/s10549-013-2488-z
63. Waugh DJ, Wilson C. The Interleukin-8 Pathway in Cancer. *Clin Cancer Res* (2008) 14(21):6735–41. doi: 10.1158/1078-0432.CCR-07-4843
64. Fujisaki K, Fujimoto H, Sangai T, Nagashima T, Sakakibara M, Shiina N, et al. Cancer-Mediated Adipose Reversion Promotes Cancer Cell Migration *Via* Il-6 and Mcp-1. *Breast Cancer Res Treat* (2015) 150(2):255–63. doi: 10.1007/s10549-015-3318-2
65. Liu S, Ginestier C, Ou SJ, Clouthier SG, Patel SH, Monville F, et al. Breast Cancer Stem Cells Are Regulated by Mesenchymal Stem Cells Through Cytokine Networks. *Cancer Res* (2011) 71(2):614–24. doi: 10.1158/0008-5472.CAN-10-0538
66. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al. Mesenchymal Stem Cells Within Tumour Stroma Promote Breast Cancer Metastasis. *Nature* (2007) 449(7162):557–63. doi: 10.1038/nature06188
67. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage Potential of Adult Human Mesenchymal Stem Cells. *Science* (1999) 284(5411):143–7. doi: 10.1126/science.284.5411.143
68. Erez N, Glanz S, Raz Y, Avivi C, Barshack I. Cancer Associated Fibroblasts Express Pro-Inflammatory Factors in Human Breast and Ovarian Tumors. *Biochem Biophys Res Commun* (2013) 437(3):397–402. doi: 10.1016/j.bbrc.2013.06.089
69. Quante M, Tu SP, Tomita H, Gonda T, Wang SS, Takashi S, et al. Bone Marrow-Derived Myofibroblasts Contribute to the Mesenchymal Stem Cell Niche and Promote Tumor Growth. *Cancer Cell* (2011) 19(2):257–72. doi: 10.1016/j.ccr.2011.01.020
70. Kinoshita H, Hirata Y, Nakagawa H, Sakamoto K, Hayakawa Y, Takahashi R, et al. Interleukin-6 Mediates Epithelial-Stromal Interactions and Promotes Gastric Tumorigenesis. *PloS One* (2013) 8(4):e60914. doi: 10.1371/journal.pone.0060914

71. Mao Y, Zhang Y, Qu Q, Zhao M, Lou Y, Liu J, et al. Cancer-Associated Fibroblasts Induce Trastuzumab Resistance in Her2 Positive Breast Cancer Cells. *Mol Biosyst* (2015) 11(4):1029–40. doi: 10.1039/c4mb00710g
72. Hendrayani SF, Al-Khalaf HH, Aboussekhra A. The Cytokine Il-6 Reactivates Breast Stromal Fibroblasts Through Transcription Factor Stat3-Dependent Up-Regulation of the Rna-Binding Protein Auf1. *J Biol Chem* (2014) 289(45):30962–76. doi: 10.1074/jbc.M114.594044
73. Kato T, Noma K, Ohara T, Kashima H, Katsura Y, Sato H, et al. Cancer-Associated Fibroblasts Affect Intratumoral Cd8(+) and Foxp3(+) T Cells *Via* Interleukin 6 in the Tumor Microenvironment. *Clin Cancer Res* (2018) 24 (19):4820–33. doi: 10.1158/1078-0432.CCR-18-0205
74. Li P, Shan JX, Chen XH, Zhang D, Su LP, Huang XY, et al. Epigenetic Silencing of Microrna-149 in Cancer-Associated Fibroblasts Mediates Prostaglandin E2/Interleukin-6 Signaling in the Tumor Microenvironment. *Cell Res* (2015) 25(5):588–603. doi: 10.1038/cr.2015.51
75. Xiong S, Wang R, Chen Q, Luo J, Wang J, Zhao Z, et al. Cancer-Associated Fibroblasts Promote Stem Cell-Like Properties of Hepatocellular Carcinoma Cells Through Il-6/Stat3/Notch Signaling. *Am J Cancer Res* (2018) 8(2):302–16.
76. Guo C, Chen Y, Gao W, Chang A, Ye Y, Shen W, et al. Liposomal Nanoparticles Carrying Anti-Il6r Antibody to the Tumour Microenvironment Inhibit Metastasis in Two Molecular Subtypes of Breast Cancer Mouse Models. *Theranostics* (2017) 7(3):775–88. doi: 10.7150/thno.17237
77. Calle EE, Thun MJ. Obesity and Cancer. *Oncogene* (2004) 23(38):6365–78. doi: 10.1038/sj.onc.1207751
78. Majed B, Moreau T, Senouci K, Salmon RJ, Fourquet A, Asselain B. Is Obesity an Independent Prognosis Factor in Woman Breast Cancer? *Breast Cancer Res Treat* (2008) 111(2):329–42. doi: 10.1007/s10549-007-9785-3
79. Dirat B, Bochet L, Escourrou G, Valet P, Muller C. Unraveling the Obesity and Breast Cancer Links: A Role for Cancer-Associated Adipocytes? *Endocr Dev* (2010) 19:45–52. doi: 10.1159/000316896
80. Zhang W, Mottillo EP, Zhao J, Gartung A, VanHecke GC, Lee JF, et al. Adipocyte Lipolysis-Stimulated Interleukin-6 Production Requires Sphingosine Kinase 1 Activity. *J Biol Chem* (2014) 289(46):32178–85. doi: 10.1074/jbc.M114.601096
81. Kim HS, Jung M, Choi SK, Woo J, Piao YJ, Hwang EH, et al. Il-6-Mediated Cross-Talk Between Human Preadipocytes and Ductal Carcinoma in Situ in Breast Cancer Progression. *J Exp Clin Cancer Res* (2018) 37(1):200. doi: 10.1186/s13046-018-0867-3
82. Walter M, Liang S, Ghosh S, Hornsby PJ, Li R. Interleukin 6 Secreted From Adipose Stromal Cells Promotes Migration and Invasion of Breast Cancer Cells. *Oncogene* (2009) 28(30):2745–55. doi: 10.1038/onc.2009.130
83. Incio J, Ligibel JA, McManus DT, Suboj P, Jung K, Kawaguchi K, et al. Obesity Promotes Resistance to Anti-Vegf Therapy in Breast Cancer by Up-Regulating Il-6 and Potentially Fgf-2. *Sci Transl Med* (2018) 10(432):1–30. doi: 10.1126/scitranslmed.aag0945
84. Santer FR, Malinowska K, Culig Z, Cavarretta IT. Interleukin-6 Trans-Signalling Differentially Regulates Proliferation, Migration, Adhesion and Maspin Expression in Human Prostate Cancer Cells. *Endocrine-related Cancer* (2010) 17(1):241–53. doi: 10.1677/ERC-09-0200
85. Suchi K, Fujiwara H, Okamura S, Okamura H, Umehara S, Todo M, et al. Overexpression of Interleukin-6 Suppresses Cisplatin-Induced Cytotoxicity in Esophageal Squamous Cell Carcinoma Cells. *Anticancer Res* (2011) 31 (1):67–75.
86. Grivennikov S, Karin M. Autocrine Il-6 Signaling: A Key Event in Tumorigenesis? *Cancer Cell* (2008) 13(1):7–9. doi: 10.1016/j.ccr.2007.12.020
87. Leslie K, Gao SP, Berishaj M, Podsypanina K, Ho H, Ivashkiv L, et al. Differential Interleukin-6/Stat3 Signaling as a Function of Cellular Context Mediates Ras-Induced Transformation. *Breast Cancer Res* (2010) 12(5):R80. doi: 10.1186/bcr2725
88. Sansone P, Storci G, Tavolari S, Guarnieri T, Giovannini C, Taffurelli M, et al. Il-6 Triggers Malignant Features in Mammospheres From Human Ductal Breast Carcinoma and Normal Mammary Gland. *J Clin Invest* (2007) 117(12):3988–4002. doi: 10.1172/JCI32533
89. Naugler WE, Karin M. The Wolf in Sheep's Clothing: The Role of Interleukin-6 in Immunity, Inflammation and Cancer. *Trends Mol Med* (2008) 14(3):109–19. doi: 10.1016/j.molmed.2007.12.007
90. Garcia-Tunon I, Ricote M, Ruiz A, Fraile B, Paniagua R, Royuela M. Il-6, Its Receptors and Its Relationship With Bcl-2 and Bax Proteins in Infiltrating and in Situ Human Breast Carcinoma. *Histopathology* (2005) 47(1):82–9. doi: 10.1111/j.1365-2559.2005.02178.x
91. Rincon M, Broadwater G, Harris L, Crocker A, Weaver D, Dressler L, et al. Interleukin-6, Multidrug Resistance Protein-1 Expression and Response to Paclitaxel in Women With Metastatic Breast Cancer: Results of Cancer and Leukemia Group B Trial 159806. *Breast Cancer Res Treat* (2006) 100(3):301–8. doi: 10.1007/s10549-006-9251-7
92. He JY, Wei XH, Li SJ, Liu Y, Hu HL, Li ZZ, et al. Adipocyte-Derived Il-6 and Leptin Promote Breast Cancer Metastasis *Via* Upregulation of Lysyl Hydroxylase-2 Expression. *Cell Commun Signal* (2018) 16(1):100. doi: 10.1186/s12964-018-0309-z
93. Fu S, Lin J. Blocking Interleukin-6 and Interleukin-8 Signaling Inhibits Cell Viability, Colony-Forming Activity, and Cell Migration in Human Triple-Negative Breast Cancer and Pancreatic Cancer Cells. *Anticancer Res* (2018) 38(11):6271–9. doi: 10.21873/anticanres.12983
94. Jafarzadeh A, Minaee K, Farsinejad AR, Nemati M, Khosravimashizi A, Daneshvar H, et al. Evaluation of the Circulating Levels of Il-12 and Il-33 in Patients With Breast Cancer: Influences of the Tumor Stages and Cytokine Gene Polymorphisms. *Iran J Basic Med Sci* (2015) 18(12):1189–98.
95. Nunez-Marrero A. Assessing the Role of the Interleukin-12/Stat4 Axis in Breast Cancer by a Bioinformatics Approach. *Int J Sci Basic Appl Res* (2019) 48(2):38–52.
96. Jacobson NG, Szabo SJ, Weber-Nordt RM, Zhong Z, Schreiber RD, Darnell JEJr., et al. Interleukin 12 Signaling in T Helper Type 1 (Th1) Cells Involves Tyrosine Phosphorylation of Signal Transducer and Activator of Transcription (Stat)3 and Stat4. *J Exp Med* (1995) 181(5):1755–62. doi: 10.1084/jem.181.5.1755
97. Green VL, Alexandropoulou A, Walker MB, Walker AA, Sharp DM, Walker LG, et al. Alterations in the Th1/Th2 Balance in Breast Cancer Patients Using Reflexology and Scalp Massage. *Exp Ther Med* (2010) 1(1):97–108. doi: 10.3892/etm_00000018
98. Hong CC, Yao S, McCann SE, Dolnick RY, Wallace PK, Gong Z, et al. Pretreatment Levels of Circulating Th1 and Th2 Cytokines, and Their Ratios, Are Associated With Er-Negative and Triple Negative Breast Cancers. *Breast Cancer Res Treat* (2013) 139(2):477–88. doi: 10.1007/s10549-013-2549-3
99. Tanaka T, Narazaki M, Kishimoto T. Il-6 in Inflammation, Immunity, and Disease. *Cold Spring Harb Perspect Biol* (2014) 6(10):a016295. doi: 10.1101/cshperspect.a016295
100. de Matos-Neto EM, Lima JD, de Pereira WO, Figueredo RG, Riccardi DM, Radloff K, et al. Systemic Inflammation in Cachexia - Is Tumor Cytokine Expression Profile the Culprit? *Front Immunol* (2015) 6:629. doi: 10.3389/fimmu.2015.00629
101. Barton BE. Il-6-Like Cytokines and Cancer Cachexia: Consequences of Chronic Inflammation. *Immunol Res* (2001) 23(1):41–58. doi: 10.1385/IR:23:1:41
102. Penet MF, Bhujwalla ZM. Cancer Cachexia, Recent Advances, and Future Directions. *Cancer J* (2015) 21(2):117–22. doi: 10.1097/PPO.0000000000000100
103. Morrow RJ, Allam AH, Yeo B, Deb S, Murone C, Lim E, et al. Paracrine Il-6 Signaling Confers Proliferation Between Heterogeneous Inflammatory Breast Cancer Sub-Clones. *Cancers (Basel)* (2022) 14(9):2292–309. doi: 10.3390/cancers14092292
104. Choy EH, De Benedetti F, Takeuchi T, Hashizume M, John MR, Kishimoto T. Translating Il-6 Biology Into Effective Treatments. *Nat Rev Rheumatol* (2020) 16(6):335–45. doi: 10.1038/s41584-020-0419-z
105. Coussens LM, Werb Z. Inflammation and Cancer. *Nature* (2002) 420 (6917):860–7. doi: 10.1038/nature01322
106. Greten FR, Grivennikov SI. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity* (2019) 51(1):27–41. doi: 10.1016/j.immuni.2019.06.025
107. Grivennikov SI, Greten FR, Karin M. Immunity, Inflammation, and Cancer. *Cell* (2010) 140(6):883–99. doi: 10.1016/j.cell.2010.01.025
108. Piotrowski I, Kulcenty K, Suchorska W. Interplay Between Inflammation and Cancer. *Rep Pract Oncol Radiother* (2020) 25(3):422–7. doi: 10.1016/j.rpor.2020.04.004

109. Ortiz-Montero P, Londono-Vallejo A, Vernot JP. Senescence-Associated Il-6 and Il-8 Cytokines Induce a Self- and Cross-Reinforced Senescence/Inflammatory Milieu Strengthening Tumorigenic Capabilities in the Mcf-7 Breast Cancer Cell Line. *Cell Commun Signal* (2017) 15(1):17. doi: 10.1186/s12964-017-0172-3
110. Clarke R, Leonessa F, Welch JN, Skaar TC. Cellular and Molecular Pharmacology of Antiestrogen Action and Resistance. *Pharmacol Rev* (2001) 53(1):25–71.
111. Clarke R, Liu MC, Bouker KB, Gu Z, Lee RY, Zhu Y, et al. Antiestrogen Resistance in Breast Cancer and the Role of Estrogen Receptor Signaling. *Oncogene* (2003) 22(47):7316–39. doi: 10.1038/sj.onc.1206937
112. Siersbaek R, Scabia V, Nagarajan S, Chernukhin I, Papachristou EK, Broome R, et al. Il6/Stat3 Signaling Hijacks Estrogen Receptor Alpha Enhancers to Drive Breast Cancer Metastasis. *Cancer Cell* (2020) 38(3):412–23 e9. doi: 10.1016/j.ccell.2020.06.007
113. Tsoi H, Man EPS, Chau KM, Khoo US. Targeting the Il-6/Stat3 Signalling Cascade to Reverse Tamoxifen Resistance in Estrogen Receptor Positive Breast Cancer. *Cancers (Basel)* (2021) 13(7):1511–32. doi: 10.3390/cancers13071511
114. Hou L, Xie S, Li G, Xiong B, Gao Y, Zhao X, et al. Il-6 Triggers the Migration and Invasion of Oestrogen Receptor-Negative Breast Cancer Cells *Via* Regulation of Hippo Pathways. *Basic Clin Pharmacol Toxicol* (2018) 123(5):549–57. doi: 10.1111/bcpt.13042
115. Benoy I, Salgado R, Colpaert C, Weytjens R, Vermeulen PB, Dirix LY. Serum Interleukin 6, Plasma Vegf, Serum Vegf, and Vegf Platelet Load in Breast Cancer Patients. *Clin Breast Cancer* (2002) 2(4):311–5. doi: 10.3816/CBC.2002.n.008
116. Shibayama O, Yoshiuchi K, Inagaki M, Matsuoka Y, Yoshikawa E, Sugawara Y, et al. Association Between Adjuvant Regional Radiotherapy and Cognitive Function in Breast Cancer Patients Treated With Conservation Therapy. *Cancer Med* (2014) 3(3):702–9. doi: 10.1002/cam4.174
117. Chavey C, Bibeau F, Gourgou-Bourgade S, Burlinchon S, Boissiere F, Laune D, et al. Oestrogen Receptor Negative Breast Cancers Exhibit High Cytokine Content. *Breast Cancer Res* (2007) 9(1):R15. doi: 10.1186/bcr1648
118. Conze D, Weiss L, Regen PS, Bhushan A, Weaver D, Johnson P, et al. Autocrine Production of Interleukin 6 Causes Multidrug Resistance in Breast Cancer Cells. *Cancer Res* (2001) 61(24):8851–8.
119. Sethi N, Dai X, Winter CG, Kang Y. Tumor-Derived Jagged1 Promotes Osteolytic Bone Metastasis of Breast Cancer by Engaging Notch Signaling in Bone Cells. *Cancer Cell* (2011) 19(2):192–205. doi: 10.1016/j.ccr.2010.12.022
120. Gao SP, Mark KG, Leslie K, Pao W, Motoi N, Gerald WL, et al. Mutations in the Egfr Kinase Domain Mediate Stat3 Activation *Via* Il-6 Production in Human Lung Adenocarcinomas. *J Clin Invest* (2007) 117(12):3846–56. doi: 10.1172/JCI31871
121. Ara T, Declerck YA. Interleukin-6 in Bone Metastasis and Cancer Progression. *Eur J Cancer* (2010) 46(7):1223–31. doi: 10.1016/j.ejca.2010.02.026
122. Korkaya H, Kim GI, Davis A, Malik F, Henry NL, Ithimakin S, et al. Activation of an Il6 Inflammatory Loop Mediates Trastuzumab Resistance in Her2+ Breast Cancer by Expanding the Cancer Stem Cell Population. *Mol Cell* (2012) 47(4):570–84. doi: 10.1016/j.molcel.2012.06.014
123. Jin K, Pandey NB, Popel AS. Simultaneous Blockade of Il-6 and Ccl5 Signaling for Synergistic Inhibition of Triple-Negative Breast Cancer Growth and Metastasis. *Breast Cancer Res* (2018) 20(1):54. doi: 10.1186/s13058-018-0981-3
124. Chung AW, Kozielski AJ, Qian W, Zhou J, Anselme AC, Chan AA, et al. Tocilizumab Overcomes Chemotherapy Resistance in Mesenchymal Stem-Like Breast Cancer by Negating Autocrine Il-1a Induction of Il-6. *NPJ Breast Cancer* (2022) 8(1):30. doi: 10.1038/s41523-021-00371-0
125. Lin S, Gan Z, Han K, Yao Y, Min D. Interleukin-6 as a Prognostic Marker for Breast Cancer: A Meta-Analysis. *Tumori* (2015) 101(5):535–41. doi: 10.5301/tj.5000357
126. Ahmad N, Ammar A, Storr SJ, Green AR, Rakha E, Ellis IO, et al. Il-6 and Il-10 Are Associated With Good Prognosis in Early Stage Invasive Breast Cancer Patients. *Cancer Immunol Immunother* (2018) 67(4):537–49. doi: 10.1007/s00262-017-2106-8
127. Shimura T, Shibata M, Gonda K, Murakami Y, Noda M, Tachibana K, et al. Prognostic Impact of Interleukin-6 and C-Reactive Protein on Patients With Breast Cancer. *Oncol Lett* (2019) 17(6):5139–46. doi: 10.3892/ol.2019.10183
128. Chiu JJ, Sgagias MK, Cowan KH. Interleukin 6 Acts as a Paracrine Growth Factor in Human Mammary Carcinoma Cell Lines. *Clin Cancer Res* (1996) 2(1):215–21.
129. Liu H, Liu K, Bodenner DL. Estrogen Receptor Inhibits Interleukin-6 Gene Expression by Disruption of Nuclear Factor Kappab Transactivation. *Cytokine* (2005) 31(4):251–7. doi: 10.1016/j.cyto.2004.12.008
130. Fertig EJ, Lee E, Pandey NB, Popel AS. Analysis of Gene Expression of Secreted Factors Associated With Breast Cancer Metastases in Breast Cancer Subtypes. *Sci Rep* (2015) 5:12133. doi: 10.1038/srep12133
131. Autenshlyus A, Davletova K, Varaksin N, Marinkin I, Lyakhovich V. Cytokines in Various Molecular Subtypes of Breast Cancer. *Int J Immunopathol Pharmacol* (2021) 35:20587384211034089. doi: 10.1177/20587384211034089
132. Cho YA, Sung MK, Yeon JY, Ro J, Kim J. Prognostic Role of Interleukin-6, Interleukin-8, and Leptin Levels According to Breast Cancer Subtype. *Cancer Res Treat* (2013) 45(3):210–9. doi: 10.4143/crt.2013.45.3.210
133. Ma Y, Ren Y, Dai ZJ, Wu CJ, Ji YH, Xu J. Il-6, Il-8 and Tnf-Alpha Levels Correlate With Disease Stage in Breast Cancer Patients. *Adv Clin Exp Med* (2017) 26(3):421–6. doi: 10.17219/acem/62120
134. Milovanovic J, Todorovic-Rakovic N, Radulovic M. Interleukin-6 and Interleukin-8 Serum Levels in Prognosis of Hormone-Dependent Breast Cancer. *Cytokine* (2019) 118:93–8. doi: 10.1016/j.cyto.2018.02.019
135. Masjedi A, Hashemi V, Hojjat-Farsangi M, Ghalamfarsa G, Azizi G, Yousefi M, et al. The Significant Role of Interleukin-6 and Its Signaling Pathway in the Immunopathogenesis and Treatment of Breast Cancer. *BioMed Pharmacother* (2018) 108:1415–24. doi: 10.1016/j.biopha.2018.09.177
136. Hartman ZC, Poage GM, den Hollander P, Tsimelzon A, Hill J, Panupinthu N, et al. Growth of Triple-Negative Breast Cancer Cells Relies Upon Coordinate Autocrine Expression of the Proinflammatory Cytokines Il-6 and Il-8. *Cancer Res* (2013) 73(11):3470–80. doi: 10.1158/0008-5472.CAN-12-4524-T
137. Liang S, Chen Z, Jiang G, Zhou Y, Liu Q, Su Q, et al. Activation of Gper Suppresses Migration and Angiogenesis of Triple Negative Breast Cancer *Via* Inhibition of Nf-Kappab/Il-6 Signals. *Cancer Lett* (2017) 386:12–23. doi: 10.1016/j.canlet.2016.11.003
138. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human Breast Cancer: Correlation of Relapse and Survival With Amplification of the Her-2/Neu Oncogene. *Science* (1987) 235(4785):177–82. doi: 10.1126/science.3798106
139. Lan KH, Lu CH, Yu D. Mechanisms of Trastuzumab Resistance and Their Clinical Implications. *Ann New York Acad Sci* (2005) 1059:70–5. doi: 10.1196/annals.1339.026
140. Kim G, Ouzounova M, Quraishi AA, Davis A, Tawakkol N, Clouthier SG, et al. Socs3-Mediated Regulation of Inflammatory Cytokines in Pten and P53 Inactivated Triple Negative Breast Cancer Model. *Oncogene* (2015) 34(6):671–80. doi: 10.1038/onc.2014.4
141. Alshaker H, Wang Q, Frampton AE, Krell J, Waxman J, Winkler M, et al. Sphingosine Kinase 1 Contributes to Leptin-Induced Stat3 Phosphorylation Through Il-6/Gp130 Transactivation in Oestrogen Receptor-Negative Breast Cancer. *Breast Cancer Res Treat* (2015) 149(1):59–67. doi: 10.1007/s10549-014-3228-8
142. Tian J, Chen X, Fu S, Zhang R, Pan L, Cao Y, et al. Bazedoxifene Is a Novel Il-6/Gp130 Inhibitor for Treating Triple-Negative Breast Cancer. *Breast Cancer Res Treat* (2019) 175(3):553–66. doi: 10.1007/s10549-019-05183-2
143. Zinzalla G, Haque MR, Basu BP, Anderson J, Kaye SL, Haider S, et al. A Novel Small-Molecule Inhibitor of Il-6 Signalling. *Bioorg Med Chem Lett* (2010) 20(23):7029–32. doi: 10.1016/j.bmcl.2010.09.117
144. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, et al. Drug Repurposing: Progress, Challenges and Recommendations. *Nat Rev Drug Discovery* (2019) 18(1):41–58. doi: 10.1038/nrd.2018.168
145. Casneuf T, Axel AE, King P, Alvarez JD, Werbeck JL, Verhulst T, et al. Interleukin-6 Is a Potential Therapeutic Target in Interleukin-6 Dependent, Estrogen Receptor-Alpha-Positive Breast Cancer. *Breast Cancer (Dove Med Press)* (2016) 8:13–27. doi: 10.2147/BCTT.S92414

146. Biggioggero M, Crotti C, Becciolini A, Favalli EG. Tocilizumab in the Treatment of Rheumatoid Arthritis: An Evidence-Based Review and Patient Selection. *Drug Des Devel Ther* (2019) 13:57–70. doi: 10.2147/DDDT.S150580
147. Wakabayashi H, Hamaguchi T, Nagao N, Kato S, Iino T, Nakamura T, et al. Interleukin-6 Receptor Inhibitor Suppresses Bone Metastases in a Breast Cancer Cell Line. *Breast Cancer* (2018) 25(5):566–74. doi: 10.1007/s12282-018-0853-9
148. Wang D, Xu J, Liu B, He X, Zhou L, Hu X, et al. Il6 Blockade Potentiates the Anti-Tumor Effects of Gamma-Secretase Inhibitors in Notch3-Expressing Breast Cancer. *Cell Death Differ* (2018) 25(2):330–9. doi: 10.1038/cdd.2017.162
149. Labovsky V, Martinez LM, Calcagno ML, Davies KM, Garcia-Rivello H, Wernicke A, et al. Interleukin-6 Receptor in Spindle-Shaped Stromal Cells, a Prognostic Determinant of Early Breast Cancer. *Tumour Biol* (2016) 37 (10):13377–84. doi: 10.1007/s13277-016-5268-7
150. Chan T-S, Shaked Y, Tsai KK. Targeting the Interplay Between Cancer Fibroblasts, Mesenchymal Stem Cells, and Cancer Stem Cells in Desmoplastic Cancers. *Front Oncol* (2019) 9:688. doi: 10.3389/fonc.2019.00688
151. Markkula A, Simonsson M, Ingvar C, Rose C, Jernstrom H. Il6 Genotype, Tumour Er-Status, and Treatment Predicted Disease-Free Survival in a Prospective Breast Cancer Cohort. *BMC Cancer* (2014) 14:759. doi: 10.1186/1471-2407-14-759
152. Yang L, Han S, Sun Y. An Il6-Stat3 Loop Mediates Resistance to Pi3k Inhibitors by Inducing Epithelial-Mesenchymal Transition and Cancer Stem Cell Expansion in Human Breast Cancer Cells. *Biochem Biophys Res Commun* (2014) 453(3):582–7. doi: 10.1016/j.bbrc.2014.09.129
153. Li H, Xiao H, Lin L, Jou D, Kumari V, Lin J, et al. Drug Design Targeting Protein-Protein Interactions (Ppis) Using Multiple Ligand Simultaneous Docking (Mlsd) and Drug Repositioning: Discovery of Raloxifene and Bazedoxifene as Novel Inhibitors of Il-6/Gp130 Interface. *J Med Chem* (2014) 57(3):632–41. doi: 10.1021/jm401144z
154. Ravishankaran P, Karunanithi R. Clinical Significance of Preoperative Serum Interleukin-6 and C-Reactive Protein Level in Breast Cancer Patients. *World J Surg Oncol* (2011) 9:18. doi: 10.1186/1477-7819-9-18
155. Yoshida N, Ikemoto S, Narita K, Sugimura K, Wada S, Yasumoto R, et al. Interleukin-6, Tumour Necrosis Factor Alpha and Interleukin-1beta in Patients With Renal Cell Carcinoma. *Br J Cancer* (2002) 86(9):1396–400. doi: 10.1038/sj.bjc.6600257
156. McKeown DJ, Brown DJ, Kelly A, Wallace AM, McMillan DC. The Relationship Between Circulating Concentrations of C-Reactive Protein, Inflammatory Cytokines and Cytokine Receptors in Patients With Non-Small-Cell Lung Cancer. *Br J Cancer* (2004) 91(12):1993–5. doi: 10.1038/sj.bjc.6602248
157. Chung YC, Chang YF. Serum Interleukin-6 Levels Reflect the Disease Status of Colorectal Cancer. *J Surg Oncol* (2003) 83(4):222–6. doi: 10.1002/jso.10269
158. Ginestier C, Liu S, Diebel ME, Korkaya H, Luo M, Brown M, et al. Cxcr1 Blockade Selectively Targets Human Breast Cancer Stem Cells *in Vitro* and in Xenografts. *J Clin Invest* (2010) 120(2):485–97. doi: 10.1172/JCI39397
159. Liao D, Luo Y, Markowitz D, Xiang R, Reisfeld RA. Cancer Associated Fibroblasts Promote Tumor Growth and Metastasis by Modulating the Tumor Immune Microenvironment in a 4t1 Murine Breast Cancer Model. *PloS One* (2009) 4(11):e7965. doi: 10.1371/journal.pone.0007965
160. Al Murri AM, Bartlett JM, Canney PA, Doughty JC, Wilson C, McMillan DC. Evaluation of an Inflammation-Based Prognostic Score (Gps) in Patients With Metastatic Breast Cancer. *Br J Cancer* (2006) 94(2):227–30. doi: 10.1038/sj.bjc.6602922
161. Al Murri AM, Wilson C, Lannigan A, Doughty JC, Angerson WJ, McArdle CS, et al. Evaluation of the Relationship Between the Systemic Inflammatory Response and Cancer-Specific Survival in Patients With Primary Operable Breast Cancer. *Br J Cancer* (2007) 96(6):891–5. doi: 10.1038/sj.bjc.6603682
162. Pasanisi P, Venturelli E, Morelli D, Fontana L, Secreto G, Berrino F. Serum Insulin-Like Growth Factor-I and Platelet-Derived Growth Factor as Biomarkers of Breast Cancer Prognosis. *Cancer Epidemiol Biomarkers Prev* (2008) 17(7):1719–22. doi: 10.1158/1055-9965.EPI-07-0654
163. Pierce BL, Ballard-Barbash R, Bernstein L, Baumgartner RN, Neuhouser ML, Wener MH, et al. Elevated Biomarkers of Inflammation Are Associated With Reduced Survival Among Breast Cancer Patients. *J Clin Oncol* (2009) 27 (21):3437–44. doi: 10.1200/JCO.2008.18.9068
164. Allin KH, Nordestgaard BG, Flyger H, Bojesen SE. Elevated Pre-Treatment Levels of Plasma C-Reactive Protein Are Associated With Poor Prognosis After Breast Cancer: A Cohort Study. *Breast Cancer Res* (2011) 13(3):R55. doi: 10.1186/bcr2891

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

REVIEW
published: 18 July 2022
doi: 10.3389/fonc.2022.910976

Immune Lymphocyte Infiltrate and its Prognostic Value in Triple-Negative Breast Cancer

Carlos Alexander Huertas-Caro[1], *Mayra Alejandra Ramirez*[1], *Henry J. Gonzalez-Torres*[2,3], *María Carolina Sanabria-Salas*[1] *and Silvia J. Serrano-Gómez*[4*]

[1] Grupo de investigación en biología del cáncer, Instituto Nacional de Cancerología, Bogotá, Colombia, [2] Doctorado en Ciencias Biomédicas, Universidad del Valle, Cali, Colombia, [3] Facultad de Ciencias de la Salud, Universidad Simón Bolívar, Barranquilla, Colombia, [4] Grupo de apoyo y seguimiento para la investigación, Instituto Nacional de Cancerología, Bogotá, Colombia

Triple-negative breast cancer (TNBC) occurs more frequently in young (<50 years) non-Hispanic black and Hispanic/Latina women. It is considered the most aggressive subtype of breast cancer, although, recently, immune infiltrate has been associated with long-term survival, lower risk of death and recurrence, and response to neoadjuvant chemotherapy. The aim of this review was to evaluate the clinical impact of the immune infiltrate in TNBC by discussing whether its prognostic value varies across different populations. A comprehensive systematic search in databases such as PubMed and Web of Science was conducted to include papers focused on tumor-infiltrating lymphocytes (TILs) in TNBC in different population groups and that were published before January 2021. TNBC patients with higher levels of TILs had longer overall survival and disease-free survival times compared with TNBC patients with low TIL levels. Similar results were observed for CD4+, CD8+ TIL populations. On the other hand, patients with high TIL levels showed a higher rate of pathological complete response regardless of the population group (Asian, European, and American). These results altogether suggest that TIL subpopulations might have a prognostic role in TNBC, but the underlying mechanism needs to be elucidated. Although the prognosis value of TILs was not found different between the population groups analyzed in the revised literature, further studies including underrepresented populations with different genetic ancestries are still necessary to conclude in this regard.

Keywords: triple-negative breast cancer, tumor-infiltrating lymphocytes, prognosis, predictive, population groups

OPEN ACCESS

Edited by:
Tomás Pascual Martínez, SOLTI breast cancer research group, Spain

Reviewed by:
Yong-Qu Zhang, Xiamen University, China
Anthony Magliocco, Protean BioDiagnostics Inc., United States

***Correspondence:**
Silvia J. Serrano-Gómez
sserrano@cancer.gov.co

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *01 April 2022*
Accepted: *20 June 2022*
Published: *18 July 2022*

Citation:
Huertas-Caro CA, Ramirez MA, Gonzalez-Torres HJ, Sanabria-Salas MC and Serrano-Gómez SJ (2022) Immune Lymphocyte Infiltrate and Its Prognostic Value in Triple-Negative Breast Cancer. Front. Oncol. 12:910976. doi: 10.3389/fonc.2022.910976

INTRODUCTION

Breast cancer (BC) is a heterogeneous disease in its phenotypic and genomic features (1). Four intrinsic subtypes, luminal A, luminal B, HER2-enriched, and triple negative, have been reported, each one characterized by differences in the transcriptional profile and clinical behavior (2–4). The prevalence of these subtypes is variable between population groups (5, 6). Several studies have agreed that the triple-negative subtype is more prevalent in NHB and in H/L compared with non-Hispanic white (NHW) women (7–10).

Triple-negative breast cancer (TNBC) is characterized by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). It constitutes 10–20% of all breast cancers and occurs more frequently in young women (<50 years) (11, 12). It is the most aggressive subtype of BC considering that it presents with a larger tumor size and a higher histological grade at the time of diagnosis and has a high expression of cell proliferation genes, which correlated with their clinical characteristics and poor prognosis (13).

TNBC has been described as a transcriptionally heterogeneous subtype (14–16). Lehmann *et al.* (14) identified 6 subtypes through gene expression analysis: basal-like 1 (BL1) characterized by a high expression of genes involved in cell cycle and cellular division, basal-like 2 (BL2) that expresses genes that enrich the signaling by growth factors such as MET and EGFR and expresses myoepithelial markers, immunomodulator (IM) subtype that expresses genes involved in the signaling of immune cells and cytokine-mediated translation pathways, and the mesenchymal (M) and mesenchymal stem-like (MSL) subtypes which display similarities in terms of the high expression of genes involved in cell motility, epithelial–mesenchymal transition pathways, and growth factors (such as, NOTCH, PDGFR, FGFR, and TGFbeta dysregulation). However, the MSL subtype differs from the M subtype as it presents a lower expression of cell proliferation genes. Finally, the luminal androgen receptor (LAR) subtype presents a high expression of genes that participate mainly in hormonally regulated pathways, for example, by the androgen receptor (AR) (14, 17–19).

An important characteristic of TNBC is that it is the most immunogenic BC subtype. Its immune infiltrate has been associated with both the control of tumor cells and with the processes of tumor growth and metastasis (20–22). It has been likewise associated with the effectiveness of neoadjuvant and adjuvant therapy, thus correlating with the clinical outcome of the disease (23).

The variability in the immune infiltrate and its clinical impact in TNBC has been studied mainly in NHW women, but it is unknown how it may vary according to the population group. The aim of this review was to systematize those studies that have evaluated the clinical impact of the immune infiltrate in TNBC, discussing whether there are differences in its prognostic value based on the population groups.

TUMOR MICROENVIRONMENT AND IMMUNE INFILTRATE IN BREAST CANCER

The neoplastic progression of BC at the cellular level depends on the interaction of the tumor microenvironment (TME) and the adjacent immune system, which can act to promote or suppress the tumor growth and invasion (24, 25).

TME is composed of tumor cells and different stromal cells, such as fibroblasts, mesenchymal cells, immune cells, and adipocytes. These stromal cells secrete growth factors, cytokines, chemokines, and exosomes, molecules that maintain a constant interaction among cells within the TME (26, 27). Tumor cells are the only ones that have mutations within the TME and can promote epigenetic modifications on non-tumor cells. These modifications facilitate tumoral invasion, survival, and growth in an autocrine and paracrine way (25) (**Figure 1**).

COMPOSITION OF TUMOR-INFILTRATING LYMPHOCYTES IN TNBC

The antitumor immune response in the TME is mainly driven by tumor-infiltrating lymphocytes (TILS) which, according to their location in the TME, are divided into stromal (sTILs) and intratumoral (iTILs). Most of the lymphocytes are sTILs, which infiltrate the tissue adjacent to the tumor and are considered the real tumor-infiltrating cells; on the other hand, iTILs are in direct contact with the tumor, actively infiltrating it

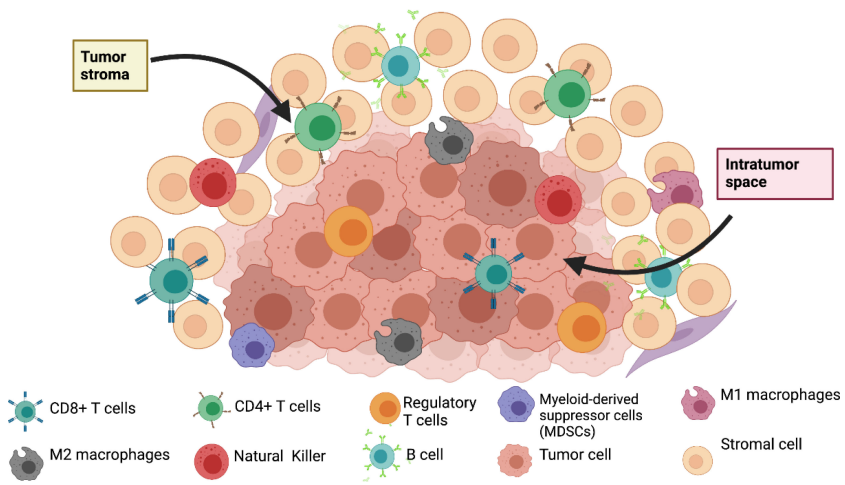


FIGURE 1 | Composition of tumor microenvironment in breast cancer.

into nests (28). It is noteworthy that different subtypes of TILs may have inhibitory or stimulatory effects on tumor progression (29)—for instance, CD8+ T cells show the highest antitumor activity that is mediated by interferon-gamma (IFN-γ), perforin, and granzyme B secretion (30). In BC, a high number of CD8+ T cells has been associated with a better prognosis and response to neoadjuvant treatment (31). On the other hand, T helper cells CD4+ have the function of enhancing the adaptive immune response by increasing the infiltration and the effector functions of CD8+ T cells and other immune cells (32). Regulatory T cells (Treg), a subpopulation of CD4+ T cells, are positive for FOXP3 and CD25 markers and participate in immune escape by suppressing the antitumor activity of CD8+ T cells (33). The presence of Treg cells within the TME is commonly associated with a poor prognosis in cancer (34). However, recent studies have demonstrated the opposite in TNBC, where the presence of Tregs in the TME was associated with longer overall survival (OS) and disease-free survival (DFS) (35, 36).

B cells can produce specific antibodies for antigens present in tumor cells; however, it has not yet been demonstrated if these cells have the same degree of clinical significance as T cells (37). The presence of B cells in the tumor stroma has been correlated with longer DFS and metastasis-free survival (MFS) in TNBC patients (38).

The role of both functionally distinct macrophage subpopulations M1 and M2 has been reported. M1 macrophages exhibit antitumoral activity by activating natural killer (NK) cells and Th1 cells (IFN- γ, IL-2, and TNF-alpha producers), which contributes to the activation of CD8+ T cells (39). In contrast, M2 macrophages or tumor-associated macrophages (TAMs) favor tumor growth and progression by facilitating tumor invasion and angiogenesis, thus being associated with a poor prognosis in patients with TNBC (40, 41).

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of cells with immunosuppressive activity, composed mainly of granulocytes and monocytes. The MDSCs have been associated with tumoral progression through the production of immunosuppressive and pro-angiogenic cytokines that inhibit the immune response of antitumor T cells (42, 43). It should be noted that the role of MDSCs specifically in TNBC patients remains relatively unexplored (44, 45).

NK cells recognize and delete tumor cells lacking MHC-1 expression on their cell surface, whose expression is necessary for the activation of CD8 + T cells (46). Recent studies have shown that NK cells are associated with a better prognosis in the early stages of TNBC (47). More studies are needed.

TILS AS PROGNOSTIC AND PREDICTIVE BIOMARKER IN TRIPLE-NEGATIVE BREAST CANCER

In the last few years, the predictive and prognostic role of TILs in TNBC have been studied. The relations between the composition of TILs subpopulations, clinico-pathological characteristics, and the survival of patients have likewise been explored (**Table 1**) (29, 62).

Studies carried out in Asian populations mostly showed that TILs, when evaluated in resected specimens, have a positive impact on the prognosis of TNBC (48) (50). Some studies have 95% CI with OS (HR: 0.493, 95% CI: 0.232–1.047, $p = 0.066$) when patients with high TILs (≥10%) *vs*. low TILs (<10%) were compared (48). Hida et al. (50) reported a poorer prognosis in TNBC patients with low TIL levels (<10%) compared with intermediate/high-TIL groups (>50%) (HR: 2.68, 95% CI: 1.13–5.95). This association remained significant in the multivariate model (HR: 2.49, 95% CI: 1.05–5.55). Moreover, TILs analyzed at the biopsy, before neoadjuvant chemotherapy, were found to be associated with pCR rate ($p = 0.024$). Despite previous results, opposite results have also been reported where TILs did not correlate with survival outcomes (52).

When TILs have been evaluated in biopsies, a lower likelihood of recurrence has been observed in patients with a high TIL infiltration (≥10%) compared with those with a low TIL infiltration (<10%) in univariate (HR: 0.18, 95% CI: 0.05–0.58) and multivariate analyses (HR: 0.24, 95% CI: 0.07–0.82). In addition, patients with higher TIL infiltration presented with higher pCR rates ($p = 0.013$) when compared with patients with low TIL infiltration (49) Similarly, Ruan *et al.* (51) reported a significant association between the percentage of TILs and pCR in a model adjusted for age, lymph–vascular invasion, and Ki67, both for iTILs (OR: 1.06, 95% CI: 1.00–1.12, $p = 0.04$, per 10% increase) and for sTILs (OR: 1.05, 95% CI: 1.02–1.09, $p = 0.006$, per 10% increase). When the optimal thresholds for TILs were analyzed, the results suggested that 20% is a better cutoff to determine high or low sTILs infiltration since it seems to be a better predictor of pCR (OR 2.85, 95% CI: 1.38–5.90, $p = 0.005$).

The differences in the prognosis impact of TILs between studies might be related to the clinical stage of the patients included. Presumably, there are lower amounts of tumor antigens among patients at earlier stages (31, 52), which could lead to misinterpretations regarding the relationship of TILs and clinico-pathological variables and outcomes of interest, as few studies have assessed the prognosis impact of TILs in early-stage TNBC patients.

Studies in a European population show similar findings to those in the Asian population. A study in France that evaluated TILs in the primary tumor reported a 15% reduction in the risk of death for every 10% of increase in sTIL levels (HR: 0.85, 95% CI: 0.74–0.99) and 18% reduction in the risk of death for every 10% of increase in iTILs (HR: 0.82, 95% CI: 0.68–0.99) in the multivariate analysis adjusted for the grade of lymph nodes (LN) (53).

In Italy, two studies were carried out in a larger number of TNBC patients and analyzed TILs in the resected specimen (54, 55). The first study included 897 women and reported TILs as an independent prognostic factor for a longer distant disease-free survival (HR: 0.76, 95% CI: 0.69–0.84, for every 10% increase in TILs) and longer OS (HR: 0.76, 95% CI: 0.68–0.84, for every 10% increase in TILs) in a model adjusted for age at diagnosis, lymph node stage, peritumoral vascular invasion, tumor size and grade, and Ki67 (54). The second study that evaluated sTILs in the resected specimen and dichotomized patients in having TILs ≥50% *vs*. patients with TILs <50% likewise found a 13% risk

TABLE 1 | Outcomes from studies that analyzed tumor-infiltrating lymphocytes (TILs) according to the region of origin.

Reference	Population	*n* (triple-negative breast cancer)	Specimen evaluated	TILs evaluated	Cut-off value	Outcomes for univariate analysis	Outcomes for multivariate analysis	Adjustment variables
(48)	Asian	308	Resected specimen	Stromal	≥10 *vs*. <10%	No specified	OS (HR: 0.493, 95% CI: 0.232–1.047) DFS (HR: 0.429, 95% CI: 0.215–0.859)	Tumor size, LN metastasis, LVI, and histologic grade
(49)	Asian	61	Biopsy	Stromal	High (≥10%) *vs*. low (<10%)	For DFS (HR: 0.18, 95% CI: 0.05–0.58)	DFS (HR: 0.24, 95% CI: 0.07–0.82)	Pathological response
(50)	Asian	381	Resected specimen	Stromal	Low (<10%) *vs*. Intermediate (10–50%) + high (>50%)	RFS (HR: 2.68, 95% CI: 1.13–5.95)	RFS (HR: 2.49, 95% CI: 1.05–5.55)	Nodal status
(51)	Asian	166	Biopsy	Stromal	Continuous (per 10% increase)	pCR for sTILS (OR: 1.07, 95% CI: 1.03–1.10)	pCR for sTILS (OR: 1.05, 95% CI: 1.02–1.09)	Age, histological grade, tumor size, nodal status, LVI, Ki67 index, and NAC
				Intratumoral		pCR for iTILS (OR: 1.10, 95% CI: 1.04–1.16)	pCR for iTILs (OR: 1.06, 95% CI: 1.00–1.12)	
(52)	Asian	121	Resected specimen	Stromal	Continuous (per 10% increase)	DFS for sTILs (HR: 0.75, 95% CI: 0.28–2.03)	DFS for sTILs (HR: 0.99, 95% CI: 0.97–1.01)	Age, T stage, and nodal status
				Intratumoral		DFS for iTILs (HR: 0.66, 95% CI: 0.24–1.83)	OS for sTILs (HR: 0.99, 95% CI: 0.97–1.02)	
(53)	European	199	Biopsy	Stromal	Continuous (per 10% increase)	OS for sTILs (HR: 0.89, 95% CI: 0.78–1.02)	OS for sTILs (HR: 0.85, 95% CI: 0.74–0.99)	Grade, LN status, and treatment arm
				Intratumoral		OS for iTILs (HR: 0.83, 95% CI: 0.69–0.99)	OS for iTILs (HR: 0.82, 95% CI: 0.68–0.99)	
(54)	European	897	Resected specimen	Stromal	Continuous (per 10% increase)	DDFS (HR: 0.79, 95% CI: 0.74–0.86) OS (HR: 0.79, 95% CI: 0.72–0.86)	DDFS (HR: 0.76, 95% CI: 0.69–0.84) OS (HR: 0.76, 95% CI: 0.68–0.84)	Age, LN status, tumor size, tumor grade, peritumoral vascular invasion, and Ki67 index
(55)	European	647	Resected specimen	Stromal	≥50 *vs*. < 50%	BCFI (HR: 0.87, 95% CI: 0.79–0.95) DFS (HR: 0.89, 95% CI: 0.82–0.97) DRFI (HR: 0.84, 95% CI: 0.74–0.94) OS (HR: 0.83, 95% CI: 0.74–0.92)	BCFI (HR: 0.87, 95% CI: 0.79–0.96) DFS (HR: 0.9, 95% CI: 0.82–0.97) DRFI (HR: 0.83, 95% CI: 0.74–0.94) OS (HR: 0.83, 95% CI: 0.74–0.93)	Age, nodal status, tumor size, and tumor grade
(56)	European	607	Biopsy	Stromal	Continuous (per 10% increase)	DFS (HR: 0.93, 95% CI: 0.87–0.98)	DFS (HR: 0.95, 95% CI: 0.89–1.01)	Age, T stage, N stage, histopathological type, tumor grading, and molecular subtype

(Continued)

TABLE 1 | Continued

Reference	Population	*n* (triple-negative breast cancer)	Specimen evaluated	TILs evaluated	Cut-off value	Outcomes for univariate analysis	Outcomes for multivariate analysis	Adjustment variables
						OS (HR: 0.92, 95% CI: 0.86–0.99) pCR (HR: 1.16, 95% CI: 1.10–1.22)	OS (HR: 0.95, 95% CI: 0.88–1.03) pCR (OR: 1.17, 95% CI: 1.11–1.24)	
(57)	European	314	Biopsy	Stromal	Continuous (per 10% increase)	pCR (HR: 1.15, 95% CI: 1.05–1.26)	pCR (HR: 1.17, 95% CI: 1.06–1.30)	LPBC, tumor grade, T stage, nodal status, therapy, and age
(58)	European	304	Residual disease	Stromal	Continuous (per 10% increase)	OS (HR: 0.79, 95% CI: 0.71–0.89) OS iTILs (HR: 0.78, 95% CI: 0.68–0.89)	OS sTILs (HR: 0.86, 95% CI: 0.77–0.97) OS iTILs (HR: 0.86, 95% CI: 0.75–0.99)	Age, stage, histotype, grade, nodal status after chemotherapy, residual tumor size, neo, and neo + adj
				Intratumoral		MFS sTILs (HR: 0.79, 95% CI: 0.71–0.88) MFS iTILs (HR: 0.77, 95% CI: 0.68–0.88)	MFS sTILs (HR: 0.86, 95% CI: 0.77–0.96) MFS iTILs (HR: 0.85, 95% CI: 0.75–0.98)	
(59)	European	375	Residual disease	Stromal	Continuous (per 10% increase)	RFS (HR: 0.83, 95% CI: 0.76–0.90) OS (HR: 0.82, 95% CI: 0.75–0.89)	RFS (HR: 0.86, 95% CI: 0.78–0.93) OS (HR: 0.85, 95% CI: 0.77–0.94)	Age, pretreatment tumor size, pretreatment nodal status, and RCB class
(21)	Australian	134	Biopsy	Stromal	Continuous (per 10% increase)	DDFS (HR: 0.79, 95% CI: 0.64–0.98) OS (HR: 0.80, 95% CI: 0.62–1.03)	DDFS (HR: 0.77, 95% CI: 0.61–0.98) OS (HR: 0.81, 95% CI: 0.61–1.1)	Tumor size, histological grade, nodal status, and age
(22)	United States	481	Resected specimen	Stromal	Continuous (per 10% increase)	DRFI (HR: 0.82, 95% CI: 0.68–0.99) OS (HR: 0.81, 95% CI: 0.69–0.95)	DFS (HR: 0.84, 95% CI: 0.74–0.95) DRFI (HR: 0.81, 95% CI: 0.68–0.97) OS (HR: 0.79, 95% CI: 0.67–0.92)	Tumor size, node status, and age
(60)	United States	157	Resected specimen	Stromal	Continuous	DFS (HR: 0.96, 95% CI: 0.93–1.00) OS (HR: 0.96, 95% CI: 0.93–1.00)	DFS (HR: 0.95, 95% CI: 0.91–1.00) OS (HR: 0.95, 95% CI: 0.91–1.00)	LV invasion and Nottingham histologic grade and stage
(61)	United States	605	Resected specimen	Stromal	Continuous (per 10% increase)	IDFS (HR: 0.89, 95% CI: 0.83–0.95)	IDFS (HR: 0.90, 95% CI: 0.86–0.94)	Age, menopausal status, tumor size, nodal status, Nottingham grade, Ki67 index, LPBC, histopathology subtypes, and type of breast surgery

OS, overall survival; DFS, disease-free survival; RFS, recurrence-free survival; pCR, pathological complete response; DDFS, distant disease-free survival; BCFI, BC-free interval; DRFI, distant recurrence-free interval; MFS, metastasis-free survival; IDFS, invasive disease-free survival; LN, lymph nodes; LVI, lymph–vascular invasion; NAC, neoadjuvant chemotherapy; LPBC, lymphocyte-predominant BC.

reduction in BC-free interval (HR: 0.87, 95% CI: 0.79–0.96, p = 0.006), 10% risk reduction for DFS (HR: 0.9, 95% CI: 0.82–0.97, p = 0.01), 17% for distance recurrence-free interval (HR: 0.83; 95% CI 0.74–0.94, p = 0.004) in a model adjusted for age, nodal status, tumor size, and tumor grade (55). A study carried out in France and Italy reported that the high presence of TILs in the residual disease after neoadjuvant treatment had a positive impact on MFS (sTIL: HR = 0.86, 95% CI: 0.77–0.96, p = 0.01; iTILs: HR: 0.85, 95% CI: 0.75–0.98, p = 0.02, per 10% increase in TILs) and longer OS (sTIL: HR: 0.86, 95% CI: 0.77–0.97, p = 0.01; iTILs: HR: 0.86, 95% CI: 0.75–0.99, p = 0.03, per 10% increase in TILs). The 5-year OS rate was 91% (95% CI, 68 to 97%) for patients with higher TILs in residual disease compared with 55% (95% CI, 48 to 61%) for patients with low TIL levels (58). Similarly, Luen *et al.* (59) found that a higher percentage of TILs in residual disease was associated with a longer recurrence-free survival (RFS) (HR: 0.86, 95% CI: 0.78–0.93, per 10% increase in TILs) and a longer OS (HR: 0.85, 95% CI: 0.77–0.94, per 10% increase in TILs).

Denkert *et al.* (56) also reported in a model adjusted for clinical parameters that patients with high TIL levels in the biopsy have longer DFS (HR: 0.93, 95% CI: 0.87–0.98, p = 0.011) and longer OS (HR: 0.92, 95% CI: 0.86–0.99, p = 0.032). However, when pCR was included in the multivariate analysis for both outcomes, the TILs were no longer significantly associated (HR: 0.95, 95% CI: 0.89–1.01, p = 0.11 for DFS, HR: 0.95, 95% CI: 0.88–1.03, p = 0.24 for OS). They also analyzed if TILs are predictors for pCR in TNBC and found a positive association for sTILs (OR: 1.17, 95% CI: 1.11–1.24, per 10% increase in sTILs). Similar results were reported by the same authors in a different study (57). A different effect of TILs according to chemotherapy regimen has been observed. TILs conferred the greatest survival benefit in patients treated with cyclophosphamide, methotrexate, and 5-fluorouracil + cyclophosphamide doxorubicin regimen (HR: 0.60, 95% CI: 0, 48 to 0.76) (54). More studies are needed to explore differences in the prognosis value of TILs according to the chemotherapy regimen.

The relationship between higher TIL levels and higher pCR rates could be explained by the degree of antitumor immune response by TILs against cancer cells that act synergistically with the natural-immunity-restoring antitumor response (20, 22). In addition, it has been demonstrated that chemotherapy treatment can promote the antitumor immune response due to the production of danger signals—danger-associated molecular patterns—during cell death. The expression of calreticulin (CALR) and release box 1 of the high mobility group (HMGB1) also boosts this antitumor immune response (63). All these could be together related to the presence of TILs in residual disease (58), and thus a good prognosis was reported for TILs in residual disease (64).

In the Australian population, an analysis that included early-stage TNBC patients showed that for every 10% increase in the presence of TILs in the primary tissue, there was a 13% decrease in the risk of distant relapse (HR: 0.77, 95% CI: 0.61 –0.98, p = 0.02) in a model adjusted for clinico-pathological characteristics. No statistically significant differences were observed for OS (21).

In the United States, Adams and colleagues (22) reported that for every 10% increase in sTILs evaluated in surgical specimens, there was a 16% reduction in the risk of recurrence (HR: 0.84, 95% CI: 0.74–0.95, p = 0.005) and a 21% reduction in the risk of death (HR: 0.79, 95% CI: 0.67–0.92). In the same direction, Krishnamurti and colleagues (60) showed that higher peripheral TILs were associated with a better survival (HR: 0.95, 95% CI: 0.91–1.00, p = 0.0354) and less chance of recurrence (HR: 0.95, 95% CI: 0.91–1.00, p = 0.0314). Leon-Ferre *et al.* (61) reported a similar association between sTILs and invasive disease-free survival in patients with TNBC diagnosed at early stages (HR: 0.90, 95% CI: 0.86–0.94, per 10% increment in TILs).

The case-only study that includes 86 Peruvian women with TNBC observed a statistically significant association between TIL density and a higher tumor grade (p = 0.006), but no significant association was found regarding the relationship between sTILs and survival (65). More studies are needed in the Latino population.

THE SUBPOPULATION OF TILS AND ITS PROGNOSTIC VALUE

Due to the relevance of TILs in TNBC, in recent years, an attempt has been made to elucidate the role of the different TIL subpopulations, in particular, the most recurrent ones such as CD8, CD4, and FOXP3 (**Table 2**).

A study conducted in the Asian population in which the number of TILs CD8+ and TILs FOXP3+ was analyzed in biopsy and residual tissue reported that a high rate of change in the CD8+/FOXP3+ ratio was an independent prognostic factor for recurrence and survival (66). In a different study, high levels of iTILs CD8+ were associated with DFS (HR: 0.48, 95% CI: 0.27–0.83) but not with OS (HR: 0.59, 95% CI: 0.32–1.07). On the other hand, patients with higher levels of sTILs CD4+ presented longer DFS (HR: 0.46, 95% CI: 0.26–0.82) and OS (HR: 0.44, 95% CI: 0.24–0,83) (67). Regarding clinico-pathological variables, a correlation between the immune infiltrate and age at diagnosis has also been reported. The highest rates of the CD8+/FOXP3+ ratio were observed more frequently in women diagnosed at an early age (p = 0.003), specifically when they are still in a premenopausal state (p = 0.002) (68). Moreover, a high CD8+/FOXP3+ ratio was found as a strong predictor of pCR (OR: 5.32, 95% CI: 1.62 to 19.98) (68).

Studies in less common subpopulations, such as B-cell (CD20+) and Tregs (FOXP3+/CD3+), have found them positively associated to better outcomes. A Kaplan–Meier analysis showed that patients with higher intratumoral Treg presented longer DFS (p = 0.001). A multivariate analysis confirmed this association (HR: 0.33, 95% CI: 0.165 to 0.659). High intratumoral Treg infiltration was also found to be associated with OS (HR: 0.49, 95% CI: 0.25–0.95). Additionally, patients with higher CD20+ B-cell infiltration in both the intratumoral (DFS: p = 0.015; OS: p = 0.020) and stromal (DFS: p = 0.012; OS: p = 0.031) compartments presented better clinical outcomes (35). Tian and colleagues (69), in a Chinese study, categorized patients according to the DFS times and reported

TABLE 2 | Outcomes from studies that analyzed tumor-infiltrating lymphocytes (TIL) subpopulations according to the region of origin.

Reference	Population	*n* (triple-negative breast cancer)	Specimen evaluated	Biomarker analyzed	Outcomes for univariate analysis	Outcomes for multivariate analysis	Adjustment variables	Methodology
(66)	Asian	39	Biopsy and residual disease	CRF	CRF low *vs*. high RFS (HR: 11.420, 95% CI: 2.215–208.742) OS (HR: 9.847, 95% CI: 1.883–180.764)	CRF low *vs*. high RFS (HR: 13.021, 95% CI: 2.241–258.136) OS (HR: 8.346, 95% CI: 1.538–155.128)	Pathological response	Tissue sections
(67)	Asian	164	Biopsy	CD8	None reported	CD8 iTILs high *vs*. low DFS (HR: 0.48, 95% CI: 0.27–0.83) OS (HR: 0.59, 95% CI: 0.32–1.07)	Tumor size, LN stage	TMA
				CD4		CD4 iTILs high *vs*. low DFS (HR: 0.62, 95% CI: 0.36–1.07) OS (HR: 0.55, 95% CI: 0.30–1.01) CD4 sTILs high *vs*. low DFS (HR: 0.46, 95% CI: 0.26–0.82) OS (HR: 0.44, 95% CI: 0.24–0.83)		
(68)	Asian	110	Biopsy	CD8 FOXP3	CD8/FOXP3 (high *vs*. low) pCR (HR: 4.93, 95% CI: 1.82–15.09)	CD8/FOXP3 (high *vs*. low) pCR (HR: 5.32, 95% CI: 1.62–19.98)	Age, menopausal status, tumor size, TNBC subtype, Ki67, CD8, and VPR	Tissue sections
(35)	Asian	164	Biopsy	Treg	Intratumoral Treg (high *vs*. low) OS (HR: 0.59, 95% CI: 0.33–1.04) DFS (HR: 0.49, 95% CI: 0.20–0.83)	Intratumoral Treg (high vs. Low) OS (HR: 0.49, 95% CI: 0.25–0.95) DFS (HR: 0.33, 95% CI: 0.17–0.66)	Tumor size, nuclear grade, and age	TMA
(69)	Asian	278	Resected specimen	FOXP3	Stromal FOXP3 (high *vs*. low) OS (HR: 1.743, 95% CI: 1.111–2.734)	Stromal FOXP3 (high *vs*. low) OS (HR: 1.712, 95% CI: 1.085–2.702)	TNM stage, p53 status, EGFR status, Scd8, TILs, Sfoxp3, and prognostic risk score	Tissue sections
(70)	European	179	Resected specimen	CD8	High *vs*. low OS (HR: 2.1, 95% CI: 1.1–4.5)	High *vs*. low OS (HR: 1.8, 95% CI: 1.1–4.4)	Tumor size	Tissue sections
(71)	European	213	Biopsy	TILs	None reported	Average TILs BCSS (HR: 0.3, 95% CI: 0.1–0.8)	CD3, CD8, FOXP3, CD20, and CD68	Tissue sections
(72)	European	175	Resected specimen	FOXP3	None reported	High *vs*. low RFS (HR: 0.371, 95% CI: 0.213–0.644)	N/A	TMA

(Continued)

TABLE 2 | Continued

Reference	Population	*n* (triple-negative breast cancer)	Specimen evaluated	Biomarker analyzed	Outcomes for univariate analysis	Outcomes for multivariate analysis	Adjustment variables	Methodology
						DSS (HR: 0.416, 95% CI: 0.231–0.750)		
(73)	United States	183	None specified	FOXP3	High *vs*. low OS (HR = 12.7, 95% CI: 4.5–35.6)	None reported	N/A	TMA
				CD163	High *vs*. low OS (HR = 3.2, 95% CI: 1.7–6.2)			
(74)	United States	160	Resected specimen	CD8	High *vs*. low in AA OS (HR: 0.51, 95% CI: 0.25–1.03)	High *vs*. low in AA OS (HR: 0.51, 95% CI: 0.25–1.04)	Age	TMA

OS, overall survival; DFS, disease-free survival; RFS, recurrence-free survival; pCR, pathological complete response; BSCC, BC-specific survival; LN, lymph nodes; AA, African American.

that patients in the DFS ≥5 years group had higher NK cell stromal infiltration ($p < 0.001$) and low stromal TAM infiltration ($p = 0.004$). Stromal FOXP3+ TILs were found as an independent prognostic factor for OS (sTILs FOXP3+ low/high HR: 1.712, 95% CI: 1.085–2.702) (69).

Regarding the studies in a European population, it was observed that patients with low TIL CD8+ infiltration were associated with a higher risk of death from BC (HR: 2.2, 95% CI: 1.0–3.8) (70). On the contrary, Althobiti and colleagues (71) only found TILs as an independent predictor of good prognosis in a model that included various immune cells, such as CD3, CD8, FOXP3, CD20, and CD68. West and colleagues (72) reported that a high infiltration of TILs FOXP3+ was strongly associated with better outcomes (RFS: HR = 0.371, 95% CI: 0.213–0.644; $p = 0.0004$) and disease-specific survival (HR = 0.416, 95%: CI 0.231–0.750; $p = 0.0036$). In contrast, a study from the United States reported that a high expression of FOXP3 and CD163 was associated to a worse OS (HR = 12.7, 95% CI: 4.5–35.6 and HR = 3.2, 95% CI: 1.7–6.2, respectively) (73).

Few studies have analyzed the differences in the tumor microenvironment between European American (EA) women and African American (AA) women, and the results have been contradictory. Preliminary data from Wright and colleagues (75) found higher levels of TILs in early-stage (I–II) tumors from AA patients compared with EA ($p = 0.019$), but this difference was not observed for late-stage (III–IV) tumors. TILs also correlated negatively with AR expression and positively with PD-L1 expression. The analysis of CD8+ T cell infiltration in AA and EA women revealed that AAs with high CD8 infiltration have a trend towards better survival compared with AA with low CD8 infiltration (HR: 0.51, 95% CI: 0.25–1.04) (74). On the other hand, a study that analyzed The Cancer Genome Atlas database and compared the immune gene expression between AA and EA women did not find large-scale immunogenic differences (76).

TILs have a useful prognostic role in TNBC based on TIL populations. Nevertheless, the immune infiltrate phenotype and its prognostic value require better understanding. Thus, it is necessary to include other immune cell populations in future studies. The association reported between the high Treg FOXP3 infiltrate and better DFS and OS in TNBC is interesting considering that Treg has been associated with a poor prognosis as it can suppress antigen-presenting cells and other immune cells, events that are regulated through the secretion of inhibitory cytokines, granzyme B, and perforin (77). On the contrary, the favorable prognosis may be explained by the positive correlation between FOXP3 infiltration and TILs CD8+ infiltration (68). There is a need to clarify the prognostic role of Treg FOXP3+ in TNBC tumors.

EXPRESSION OF MEMBRANE MARKERS IN THE IMMUNE INFILTRATE

In addition to the different immune cell's populations mentioned before, there are other biomarkers of special interest, such as the expression of PD-L1. Studies in different populations have consistently showed a correlation between a high expression of PD-L1 in tumor cells and higher levels of sTILs (78–80).

Regarding the impact of PD-L1 in a patient's prognosis, controversial results have been published. A study from Japan found PD-L1 positive/TILs low expression as an independent negative prognostic factor for RFS (HR = 4.7, 95% CI: 1.6–12.7) and OS (HR = 8.4, 95% CI: 2.3–30.3) (79). AiErken and colleagues (80) conducted a study that included Chinese patients diagnosed with TNBC and reported a positive PD-L1 expression as an independent prognosis factor for OS (HR: 0.302, 95% CI: 0.127–0.721) and DFS (HR: 0.451, 95% CI: 0.211–0.963). A study from the United States reported that elevated levels of PD-L1 were associated with decreased OS compared with a low expression (HR: 10.4, 95% CI: 3.6–29.6) (73). On the contrary, Li and colleagues found that any stromal PD-L1 expression was associated with better DFS but not OS (81).

The association between the expression of PD-L1 and a high percentage of TILs could be explained by activated T cells, which produce IFNγ (82). It has been proposed that IFNγ induce PD-L1

expression as an immune evasion mechanism by the tumor (83). Additionally, the relationship of high TIL levels and PD-L1 expression could also explain the association between PD-L1 expression and DFS and OS in Asian populations (83) and the pCR rates in European populations (80).

Cerbelli *et al.* (78) analyzed 54 TNBC biopsies taken from different institutions in Rome, Italy, and found a statistically significant association between PD-L1 expression in ≥25% of neoplastic cells and pCR (OR: 1.13, 95% CI: 1.01–1.27). Additionally, it was observed that 100% of the patients who achieved a pCR presented jointly a higher percentage of TILs and PD-L1 expression in ≥25% of tumor cells (p = 0.011). These results suggest that PD-L1 expression could be a marker of response to neoadjuvant chemotherapy in patients with TNBC. However, to reach this conclusion, more and larger studies that focus on the expression of PD-L1 in TNBC patients treated or not with neoadjuvant chemotherapy are needed—for instance, PD-L1 is described to be more commonly expressed in primary tumors than metastatic tumors (p = 0.002) (84), although some controversial results have also been published (85).

TIM3 is an immune checkpoint molecule that is expressed on CD4+ helper 1 (Th1) cells, CD8+ T cells, dendritic cells, and other subpopulations of lymphocytes, macrophages, and monocytes (86). The high expression of PD-1 and PD-L1 was each associated with a high expression of TIM-3 (p = 0.0001 and p = 0.0019, respectively). Patients with a higher TIM-3 expression presented better DFS (HR: 0.1072, 95% CI: 0.0319–0.3603) and longer OS (HR: 0.1129, 95% CI: 0.0323–0.3948) (86).

Interestingly, a German study analyzed the expression levels of 12 immune genes that included T cells, B cells, cytokines, and immune checkpoints markers (CXCL9, CCL5, CD8A, CD80, CXCL13, IGKC, CD21, IDO1, PD-1, PD -L1, CTLA4, and FOXP3). Based on their gene expression, they categorized the patients in three immune groups: low expression (A), intermediate expression (B), and high expression (C). They observed differences in the pCR rates among the three groups: 24% for A, 37.4% for B, and 50.4% for C (p <0.001). All 12 immune genes at the mRNA level were significantly linked to pCR; the best predictors were PD-L1 (OR: 1.44, 95% CI: 1.18 to 1.77, per ΔCt) and CD80 (OR: 1.74, 95% CI: 1.28 to 2.38, per ΔCt) (57).

CONCLUSIONS

Although it is not doubted that TILs play an essential role in tumor development, the methods used across studies to measure the infiltrate are heterogeneous (87)—for example, it has been recommended to consider as high an infiltration value >50% (88) or a cutoff point >60% (89) or even to consider three cutoff points (<10%, between 10 and 50%, and >50%) (90). Moreover, studies differ in their sample sizes and inclusion criteria. Some studies evaluate TILs in biopsies and others in the resected specimens of patients that received neoadjuvant chemotherapy or not. Other studies included only early-stage patients. Therefore, all these circumstances make it difficult to provide an assertive comparison between studies to conclude on the role of TILs in carcinogenesis.

The classification of triple-negative breast cancer by immunohistochemical techniques could also be a source of heterogeneity. As mentioned above, some studies included biopsies and others resected specimens. The heterogeneity in the expression of immunohistochemistry markers such as ER, PR, and HER2, when evaluated in core needle biopsies or in a resected specimen, could lead to the misclassification of breast cancer into intrinsic subtypes (91–93). We cannot rule out that there may be misclassified cases among studies and that this may explain, in part, why some studies did not find statistically significant differences in some of the outcomes evaluated. It is also important to consider if TILs were evaluated in resected specimens from patients who previously received neoadjuvant chemotherapy since it is well known that chemotherapy can modify the panorama of the immune infiltrate, and this could impact the results of TIL characterization (94–96).

Germline *BRCA1/2* mutations range between 9 and 21% in unselected TNBC patients (97, 98). The presence of mutations in repair genes could lead to a greater formation of neoantigens, which would translate into an increase in immune infiltrate in these cases (99–102). For this reason, it is important to analyze the results of the studies considering the germinal component to avoid bias in the results.

In any case, the results presented below on the prognostic and predictive value of TILs in different populations such as Asian, European, Australian, and American present similar risk directions highlighting that TILs might be an independent prognostic factor for recurrence and survival and an independent predictor factor for pCR regardless on the origin of the patients.

AUTHOR CONTRIBUTIONS

Writing—review of the draft: CH-C, MR, and HG-T. Conception and study design: SS-G, CH-C, and MS-S. Manuscript preparation: CH-C, MR, HG-T, and SS-G. Writing—reviewing and editing: SS-G and MS-S. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Minciencias (Contrato 838-2018 to SS-G) and the Colombian National Cancer Institute (C-19010300431 to SS-G).

REFERENCES

1. Turashvili, G, and Brogi, E. Tumor Heterogeneity in Breast Cancer. *Front Med* (2017) 4:227. doi: 10.3389/fmed.2017.00227
2. Rakha, EA, and Green, AR. Molecular Classification of Breast Cancer: What the Pathologist Needs to Know. *Pathology* (2017) 49(2):111–119. doi: 10.1016/j.pathol.2016.10.012
3. Sachs, N, de Ligt, J, Kopper, O, Gogola, E, Bounova, G, Weeber, F, et al. A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. *Cell* (2018) 172(1-2):373–386.e10. doi: 10.1016/j.cell.2017.11.010
4. Barnard, ME, Boeke, CE, and Tamimi, RM. Established Breast Cancer Risk Factors and Risk of Intrinsic Tumor Subtypes. *Biochim Biophys Acta - Rev Cancer* (2015) 1856(1):73–85. doi: 10.1016/j.bbcan.2015.06.002
5. DeSantis, CE, Ma, J, Gaudet, MM, Newman, LA, Miller, KD, Goding Sauer, A, et al. Breast Cancer Statistics, 2019. *CA: A Cancer J Clin* (2019) 69(6):438–451. doi: 10.3322/caac.21583
6. Siegel, RL, Miller, KD, and Jemal, A. Cancer Statistics, 2019. *CA: A Cancer J Clin* (2019) 69(1):7–34. doi: 10.3322/caac.21551
7. Yip, CH, Evans, DG, Agarwal, G, Buccimazza, I, Kwong, A, Morant, R, et al. Global Disparities in Breast Cancer Genetics Testing, Counselling and Management. *World J Surg* (2019) 43(5):1264–1270. doi: 10.1007/s00268-018-04897-6
8. Parise, CA, and Caggiano, V. The Influence of Socioeconomic Status on Racial/Ethnic Disparities Among the ER/PR/HER2 Breast Cancer Subtypes. *J Cancer Epidemiol* (2015) 2015:813456. doi: 10.1155/2015/813456
9. Carvalho, FM, Bacchi, LM, Pincerato, KM, Van de Rijn, M, and Bacchi, CE. Geographic Differences in the Distribution of Molecular Subtypes of Breast Cancer in Brazil. *BMC Women's Health* (2014) 14:102. doi: 10.1186/1472-6874-14-102
10. Jiagge, E, Jibril, AS, Chitale, D, Bensenhaver, JM, Awuah, B, Hoenerhoff, M, et al. Comparative Analysis of Breast Cancer Phenotypes in African American, White American, and West Versus East African Patients: Correlation Between African Ancestry and Triple-Negative Breast Cancer. *Ann Surg Oncol* (2016) 23(12):3843–3849. doi: 10.1245/s10434-016-5420-z
11. Telli, ML. Triple-Negative Breast Cancer. In: S. Badve and Y Gökmen-Polar (eds) *Molecular Pathology of Breast Cancer* (2016), Springer, Cham 71–80. doi: 10.1007/978-3-319-41761-5_6
12. Foulkes, WD, Smith, IE, and Reis-Filho, JS. Triple-Negative Breast Cancer. *N Engl J Med* (2010) 363:1938–48. doi: 10.1056/NEJMra1001389
13. López-Ozuna, VM, Hachim, IY, Hachim, MY, Lebrun, JJ, and Ali, S. Prolactin Modulates TNBC Aggressive Phenotype Limiting Tumorigenesis. *Endocr Relat Cancer* (2019) 26(3):321–337. doi: 10.1530/ERC-18-0523
14. Lehmann, BD, Bauer, JA, Chen, X, Sanders, ME, Chakravarthy, AB, Shyr, Y, et al. Identification of Human Triple-Negative Breast Cancer Subtypes and Preclinical Models for Selection of Targeted Therapies. *J Clin Invest* (2011) 121(7):2750–67. doi: 10.1172/JCI45014
15. Lehmann, BD, Jovanović, B, Chen, X, Estrada, MV, Johnson, KN, Shyr, Y, et al. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. *PLoS One* (2016) 11: e0157368. doi: 10.1371/journal.pone.0157368
16. Burstein, MD, Tsimelzon, A, Poage, GM, Covington, KR, Contreras, A, Fuqua, SAW, et al. Comprehensive Genomic Analysis Identifies Novel Subtypes and Targets of Triple-Negative Breast Cancer Analysis and Interpretation of Data: HHS Public Access. *Clin Cancer Res* (2015) 21:1688–98. doi: 10.1158/1078-0432.CCR-14-0432
17. Santonja, A, Sánchez-Muñoz, A, Lluch, A, Chica-Parrado, MR, Albanell, J, Chacón, JI, et al. Triple Negative Breast Cancer Subtypes and Pathologic Complete Response Rate to Neoadjuvant Chemotherapy. *Oncotarget* (2018) 9(41):26406–26416. doi: 10.18632/oncotarget.25413
18. Gong, Y, Ji, P, Yang, YS, Xie, S, Yu, TJ, Xiao, Y, et al. Metabolic-Pathway-Based Subtyping of Triple-Negative Breast Cancer Reveals Potential Therapeutic Targets. *Cell Metab* (2021) 33:51–64.e9. doi: 10.1016/J.CMET.2020.10.012
19. Angajala, A, Mothershed, E, Davis, MB, Tripathi, S, He, Q, Bedi, D, et al. Quadruple Negative Breast Cancers (QNBC) Demonstrate Subtype Consistency Among Primary and Recurrent or Metastatic Breast Cancer. *Transl Oncol* (2019) 12:493–501. doi: 10.1016/j.tranon.2018.11.008
20. Loi, S. Tumor-Infiltrating Lymphocytes, Breast Cancer Subtypes and Therapeutic Efficacy. *OncoImmunology* (2013) 2:e24720. doi: 10.4161/onci.24720
21. Loi, S, Michiels, S, Salgado, R, Sirtaine, N, Jose, V, Fumagalli, D, et al. Tumor Infiltrating Lymphocytes are Prognostic in Triple Negative Breast Cancer and Predictive for Trastuzumab Benefit in Early Breast Cancer: Results From the FinHER Trial. *Ann Oncol* (2014) 25:1544–50. doi: 10.1093/annonc/mdu112
22. Adams, S, Gray, RJ, Demaria, S, Goldstein, L, Perez, EA, Shulman, LN, et al. Prognostic Value of Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancers From Two Phase III Randomized Adjuvant Breast Cancer Trials: ECOG 2197 and ECOG 1199. *J Clin Oncol* (2014) 32:2959–66. doi: 10.1200/JCO.2013.55.0491
23. Pruneri, G, Vingiani, A, and Denkert, C. Tumor Infiltrating Lymphocytes in Early Breast Cancer. *Breast* (2018) 37:207–214. doi: 10.1016/j.breast.2017.03.010
24. Chen, DS, and Mellman, I. Elements of Cancer Immunity and the Cancer-Immune Set Point. *Nature* (2017) 541(7637):321–330. doi: 10.1038/nature21349
25. Mao, Y, Keller, ET, Garfield, DH, Shen, K, and Wang, J. Stromal Cells in Tumor Microenvironment and Breast Cancer. *Cancer Metastasis Rev* (2013) 32(1-2):303–15. doi: 10.1007/s10555-012-9415-3
26. Bahrami, A, Hassanian, SM, Khazaei, M, Hasanzadeh, M, Shahidsales, S, Maftouh, M, et al. The Therapeutic Potential of Targeting Tumor Microenvironment in Breast Cancer: Rational Strategies and Recent Progress. *J Cell Biochem* (2018) 119(1):111–122. doi: 10.1002/jcb.26183
27. Xie, HY, Shao, ZM, and Li, DQ. Tumor Microenvironment: Driving Forces and Potential Therapeutic Targets for Breast Cancer Metastasis. *Chin J Cancer* (2017) 36(1):36. doi: 10.1186/s40880-017-0202-y
28. Salgado, R, Denkert, C, Demaria, S, Sirtaine, N, Klauschen, F, Pruneri, G, et al. The Evaluation of Tumor-Infiltrating Lymphocytes (TILS) in Breast Cancer: Recommendations by an International TILS Working Group 2014. *Ann Oncol* (2015) 26(2):259–71. doi: 10.1093/annonc/mdu450
29. Ibrahim, EM, Al-Foheidi, ME, Al-Mansour, MM, and Kazkaz, GA. The Prognostic Value of Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancer: A Meta-Analysis. *Breast Cancer Res Treat* (2014) 148(3):467–76. doi: 10.1007/s10549-014-3185-2
30. Prizment, AE, Vierkant, RA, Smyrk, TC, Tillmans, LS, Nelson, HH, Lynch, CF, et al. Cytotoxic T Cells and Granzyme B Associated With Improved Colorectal Cancer Survival in a Prospective Cohort of Older Women. *Cancer Epidemiol Biomarkers Prev* (2017) 26(4):622–631. doi: 10.1158/1055-9965.EPI-16-0641
31. Savas, P, Salgado, R, Denkert, C, Sotiriou, C, Darcy, PK, Smyth, MJ, et al. Clinical Relevance of Host Immunity in Breast Cancer: From TILs to the Clinic. *Nat Rev Clin Oncol* (2016) 13(4):228–41. doi: 10.1038/nrclinonc.2015.215
32. Zander, R, Schauder, D, Xin, G, Nguyen, C, Wu, X, Zajac, A, et al. CD4+ T Cell Help Is Required for the Formation of a Cytolytic CD8+ T Cell Subset That Protects Against Chronic Infection and Cancer. *Immunity* (2019) 51 (6):1028–1042.e4. doi: 10.1016/j.immuni.2019.10.009
33. Ma, C, Zhang, Q, Ye, J, Wang, F, Zhang, Y, Wevers, E, et al. Tumor-Infiltrating γδ T Lymphocytes Predict Clinical Outcome in Human Breast Cancer. *J Immunol* (2012) 189(10):5029–36. doi: 10.4049/jimmunol.1201892
34. Joshi, NS, Akama-Garren, EH, Lu, Y, Lee, DY, Chang, GP, Li, A, et al. Regulatory T Cells in Tumor-Associated Tertiary Lymphoid Structures Suppress Anti-Tumor T Cell Responses. *Immunity* (2015) 43(3):579–90. doi: 10.1016/j.immuni.2015.08.006
35. Yeong, J, Thike, AA, Lim, JCT, Lee, B, Li, H, Wong, SC, et al. Higher Densities of Foxp3+ Regulatory T Cells are Associated With Better Prognosis in Triple-Negative Breast Cancer. *Breast Cancer Res Treat* (2017) 163:21–35. doi: 10.1007/s10549-017-4161-4
36. Lee, S, Cho, EY, Park, YH, Ahn, JS, and Im, YH. Prognostic Impact of FOXP3 Expression in Triple-Negative Breast Cancer. *Acta Oncol* (2013) 52 (1):73–81. doi: 10.3109/0284186X.2012.731520
37. Mahmoud, SMA, Lee, AHS, Paish, EC, MacMillan, RD, Ellis, IO, and Green, AR. The Prognostic Significance of B Lymphocytes in Invasive Carcinoma of the Breast. *Breast Cancer Res Treat* (2012) 132(2):545–53. doi: 10.1007/s10549-011-1620-1

38. Iglesia, MD, Vincent, BG, Parker, JS, Hoadley, KA, Carey, LA, Perou, CM, et al. Prognostic B-Cell Signatures Using mRNA-Seq in Patients With Subtype-Specific Breast and Ovarian Cancer. *Clin Cancer Res* (2014) 20 (14):3818–3829. doi: 10.1158/1078-0432.CCR-13-3368
39. Yang, M, Ma, B, Shao, H, Clark, AM, and Wells, A. Macrophage Phenotypic Subtypes Diametrically Regulate Epithelial-Mesenchymal Plasticity in Breast Cancer Cells. *BMC Cancer* (2016) 16:419. doi: 10.1186/s12885-016-2411-1
40. Zhang, W-J, Wang, X-H, Gao, S-T, Cheng, C, Xu, X-Y, Sun, Q, et al. Tumor-Associated Macrophages Correlate With Phenomenon of Epithelial-Mesenchymal Transition and Contribute to Poor Prognosis in Triple-Negative Breast Cancer Patients. *J Surg Res* (2018) 222:93–101. doi: 10.1016/j.jss.2017.09.035
41. Zhao, X, Qu, J, Sun, Y, Wang, J, Liu, X, Wang, F, et al. Prognostic Significance of Tumor-Associated Macrophages in Breast Cancer: A Meta-Analysis of the Literature. *Oncotarget* (2017) 8(18):30576–30586. doi: 10.18632/oncotarget.15736
42. Bergenfelz, C, Roxå, A, Mehmeti, M, Leandersson, K, and Larsson, AM. Clinical Relevance of Systemic Monocytic-MDSCs in Patients With Metastatic Breast Cancer. *Cancer Immunol Immunother* (2020) 69(3):435–448. doi: 10.1007/s00262-019-02472-z
43. Bergenfelz, C, Larsson, AM, Von Stedingk, K, Gruvberger-Saal, S, Aaltonen, K, Jansson, S, et al. Systemic Monocytic-MDSCs are Generated From Monocytes and Correlate With Disease Progression in Breast Cancer Patients. *PLoS One* (2015) 10(5):e0127028. doi: 10.1371/journal.pone.0127028
44. Gabrilovich, DI. Myeloid-Derived Suppressor Cells. *Cancer Immunol Res* (2017) 5:3–8. doi: 10.1158/2326-6066.CIR-16-0297
45. Dysthe, M, and Parihar, R. Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. *Adv Exp Med Biol* (2020) 1224:117–40. doi: 10.1007/978-3-030-35723-8_8
46. Mamessier, E, Bertucci, F, Sabatier, R, Birnbaum, D, and Olive, D. "Stealth" Tumors: Breast Cancer Cells Shun NK-Cells Anti-Tumor Immunity. *OncoImmunology* (2012) 1(3):366–368. doi: 10.4161/onci.18528
47. Stovgaard, ES, Nielsen, D, Hogdall, E, and Balslev, E. Triple Negative Breast Cancer–Prognostic Role of Immune-Related Factors: A Systematic Review. *Acta Oncol* (2018) 57(1):74–82. doi: 10.1080/0284186X.2017.1400180
48. Jang, N, Kwon, HJ, Park, MH, Kang, SH, and Bae, YK. Prognostic Value of Tumor-Infiltrating Lymphocyte Density Assessed Using a Standardized Method Based on Molecular Subtypes and Adjuvant Chemotherapy in Invasive Breast Cancer. *Ann Surg Oncol* (2018) 25:937–46. doi: 10.1245/s10434-017-6332-2
49. Asano, Y, Kashiwagi, S, Goto, W, Takada, K, Takahashi, K, Hatano, T, et al. Prediction of Treatment Response to Neoadjuvant Chemotherapy in Breast Cancer by Subtype Using Tumor-Infiltrating Lymphocytes. *Anticancer Res* (2018) 38:2311–21. doi: 10.21873/anticanres.12476
50. Hida, AI, Sagara, Y, Yotsumoto, D, Kanemitsu, S, Kawano, J, Baba, S, et al. Prognostic and Predictive Impacts of Tumor-Infiltrating Lymphocytes Differ Between Triple-Negative and HER2-Positive Breast Cancers Treated With Standard Systemic Therapies. *Breast Cancer Res Treat* (2016) 158:1–9. doi: 10.1007/s10549-016-3848-2
51. Ruan, M, Tian, T, Rao, J, Xu, X, Yu, B, Yang, W, et al. Predictive Value of Tumor-Infiltrating Lymphocytes to Pathological Complete Response in Neoadjuvant Treated Triple-Negative Breast Cancers. *Diagn Pathol* (2018) 13(1):66. doi: 10.1186/s13000-018-0743-7
52. Park, HS, Heo, I, Kim, JY, Kim, S, Nam, S, Park, S, et al. No Effect of Tumor-Infiltrating Lymphocytes (TILs) on Prognosis in Patients With Early Triple-Negative Breast Cancer: Validation of Recommendations by the International TILs Working Group 2014. *J Surg Oncol* (2016) 114:17–21. doi: 10.1002/jso.24275
53. Dieci, MV, Mathieu, MC, Guarneri, V, Conte, P, Delaloge, S, Andre, F, et al. Prognostic and Predictive Value of Tumor-Infiltrating Lymphocytes in Two Phase III Randomized Adjuvant Breast Cancer Trials. *Ann Oncol* (2015) 26:1698–704. doi: 10.1093/annonc/mdv239
54. Pruneri, G, Vingiani, A, Bagnardi, V, Rotmensz, N, de Rose, A, Palazzo, A, et al. Clinical Validity of Tumor-Infiltrating Lymphocytes Analysis in Patients With Triple-Negative Breast Cancer. *Ann Oncol* (2016) 27:249–56. doi: 10.1093/annonc/mdv571
55. Pruneri, G, Gray, KP, Vingiani, A, Viale, G, Curigliano, G, Criscitiello, C, et al. Tumor-Infiltrating Lymphocytes (TILs) are a Powerful Prognostic Marker in Patients With Triple-Negative Breast Cancer Enrolled in the IBCSG Phase III Randomized Clinical Trial 22-00. *Breast Cancer Res Treat* (2016) 158:323–31. doi: 10.1007/s10549-016-3863-3
56. Denkert, C, von Minckwitz, G, Darb-Esfahani, S, Lederer, B, Heppner, BI, Weber, KE, et al. Tumour-Infiltrating Lymphocytes and Prognosis in Different Subtypes of Breast Cancer: A Pooled Analysis of 3771 Patients Treated With Neoadjuvant Therapy. *Lancet Oncol* (2018) 19:40–50. doi: 10.1016/S1470-2045(17)30904-X
57. Denkert, C, Von Minckwitz, G, Brase, JC, Sinn, BV, Gade, S, Kronenwett, R, et al. Tumor-Infiltrating Lymphocytes and Response to Neoadjuvant Chemotherapy With or Without Carboplatin in Human Epidermal Growth Factor Receptor 2-Positive and Triple-Negative Primary Breast Cancers. *J Clin Oncol* (2015) 33:983–91. doi: 10.1200/JCO.2014.58.1967
58. Dieci, MV, Criscitiello, C, Goubar, A, Viale, G, Conte, P, Guarneri, V, et al. Prognostic Value of Tumor-Infiltrating Lymphocytes on Residual Disease After Primary Chemotherapy for Triple-Negative Breast Cancer: A Retrospective Multicenter Study. *Ann Oncol* (2014) 25:611–8. doi: 10.1093/annonc/mdt556
59. Luen, SJ, Salgado, R, Dieci, MV, Vingiani, A, Curigliano, G, Gould, RE, et al. Prognostic Implications of Residual Disease Tumor-Infiltrating Lymphocytes and Residual Cancer Burden in Triple-Negative Breast Cancer Patients After Neoadjuvant Chemotherapy. *Ann Oncol* (2019) 30:236–42. doi: 10.1093/annonc/mdy547
60. Krishnamurti, U, Wetherilt, CS, Yang, J, Peng, L, and Li, X. Tumor-Infiltrating Lymphocytes are Significantly Associated With Better Overall Survival and Disease-Free Survival in Triple-Negative But Not Estrogen Receptor–Positive Breast Cancers. *Hum Pathol* (2017) 64:7–12. doi: 10.1016/j.humpath.2017.01.004
61. Leon-Ferre, RA, Polley, MY, Liu, H, Gilbert, JA, Cafourek, V, Hillman, DW, et al. Impact of Histopathology, Tumor-Infiltrating Lymphocytes, and Adjuvant Chemotherapy on Prognosis of Triple-Negative Breast Cancer. *Breast Cancer Res Treat* (2018) 167:89–99. doi: 10.1007/s10549-017-4499-7
62. Castaneda, CA, Mittendorf, E, Casavilca, S, Wu, Y, Castillo, M, Arboleda, P, et al. Tumor Infiltrating Lymphocytes in Triple Negative Breast Cancer Receiving Neoadjuvant Chemotherapy. *World J Clin Oncol* (2016) 7(5):387–394. doi: 10.5306/wjco.v7.i5.387
63. Galluzzi, L, Buqué, A, Kepp, O, Zitvogel, L, and Kroemer, G. Immunogenic Cell Death in Cancer and Infectious Disease. *Nat Rev Immunol 2016 17:2* (2016) 17:97–111. doi: 10.1038/nri.2016.107
64. Ladoire, S, Mignot, G, Dabakuyo, S, Arnould, L, Apetoh, L, Rébé, C, et al. *In Situ* Immune Response After Neoadjuvant Chemotherapy for Breast Cancer Predicts Survival. *J Pathol* (2011) 224:389–400. doi: 10.1002/PATH.2866
65. Galvez, M, Castaneda, CA, Sanchez, J, Castillo, M, Rebaza, LP, Calderon, G, et al. Clinicopathological Predictors of Long-Term Benefit in Breast Cancer Treated With Neoadjuvant Chemotherapy. *World J Clin Oncol* (2018) 9 (2):33–41. doi: 10.5306/wjco.v9.i2.33
66. Goto, W, Kashiwagi, S, Asano, Y, Takada, K, Takahashi, K, Hatano, T, et al. Predictive Value of Improvement in the Immune Tumour Microenvironment in Patients With Breast Cancer Treated With Neoadjuvant Chemotherapy. *ESMO Open* (2018) 3(6):e000305. doi: 10.1136/esmoopen-2017-000305
67. Matsumoto, H, Thike, AA, Li, H, Yeong, J, Koo, SL, Dent, RA, et al. Increased CD4 and CD8-Positive T Cell Infiltrate Signifies Good Prognosis in a Subset of Triple-Negative Breast Cancer. *Breast Cancer Res Treat* (2016) 156:237–47. doi: 10.1007/s10549-016-3743-x
68. Miyashita, M, Sasano, H, Tamaki, K, Chan, M, Hirakawa, H, Suzuki, A, et al. Tumor-Infiltrating CD8+ and FOXP3+ Lymphocytes in Triple-Negative Breast Cancer: Its Correlation With Pathological Complete Response to Neoadjuvant Chemotherapy. *Breast Cancer Res Treat* (2014) 148:525–34. doi: 10.1007/s10549-014-3197-y
69. Tian, W, Wang, L, Yuan, L, Duan, W, Zhao, W, Wang, S, et al. A Prognostic Risk Model for Patients With Triple Negative Breast Cancer Based on Stromal Natural Killer Cells, Tumor-Associated Macrophages and Growth-Arrest Specific Protein 6. *Cancer Sci* (2016) 107:882–9. doi: 10.1111/cas.12964
70. Vihervuori, H, Autere, TA, Kurki, S, Kallio, L, Lintunen, MM, Talvinen, K, et al. Tumor-Infiltrating Lymphocytes and CD8+ T Cells Predict Survival of Triple-Negative Breast Cancer. *J Cancer Res Clin Oncol* (2019) 145 (12):3105–3114. doi: 10.1007/s00432-019-03036-5

71. Althobiti, M, Aleskandarany, MA, Joseph, C, Toss, M, Mongan, N, Diez-Rodriguez, M, et al. Heterogeneity of Tumour-Infiltrating Lymphocytes in Breast Cancer and its Prognostic Significance. *Histopathology* (2018) 73:887–96. doi: 10.1111/his.13695
72. West, N, Kost, S, Martin, S, Milne, K, Deleeuw, R, and Nelson, B. Tumour-Infiltrating FOXP3(+) Lymphocytes are Associated With Cytotoxic Immune Responses and Good Clinical Outcome in Oestrogen Receptor-Negative Breast Cancer. *Br J Cancer* (2013) 108:155–62. doi: 10.1038/bjc.2012.524
73. Adams, TA, Vail, PJ, Ruiz, A, Mollaee, M, McCue, PA, Knudsen, ES, et al. Composite Analysis of Immunological and Metabolic Markers Defines Novel Subtypes of Triple Negative Breast Cancer. *Modern Pathol* (2017) 31:288–98. doi: 10.1038/modpathol.2017.126
74. Abdou, Y, Attwood, K, Cheng, TYD, Yao, S, Bandera, EV, Zirpoli, GR, et al. Racial Differences in CD8+ T Cell Infiltration in Breast Tumors From Black and White Women. *Breast Cancer Research : BCR* (2020) 22(1):62. doi: 10.1186/S13058-020-01297-4
75. Wright, N, Lee, C, Guanhao, W, Krishnamurti, U, Li, X, Rida, PCG, et al. Abstract PR06: Differences in Tumor-Infiltrating Lymphocytes Between Racially Distinct Triple-Negative Breast Tumors. *Cancer Epidemiol Prev Biomarkers* (2018) 27(7 Suppl). doi: 10.1158/1538-7755.DISP17-PR06
76. O'Meara, T, Safonov, A, Casadevall, D, Qing, T, Silber, A, Killelea, B, et al. Immune Microenvironment of Triple-Negative Breast Cancer in African-American and Caucasian Women. *Breast Cancer Res Treat* (2019) 175 (1):247–259. doi: 10.1007/s10549-019-05156-5
77. Takeuchi, Y, and Nishikawa, H. Roles of Regulatory T Cells in Cancer Immunity. *Int Immunol* (2016) 28:401–9. doi: 10.1093/INTIMM/DXW025
78. Cerbelli, B, Pernazza, A, Botticelli, A, Fortunato, L, Monti, M, Sciattella, P, et al. PD-L1 Expression in TNBC: A Predictive Biomarker of Response to Neoadjuvant Chemotherapy? *BioMed Res Int* (2017) 2017:1750925. doi: 10.1155/2017/1750925
79. Mori, H, Kubo, M, Yamaguchi, R, Nishimura, R, Osako, T, Arima, N, et al. The Combination of PD-L1 Expression and Decreased Tumorinfiltrating Lymphocytes is Associated With a Poor Prognosis in Triple-Negative Breast Cancer. *Oncotarget* (2017) 8(9):15584–15592. doi: 10.18632/oncotarget.14698
80. AiErken, NJ, Shi, HJ, Zhou, Y, Shao, N, Zhang, J, Shi, Y, et al. High PD-L1 Expression is Closely Associated With Tumor-Infiltrating Lymphocytes and Leads to Good Clinical Outcomes in Chinese Triple Negative Breast Cancer Patients. *Int J Biol Sci* (2017) 13:1172–9. doi: 10.7150/ijbs.20868
81. Li, X, Wetherilt, CS, Krishnamurti, U, Yang, J, Ma, Y, Styblo, TM, et al. Stromal PD-L1 Expression is Associated With Better Disease-Free Survival in Triple-Negative Breast Cancer. *Am J Clin Pathol* (2016) 146(4):496–502. doi: 10.1093/ajcp/aqw134
82. Street, D, Kaufmann, AM, Vaughan, A, Fisher, SG, Hunter, M, Schreckenberger, C, et al. Interferon-Gamma Enhances Susceptibility of Cervical Cancer Cells to Lysis by Tumor-Specific Cytotoxic T Cells. *Gynecol Oncol* (1997) 65:265–72. doi: 10.1006/gyno.1997.4667
83. Mandai, M, Hamanishi, J, Abiko, K, Matsumura, N, Baba, T, and Konishi, I. Dual Faces of Ifnγ in Cancer Progression: A Role of PD-L1 Induction in the Determination of Pro- and Antitumor Immunity. *Clin Cancer Res* (2016) 22:2329–34. doi: 10.1158/1078-0432.CCR-16-0224
84. Hoda, RS, Brogi, E, dos Anjos, CH, Grabenstetter, A, Ventura, K, Patil, S, et al. Clinical and Pathologic Features Associated With PD-L1 (SP142) Expression in Stromal Tumor-Infiltrating Immune Cells of Triple-Negative Breast Carcinoma. *Modern Pathol* (2020) 33:2221–32. doi: 10.1038/s41379-020-0606-0
85. Boman, C, Zerdes, I, Mårtensson, K, Bergh, J, Foukakis, T, Valachis, A, et al. Discordance of PD-L1 Status Between Primary and Metastatic Breast Cancer: A Systematic Review and Meta-Analysis. *Cancer Treat Rev* (2021) 99:102257. doi: 10.1016/j.ctrv.2021.102257
86. Byun, K, Hwang, HJ, Park, KJ, Kim, MC, Cho, SH, Ju, MH, et al. T-Cell Immunoglobulin Mucin 3 Expression on Tumor Infiltrating Lymphocytes as a Positive Prognosticator in Triple-Negative Breast Cancer. *J Breast Cancer* (2018) 21(4):406–414. doi: 10.4048/jbc.2018.21.e61
87. Herrero-Vicent, C, Guerrero, A, Gavilá, J, Gozalbo, F, Hernández, A, Sandiego, S, et al. Predictive and Prognostic Impact of Tumour-Infiltrating Lymphocytes in Triple-Negative Breast Cancer Treated With Neoadjuvant Chemotherapy. *Ecancermedicalscience* (2017) 11:759. doi: 10.3332/ECANCER.2017.759
88. Loi, S, Sirtaine, N, Piette, F, Salgado, R, Viale, G, Van Eenoo, F, et al. Prognostic and Predictive Value of Tumor-Infiltrating Lymphocytes in a Phase III Randomized Adjuvant Breast Cancer Trial in Node-Positive Breast Cancer Comparing the Addition of Docetaxel to Doxorubicin With Doxorubicin-Based Chemotherapy: BIG 02-98. *J Clin Oncol* (2013) 31 (7):860–7. doi: 10.1200/JCO.2011.41.0902
89. Denkert, C, Loibl, S, Noske, A, Roller, M, Müller, BM, Komor, M, et al. Tumor-Associated Lymphocytes as an Independent Predictor of Response to Neoadjuvant Chemotherapy in Breast Cancer. *J Clin Oncol* (2010) 28:105–13. doi: 10.1200/JCO.2009.23.7370
90. Ono, M, Tsuda, H, Shimizu, C, Yamamoto, S, Shibata, T, Yamamoto, H, et al. Tumor-Infiltrating Lymphocytes are Correlated With Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer. *Breast Cancer Res Treat* (2012) 132:793–805. doi: 10.1007/S10549-011-1554-7
91. Rey-Vargas, L, Mejía-Henao, JC, Sanabria-Salas, MC, and Serrano-Gomez, SJ. Effect of Neoadjuvant Therapy on Breast Cancer Biomarker Profile. *BMC Cancer* (2020) 20:675. doi: 10.1186/s12885-020-07179-4
92. Parinyanitikul, N, Lei, X, Chavez-MacGregor, M, Liu, S, Mittendorf, EA, Litton, JK, et al. Receptor Status Change From Primary to Residual Breast Cancer After Neoadjuvant Chemotherapy and Analysis of Survival Outcomes. *Clin Breast Cancer* (2015) 15:153–60. doi: 10.1016/j.clbc.2014.09.006
93. Gahlaut, R, Bennett, A, Fatayer, H, Dall, BJ, Sharma, N, Velikova, G, et al. Effect of Neoadjuvant Chemotherapy on Breast Cancer Phenotype, ER/PR and HER2 Expression – Implications for the Practising Oncologist. *Eur J Cancer* (2016) 60:40–8. doi: 10.1016/j.ejca.2016.03.006
94. Hamy, A-S, Bonsang-Kitzis, H, de Croze, D, Laas, E, Darrigues, L, Topciu, L, et al. Interaction Between Molecular Subtypes and Stromal Immune Infiltration Before and After Treatment in Breast Cancer Patients Treated With Neoadjuvant Chemotherapy. *Clin Cancer Res* (2019) 25:6731–41. doi: 10.1158/1078-0432.CCR-18-3017
95. Pelekanou, V, Barlow, WE, Nahleh, ZA, Wasserman, B, Lo, Y-C, von Wahlde, M-K, et al. Tumor-Infiltrating Lymphocytes and PD-L1 Expression in Pre- and Posttreatment Breast Cancers in the SWOG S0800 Phase II Neoadjuvant Chemotherapy Trial. *Mol Cancer Ther* (2018) 17:1324–31. doi: 10.1158/1535-7163.MCT-17-1005
96. Urueña, C, Lasso, P, Bernal-Estevez, D, Rubio, D, Salazar, AJ, Olaya, M, et al. The Breast Cancer Immune Microenvironment is Modified by Neoadjuvant Chemotherapy. *Sci Rep* (2022) 12:7981. doi: 10.1038/s41598-022-12108-5
97. Gonzalez-Angulo, AM, Timms, KM, Liu, S, Chen, H, Litton, JK, Potter, J, et al. Incidence and Outcome of BRCA Mutations in Unselected Patients With Triple Receptor-Negative Breast Cancer. *Clin Cancer Res* (2011) 17:1082–9. doi: 10.1158/1078-0432.CCR-10-2560
98. Mansouri, M, Derkaoui, T, Bakkach, J, Loudiyi, A, Ghailani Nourouti, N, Barakat, A, et al. Screening of BRCA1 and BRCA2 Germline Mutations in Unselected Triple-Negative Breast Cancer Patients: A Series From North of Morocco. *Precis Med Sci* (2020) 9:43–8. doi: 10.1002/prm2.12009
99. de Boo, L, Cimino-Mathews, A, Lubeck, Y, Daletzakis, A, Opdam, M, Sanders, J, et al. Tumour-Infiltrating Lymphocytes (TILs) and BRCA-Like Status in Stage III Breast Cancer Patients Randomised to Adjuvant Intensified Platinum-Based Chemotherapy Versus Conventional Chemotherapy. *Eur J Cancer* (2020) 127:240–50. doi: 10.1016/j.ejca.2019.12.003
100. Liu, Z, Li, M, Jiang, Z, and Wang, X. A Comprehensive Immunologic Portrait of Triple-Negative Breast Cancer. *Trans Oncol* (2018) 11:311–29. doi: 10.1016/j.tranon.2018.01.011
101. Jiang, T, Shi, W, Wali, VB, Pongor, LS, Li, C, Lau, R, et al. Predictors of Chemosensitivity in Triple Negative Breast Cancer: An Integrated Genomic Analysis. *PLoS Med* (2016) 13:e1002193. doi: 10.1371/journal.pmed.1002193
102. van Vugt, MATM, and Parkes, EE. When Breaks Get Hot: Inflammatory Signaling in BRCA1/2-Mutant Cancers. *Trends Cancer* (2022) 8:174–89. doi: 10.1016/j.trecan.2021.12.003

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 02 August 2022
DOI 10.3389/fonc.2022.955057

OPEN ACCESS

EDITED BY
Maria Rosaria De Miglio,
University of Sassari, Italy

REVIEWED BY
Elise Radiname,
Miami University, United States

*CORRESPONDENCE
María Cristina Martínez-Ávila
maria.martinez@chsm.com

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 28 May 2022
ACCEPTED 07 July 2022
PUBLISHED 02 August 2022

CITATION
Hernández-Blanquisett A, Quintero-Carreño V, Álvarez-Londoño A, Martínez-Ávila MC and Diaz-Cáceres R (2022) Sexual dysfunction as a challenge in treated breast cancer: in-depth analysis and risk assessment to improve individual outcomes. *Front. Oncol.* 12:955057. doi: 10.3389/fonc.2022.955057

Sexual dysfunction as a challenge in treated breast cancer: in-depth analysis and risk assessment to improve individual outcomes

Abraham Hernández-Blanquisett[1], Valeria Quintero-Carreño[1], Angelina Álvarez-Londoño[1], María Cristina Martínez-Ávila[1*] and Raissa Diaz-Cáceres[2]

[1]Cancer Institute, Centro Hospitalario Serena del Mar, Cartagena, Colombia, [2]Department of Psychology Oncology, Centro Hospitalario Serena del Mar, Cartagena, Colombia

The increasing number of breast cancer survivors has led to a greater emphasis on issues related to quality of life (QoL). Up to 75% of women treated for breast cancer (BC) report sexual disorders. However, most oncologists are not trained to recognize which patients are at high-risk of developing sexual disorders. Female sexual dysfunction (FSD) is common in patients with BC; we found that patients without FSD prior to BC treatment are at risk of developing FSD after treatment. Treatment of early BC relies on the combination of chemotherapy, surgery, and radiation therapy. All these treatments have side effects or sequelae identified as high-risk factors for the development of FSD. The choice of less toxic treatments in each modality could reduce the risk of FSD in some cases, without affecting the risk of recurrence or effectiveness. A comprehensive approach of BC must consider FSD as a determinant factor of QoL in survivors.

KEYWORDS

breast cancer, female sexual dysfunction, breast cancer survivor, risk factors, sexology, risk assessment, patient stratification

Introduction

Breast cancer (BC) is the most common cancer in women worldwide, with more than 2.2 million cases in 2020 (1), it is the leading cause of cancer mortality in women in the world. According to figures from the World Health Organization (WHO) in 2020, around 685,000 women died as a result of BC, with most deaths occurring in low and middle-income countries (1). Fortunately, with early diagnosis and proper treatment, BC patients can have 5-

year survival rates of 80-90% (2). The increasing number of survivors has led to a greater emphasis on issues related to quality of life (QoL) in BC survivors.

The treatments of BC have particular consequences for the female body, which can directly influence a woman's self-esteem, appearance and sexual desire (3, 4). Despite efforts to comprehensively address survivorship issues in these patients, several concerns remain unaddressed, particularly those related to sexual function (3, 4). Most oncologists are not trained to advise, carry out preventive interventions or treat patients with disorders related to alterations in sexual function, mainly because of the lack of education on this topic during oncology training. Reports indicated that many doctors are uncomfortable or ill-prepared to address female sexual dysfunction (FSD) (3). Up to 75% of people treated for BC report temporary or permanent physical, psychological, or interpersonal sexual concerns (4) and sexual disorders, as well as disorders related to anxiety, pain, fatigue, marital life, and their overall QoL (3, 5). However, in daily practice it is unusual for BC survivors to be referred to sexologist to assess these aspects at the end of treatment.

?>In short, addressing concerns about sexuality and intimacy are paramount issues in the care of BC survivors, who must be in the care of a specialist in sexologist for the comprehensive management of FSD (5).

In this manuscript, we review the literature on BC and sexuality to describe the risk factors most frequently related to FSD after breast cancer diagnosis and treatment. Based on this research, we developed a checklist that could be helpful in identifying patients at high-risk for FSD and could help oncologists refer high-risk patients accurately.

Early BC current treatments

The fundamental pillars of local treatment for BC are surgery, chemotherapy, and radiotherapy. Over the course of several decades, the surgical treatment of BC has evolved significantly, from radical mastectomy to breast conserving surgery (lumpectomy), with the intention to minimize the cosmetic and functional sequelae (6). The main goal of breast conservation therapy is to provide primary tumor control and to preserve an acceptable cosmetic appearance of the breast comparable to mastectomy (6).

Today, the standard management of early-stage invasive BC and carcinoma *in situ* is lumpectomy with whole breast radiotherapy (WBRT), which is equivalent in terms of overall survival and local recurrence to radical mastectomy. In these patients, radiotherapy reduces the risk of local recurrence by 60-70% in invasive tumors and by 50-60% in tumors in situ (7–9). This is considered a remarkable achievement of modern oncology since previously, all women with BC, regardless of the stage of their disease, were treated with a radical mastectomy, which increases the incidence and severity of depression and anxiety in these patients (10). Additionally, studies have shown that women undergoing lumpectomy are more likely to sustain feelings for their physical attractiveness (11). The remaining percentage of breast tissue contributes to better breast-specific sensuality; being an important part of sexual desire during intercourse, intimacy and/or the experience of pleasurable breast caressing (11). This seems to correlate with improved sexual function postoperatively (11).

Regarding BC in localized advance-stage cancer, scientific evidence supports the use of neoadjuvant chemotherapy (NCT) mainly based on Anthracyclines and Taxanes, to reduce the size of the tumor so that the patient can be managed with conservative surgery and WBRT (12–14). If this is not achieved, the indicated treatment will be mastectomy and chest wall radiation, since the latter provides a benefit in terms of local control with a decrease in the relative risk of recurrence of 60-70% and an improvement in terms of overall survival of 10% (12–14). Despite the indisputable clinical benefits of NCT, unfortunately, in most cases there are significant toxicities such as alopecia, neutropenia, nausea, vomiting, anemia and premature ovarian failure. In addition, some side effects such as chronic fatigue, neurotoxicity, cardiotoxicity or FSD can appear months after treatment. In this context, Anthracycline-free regimens are an interesting option to consider, since they have shown less toxicity with equal efficacy (15).

On the other hand, the most commonly injured organs during radiation therapy treatment in BC patients are skin, heart, and lung; and, if regional nodal irradiation is added, secondary effects may occur in the brachial plexus and shoulder (6). At present, with the development of modern techniques, radiotherapy generally does not generate significant acute and late toxicity that impairs QoL. However, the cosmetic changes that this can generate on the irradiated breast continue to be a great concern due to the subsequent emotional impact that patients can experience and report. Worse cosmetic results have been described with treatment decisions as doses greater than 50 Gy, addition of boost, regional nodal irradiation, inhomogeneity of the radiation dose, more than two fields and no use of compensation filters (6). Likewise, other characteristics such as breast size, age, race, extent of surgical resection, scar orientation and chemotherapy can also affect aesthetic results and self-perception of the body, and therefore must be considered in treatment selection (6).

Sexual education in the medical school

For medicine faculties, sexual education focuses on birth control, anatomy, and physiology of the reproductive organs (16). Studies have shown that sexual health education among undergraduate medical students predominantly focuses on reproduction and organic diseases (70%) (17). Likewise, a survey carried out with 125 medical students reveals that 53.8% were afraid

of offending the patient asking about sexual health topics and 33.4% admitted insufficient knowledge on the topic (17).

Sexual supportive care should be discussed and taught as an integral part of training in medical school concerning sexual education and rights in all patients, especially in oncological patients. Sexual education must be taught during medical school and up to all the specialists dealing with sexual health problems in clinical practice (urology, obstetrics and gynecology, psychiatry, endocrinology, oncologist) (16, 18). However, this does not happen in most medical schools and training programs in most countries around the world. In Latin America for example, a survey demonstrated no more than 18% medical schools provide some type of modern instruction on sexual education (19). Among 366 participants from 40 countries in Europe, 62.3% surveyed had not received any training in sexual health and 48,1% did not have it as a part of their curriculum (18). Literature corroborates the lack of sexual education as a tangible reality.

Sexual functioning and BC patients

The WHO defines human sexuality as a permanent and continuous variable throughout the life cycle (20). It includes sex, gender identity, sexual orientations, eroticism, subjective sexual arousal, pleasure, intercourse, intimacy, and reproduction as main categories, which are reinforced through social, historical, cultural, psychological, ethical, legal, historical, spiritual, religious construction, experienced through the belief system of each individual immersed in a culture or social group (20, 21).

From the traditional physiological model, Master and Johnson represented sexual response as a linear state that includes the phases of arousal, plateau, orgasm, and resolution (22). In men, there is also a refractory period (22). Later, Kaplan (1979), recognized a new phase in this interpretation of sexual functioning by assigning the variable desire or sexual interest defined as an emotion, an impulse that causes motivation to initiate and continue establishing interpersonal relationships accompanied by the sensation of intimacy and affection for the full enjoyment of pleasure through the human potentiality of eroticism (23). Sexual intimacy and consummating erotic desire are then phases associated with the perception of affective responses as a fundamental part of sexual satisfaction, the latter being the result or state of gratification reinforced through sexual communication between the couple (20, 24).

Another model of sexual response that strays from a purely biological explanation, which includes desire, is Basson's cycle of sexual response in women (25). According to Basson's model, female sexual function is found in the context of awareness of non-sexual needs that are explained through the bond of emotional intimacy, affection, commitment, tolerance, and perhaps sexual activity (25). Thus, sexual functioning is interpreted through a deliberate decision to choose a sexual stimulus that could lead to physical experience, subjective arousal, and the possibility or not of having an orgasm (25).

As has been observed so far, sexual functioning requires several variables that lead to the recognition of a free enjoyment of sexuality, requiring a synchrony between organic, psychological, affective, behavioral, cultural, and other dimensions. The diagnosis of BC, its symptomatology, and the respective treatments, influence the patient's perception of their QoL (25). Concern about the possibility of being mutilated, the risk of dying, the prolonged presence of pain and the distortion of their body image are risk factors that trigger anxious and depressive symptoms, suffering or distress (25).

In this aspect, the diagnosis, and some type of treatments for BC will be risk factors for sexual health impairment and FSD, in addition to physical or mental health alterations. The Netherlands Cancer Institute in Amsterdam conducted a study with 169 BC survivors and 67 partners to identify the correlation between sexual dysfunctions and BC in women, showing a prevalence of hypoactive sexual desire disorder in 83% of cases, followed by sexual arousal disorder with 40% and finally a 33% incidence for a diagnosis of dyspareunia (26). Dyspareunia affects female sexual health by alterations in sexual satisfaction, orgasmic functioning, and the appearance of pain during sexual intercourse (26). The perception of declining QoL is interpreted with the presence of sexual dysfunctions. For this reason, sexuality should be considered fundamental in therapeutic decisions following a diagnosis of BC. The question is timing: when should we refer to the specialist? Most patients do not complain of sexual health problems at the time of diagnosis (27, 28), conversely the simultaneous monitoring of a multidisciplinary care group that includes sex therapists, sexologists or clinical sexologists could prevent the risk of sexual dysfunction (29, 30).

The recommendation for the integral management of female sexuality and BC seeks to promote an intervention for the reinterpretation of the sexual response based on sexual desire and satisfaction, its reorganization before the possibility of sexual activity without penetration, the use of lubricants and vibrators as non-pharmacological strategies, providing counseling and couples therapy as protective factors to resume intimacy and sexual communication (27).

Identifying which patients are in high-risk of sexual dysfunction

BC patients face great challenges in their lives. Once their BC doctor utters those terrible words, their world is turned upside down. As clinicians, our goal is to fight the disease in all its

aspects; modern oncology goes beyond the simple prescription of chemotherapeutic agents. The multidisciplinary approach is the gold standard for the treatment of BC, which involves specialists from multiple areas in the diagnosis, treatment, rehabilitation, and follow-up. Nevertheless, there are some holdovers from the old days where BC doctors remain oblivious to some situations that are vital to patients. Sometimes sexuality is the last topic to be addressed in follow-ups, but FSD directly impacts a patient's mood and subsequent recovery from cancer (17, 28). As mentioned above, many BC specialists are not adequately trained to recognize and treat FSD associated with BC treatments. In this context, we reviewed the literature to find what factors could identify those patients at high-risk for sexual dysfunction and we put them together into one simple checklist that could help clinicians address the problem easily and in a timely manner.

Below, we present the main risk factors related to sexual dysfunction in BC patients in a checklist format (Table 1). Other widely known risk factors for sexual dysfunctions, such as chronic severe diseases, endocrine disorders, obesity, smoking, hypertension, psychiatric disorders, hysterectomy, age, marital status, sexual orientation, or sociocultural factors were grouped under the category of others risk factor for FSD not related to BC (29, 30). Because these factors are not directly related with BC treatment.

Selected Risk factors

Mastectomy

In 2014, L Aerts et al. conducted a prospective controlled study comparing the impact of mastectomy versus breast-conserving surgery on the sexual functioning of 149 BC patients compared to 149 age-matched controls. The median age was 57 years and most of the patients were cohabiting or married patients. In this study, 68 patients were treated with mastectomy and 81 with conservative surgery. In the month prior to surgery, 76% of the breast-conserving surgery group and 50% of the mastectomy group were sexually active. The Dyadic Adjustment Scale (DAS) was used to assess the quality of the couple's relationship, to measure the impact on sexual functioning, two questionnaires developed by the authors were used. The results showed no difference between healthy patients and those who underwent conservative surgery for BC. However, compared to healthy women, women in the mastectomy group show more problems in sexual desire and sexual arousal six months after surgery and more problems in sexual desire, the ability to achieve orgasm, and a lower intensity of orgasm 1-year after surgery. These differences were statistically significant (31).

Chemotherapy

Premature ovarian failure caused by chemotherapy results in decreased estrogen levels and is a known cause of vaginal dryness and dyspareunia. In 2002, Ganz et al. showed the results of a retrospective follow-up of 817 BC survivors, where sex life was significantly worse in women who received chemotherapy compared to women who received tamoxifen (32). Another study in 2017 reported that anti-Müllerian hormone levels are undetectable in most women receiving chemotherapy and, more importantly, remain at low levels after completion of treatment in most women (33). In addition, in 2021, Qi et al. published a retrospective study of 201 women <50 years without FSD prior to treatment, who were evaluated after finishing their treatment for BC. Unfortunately, 83% documented the appearance of sexual dysfunction. In the multivariate analysis, chemotherapy was found as an independent risk factor for FSD (OR 11.876). In addition to total mastectomy (OR 7.84) and endocrine therapy (OR 19.688) (34).

Aromatase inhibitors

In 2011, Panjari et al, conducted a retrospective study of 1,684 BC patients enrolled in the BUPA trial and assessed their sexual function using the Menopause-Specific Quality of Life Questionnaire, a set of 5 questions "yes/no" scores on libido were included to determine whether low libido was prior or secondary to breast cancer and treatment. Patients older than 70 years, with active disease, widowers and without a partner were excluded from the final analysis. The authors found that prior to diagnosis, 82.7%

TABLE 1 Risk factors for female sexual dysfunction in breast cancer survivors.

	YES	NO
Mastectomy		
Chemotherapy		
Aromatase inhibitors		
Use of concomitant medication (SSRis/SNRis)		
Chronic pain		
Other risk factors for FSD not related to breast cancer		

of the patients had good and satisfactory sexual function. At the time of the questionnaire, 70% of the patients reported sexual function problems. In this study, women taking aromatase inhibitors were 1.5 times more likely to report sexual function problems (OR 1.50, 95% CI 1.0, 2.2, P = 0.04), while women using tamoxifen did not (OR 1.1, 95% CI 0.8, 1.5, P = 0.6) (35).

Use of concomitant medication

Selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) used for hot flashes secondary to tamoxifen or anti-estrogen therapies can cause reduced libido, altered excitement and anorgasmia. Reducing the dose or changing to a different drug could be helpful in these cases, because the effect may be dependent on the dose of the drug (36).

Chronic pain

With advances and greater effectiveness in the treatment of BC, there are more survivors of this disease, and because of this, many women are left with a lasting legacy of chronic pain, which has a significant impact on their functionality, physical health, sexual, emotional and in general in their QoL. Chronic pain, generally of the neuropathic type, is reported in the literature as the most frequent complication in BC survivors (37, 38). Chronic pain after BC surgery has traditionally been called Postmastectomy Pain Syndrome (PMPS) (39). However, this term can be misleading, since persistent pain can also develop after breast-conserving surgeries, therefore nomenclatures such as postoperative breast pain or persistent pain after BC surgery are also used (39). The International Association for the Study of Pain defines PMPS as chronic pain (greater than three months of evolution), non-malignant and that does not stop immediately after BC surgery, affecting the anterior chest, armpit and/or the upper medial aspect of the arm (37, 40). Incidence rates for persistent pain after BC surgery vary in the literature, with reports ranging in their estimates from 11-57% (39).

Another type of painful condition described after breast surgery is phantom breast syndrome, which comprises a set of symptoms that occur in the absent breast (37, 41). These symptoms range from intensely painful phenomena to simple discomfort or non-painful sensations such as itching, throbbing, pressure, or a tingling sensation, which occur in 30-80% of women after mastectomy (41). Anxiety or stress can worsen this clinical picture. Therefore, it is very important that the doctor inquiries about these symptoms in order to offer therapeutic options to patients that allow them to improve their QoL.

The therapeutic approach to these types of pain described should be based on multimodal pain treatment, generally, it is carried out according to the analgesic ladder of the WHO (42), associating adjuvant treatment, neuromodulators, physical therapy and in some cases interventional management.

Discussion

Education and sexual health should be considered a mainstay in the care of cancer patients in general. It is necessary to ask, instruct and encourage sexual practices and provide safe environments to freely discuss this issue during the consultation. Just as we take the time to explain to our patients how and when to take their oral chemotherapy pills, we must also prescribe, counsel, and encourage safe sexual practices. We must always remember that sexuality is an important issue for the health and QoL of women.

FSD is common in patients with BC; in our review we found robust evidence that patients without FSD prior to BC treatment are at risk of developing FSD after treatment. Treatment of early BC currently relies on the combination of chemotherapy, surgery, and radiation therapy. All these treatments have side effects or sequelae that have been identified as high-risk factors for the development of FSD. Nonetheless, when deciding the ideal treatment for each patient, the risk of FSD is not normally considered, nor is it specifically recommended in international clinical practice guidelines.

Treatment course sometimes use the combination of 3 or more options that are considered of high-risk for the development of FSD. For example, NCT regimens, followed by mastectomy, radiotherapy, hormonal therapy with aromatase inhibitors is common. What, if it is worth discussing, the choice of less toxic treatments in each modality? For instance, consider the use of short and Anthracycline-free chemotherapy regimens, chosing conservative surgery, when possible, prescribe less toxic radiotherapy techniques such as IMRT and avoiding the risk factors associated with worse cosmetic result, described in the treatment section.

We believe that a better selection of treatment options could, in some cases, reduce the risk of FSD. In addition, early referral to an expert in sexology could reduce the impact on QoL and sexual life of those patients who already have high-risk factors at the initial visit or post-treatment visit. However, in our daily practice it is rare for patients with high-risk factors to be referred to an expert in sexology, unless they developed FSD. We believe that this is mainly due to two factors:

1) The lack of proper training for doctors in matters of sexology that leads to the non-recognition of sexual health as a vital important issue. Bearing this in mind, it would be important for the health-care institutions to include in their educational programs in medicine and areas related to oncology more training on issues of sexology.

2) The non-perception of the BC treatments carried out as high-risk factors for FSD. This point was what motivated us to design a simple checklist where doctors can quickly consult if the patient has a high-risk of FSD and with this select a better treatment strategy (if possible) in addition to referring the BC survivor to a specialist in sexology at the right time.

Conclusions

The evaluation of FSD is of great relevance. The identification of specific needs for the cancer patient will improve the QoL in that difficult stage. Education and sexual health should be considered a pillar in the care of patients with cancer. The right moment to approach sexuality is a great challenge in daily practice and a good relationship with the patient is essential, knowing risk factors could help oncologists refer high-risk patients on a timely basis. We need to learn to approach both cancer and sexuality with compassion.

Author contributions

MCMÁ and RDC reviewed the literature and drafted the article. VQC and AAL conceived the review and drew the tables. AHB revised the manuscript. All authors read and approved the final manuscript.

Funding

Author's resources.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. World Health Organization. GLOBOCAN 2020 WORLD. *Global Cancer Observatory: Cancer Today* (2021). Available at: https://gco.iarc.fr.

2. OPS. Cáncer de mama. *Hojas informativas para los profesionales de salud*. Pan American Health Organization/World Health Organization (2019). Available at: https://www.paho.org/es/temas/cancer/cancer-mama-hojas-informativaspara-profesionales-salud.

3. Runowicz CD, Leach CR, Henry NL, Henry KS, Mackey HT, Cowens-Alvarado RL, et al. American Cancer Society/American society of clinical oncology breast cancer survivorship care guideline. *CA: A Cancer J Clin* (2016) 66. doi: 10.3322/caac.21319

4. Streicher L, Simon JA. Sexual function post-breast cancer. In: *Cancer treatment and research*. Springer Cham Copyright Information: Springer International Publishing AG (2018). doi: 10.1007/978-3-319-70197-4_11

5. Miaja M, Platas A, Martinez-Cannon BA. Psychological impact of alterations in sexuality, fertility, & body image in young breast cancer patients & their partners. *Rev Investigacion Clinica* (2017) 69:204–9. doi: 10.24875/RIC.17002279

6. White J, Joiner MC. Toxicity from radiation in breast cancer. *Cancer Treat Res* (2006) 128:65–109. doi: 10.1007/0-387-25354-8_5

7. Veronesi U, Cascinelli N, Mariani L, Greco M, Saccozzi R, Luini A, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. *New Engl J Med* (2002) 347:1227–32. doi: 10.1056/nejmoa020989

8. Fisher B. Twenty-year follow-up of a randomized trial comparing total for the treatment of invasive breast cancer. *English J* (2002) 347. doi: 10.1056/NEJMoa022152

9. Van Dongen JA, Voogd AC, Fentiman IS, Legrand C, Sylvester RJ, Tong D, et al. Long-term results of a randomized trial comparing breast-conserving therapy with mastectomy: European organization for research and treatment of cancer 10801 trial. *J Natl Cancer Inst* (2000) 92:1143–50. doi: 10.1093/jnci/92.14.1143

10. Chen W, Lv X, Xu X, Gao X, Wang B. Meta-analysis for psychological impact of breast reconstruction in patients with breast cancer. *Breast Cancer* (2018) 25:464–9. doi: 10.1007/s12282-018-0846-8

11. Gass JS, Onstad M, Pesek S, Rojas K, Fogarty S, Stuckey A, et al. Breast-specific sensuality and sexual function in cancer survivorship: Does surgical modality matter? *Ann Surg Oncol* (2017) 24:3133–40. doi: 10.1245/s10434-017-5905-4

12. Overgaard M, Hansen PS, Overgaard J, Rose C, Andersson M, Bach F, et al. Postoperative radiotherapy in high-risk premenopausal women with breast cancer who receive adjuvant chemotherapy. Danish breast cancer cooperative group 82b trial. *N Engl J Med* (1997) 337. doi: 10.1056/NEJM199710023371401

13. Overgaard M, Jensen MB, Overgaard J, Hansen PS, Rose C, Andersson M, et al. Postoperative radiotherapy in high-risk postmenopausal breast-cancer patients given adjuvant tamoxifen: Danish breast cancer cooperative group DBCG 82c randomised trial. *Lancet* (1999) 353:1641–8. doi: 10.1016/S0140-6736(98)09201-0

14. Ragaz J, Olivotto IA, Spinelli JJ, Phillips N, Jackson SM, Wilson KS, et al. Locoregional radiation therapy in patients with high-risk breast cancer receiving adjuvant chemotherapy: 20-year results of the British Columbia randomized trial. *J Natl Cancer Inst* (2005) 97:116–26. doi: 10.1093/jnci/djh297

15. De Iuliis F, Salerno G, Corvino R, D'Aniello D, Cefalì K, Taglieri L, et al. Anthracycline-free neoadjuvant chemotherapy ensures higher rates of pathologic complete response in breast cancer. *Clin Breast Cancer* (2017) 17:34–40. doi: 10.1016/j.clbc.2016.06.010

16. Farmer E, Fleming N, Black A, Dumont T. Where are we in terms of sexual health education? an Ontario perspective. *J Obstetrics Gynaecol Canada* (2019) 41:835–7. doi: 10.1016/j.jogc.2018.11.001

17. Olimpio LM, Spessoto LCF, Fácio FN. Sexual health education among undergraduate students of medicine. *Trans Androl Urol* (2020) 9:510–5. doi: 10.21037/tau.2020.02.13

18. Kristufkova A, Pinto Da Costa M, Mintziori G, Vásquez JL, Aabakke AJM, Fode M, et al. Sexual health during postgraduate training–European survey across medical specialties. *Sexual Med* (2018) 6:255–62. doi: 10.1016/j.esxm.2018.04.001

19. Alzate H. La educación sexual médica. *Rev Colombiana Obstetricia y Ginecología* (1976) 27:27–33. doi: 10.18597/rcog.1964

20. World Health Organization. *Sexual health and its linkages to reproductive health: an operational approach*. World Health Organization (2017). Available at: https://apps.who.int/iris/handle/10665/258738.

21. Montgomery P, Knerr W. Review of the evidence on sexuality education. In: *Report to inform the update of the UNESCO international technical guidance on sexuality education*. Unesco (2018). Available at: https://healtheducationresources.unesco.org/library/documents/reviewevidence-sexuality-education-reportinform-update-unesco-international.

22. Gutiérrez Teira B. La respuesta sexual humana. In: *Actualización en medicina de familia* Ars Medica (2001). p. 6.

23. Miranda Baquedano VM, Lara Lara MV. DISFUNCIÓN SEXUAL de la MUJER EN EDAD REPRODUCTIVA. *Rev Científica la Escuela Universitaria las Cienc la Salud* (2019) 4:22–6. doi: 10.5377/rceucs.v4i1.7065

24. Iglesias Campos P, Morell-Mengual V, Caballero-Gascón L, Ceccato R, Gil-Llario MD. Satisfacción sexual femenina: influencia de la edad y variedad de prácticas sexuales. *Int J Dev Educ Psychol Rev INFAD Psicología* (2018) 1:85–92. doi: 10.17060/ijodaep.2018.n1.v1.1163

25. de la Hoz FE, Ospina DDL. Evaluación de la función sexual en mujeres con cáncer de mama, en el quindío TT - evaluation of sexual function in women with breast cancer in quindío. *Rev Avances en Salud* (2019) 3. doi: 10.21897/25394622.1754

26. Hummel SB, Hahn DEE, van Lankveld JJDM, Oldenburg HSA, Broomans E, Aaronson NK. Factors associated with specific diagnostic and statistical manual of mental disorders, fourth edition sexual dysfunctions in breast cancer survivors: A study of patients and their partners. *J Sexual Med* (2017) 14:1248–59. doi: 10.1016/j.jsxm.2017.08.004

27. Mendoza N, Molero F, Criado F, Cornellana MJ, González E E. Sexuality In Breast Cancer Survivors Group. Sexual health after breast cancer: Recommendations from the Spanish menopause society, federación española de sociedades de sexología, sociedad española de médicos de atención primaria and sociedad española de oncología médica. *Maturitas* (2017) 105:126–31. doi: 10.1016/j.maturitas.2017.02.010

28. Komlenac N, Hochleitner M. Predictors for low frequencies of patient-physician conversations concerning sexual health at an Austrian university hospital. *Sexual Med* (2020) 8:100–6. doi: 10.1016/j.esxm.2019.09.006

29. McCabe MP, Sharlip ID, Lewis R, Atalla E, Balon R, Fisher AD, et al. Risk factors for sexual dysfunction among women and men: A consensus statement from the fourth international consultation on sexual medicine 2015. *J Sexual Med* (2016) 13:153–67. doi: 10.1016/j.jsxm.2015.12.015

30. Desimone M, Spriggs E, Gass JS, Carson SA, Krychman ML, Dizon DS, et al. Sexual dysfunction in female cancer survivors. *Am J Clin Oncol: Cancer Clin Trials* (2014) 37:101–6. doi: 10.1097/COC.0b013e318248d89d

31. Aerts L, Christiaens MR, Enzlin P, Neven P, Amant F. Sexual functioning in women after mastectomy versus breast conserving therapy for early-stage breast cancer: A prospective controlled study. *Breast* (2014) 23:629–36. doi: 10.1016/j.breast.2014.06.012

32. Ganz PA, Desmond KA, Leedham B, Rowland JH, Meyerowitz BE, Belin TR. Quality of life in long-term, disease-free survivors of breast cancer: A follow-up study. *J Natl Cancer Inst* (2002) 94. doi: 10.1093/jnci/94.1.39

33. Fréour T, Barrière P, Masson D. Anti-müllerian hormone levels and evolution in women of reproductive age with breast cancer treated with chemotherapy. *Eur J Cancer* (2017) 74:1–8. doi: 10.1016/j.ejca.2016.12.008

34. Qi A, Li Y, Sun H, Jiao H, Liu Y, Chen Y. Incidence and risk factors of sexual dysfunction in young breast cancer survivors. *Ann Palliat Med* (2021) 10:4428–34. doi: 10.21037/apm-21-352

35. Panjari M, Bell RJ, Davis SR. Sexual function after breast cancer. *J Sexual Med* (2011) 8:294–302. doi: 10.1111/j.1743-6109.2010.02034.x

36. Taylor CE, Meisel JL. Management of breast cancer therapy–related sexual dysfunction. *ONCOLOGY* (2017) 31. Available at: https://www.cancernetwork.com/view/management-breast-cancertherapyrelated-sexual-dysfunction.

37. Lovelace DL, McDaniel LR, Golden D. Long-term effects of breast cancer surgery, treatment, and survivor care. *J Midwifery Women's Health* (2019) 64:713–24. doi: 10.1111/jmwh.13012

38. Feeney LR, Tormey SM, Harmon DC. Breast cancer and chronic pain: a mixed methods review. *Irish J Med Sci* (2018) 187:877–85. doi: 10.1007/s11845-018-1760-y

39. Khan JS, Ladha KS, Abdallah F, Clarke H. Treating persistent pain after breast cancer surgery. *Drugs* (2020) 80:23–31. doi: 10.1007/s40265-019-01227-5

40. Scholz J, Finnerup NB, Attal N, Aziz Q, Baron R, Bennett MI, et al. The IASP classification of chronic pain for ICD-11: Chronic neuropathic pain. *Pain* (2019) 160:53–9. doi: 10.1097/j.pain.0000000000001365

41. Waltho D, Rockwell G. Post-breast surgery pain syndrome: Establishing a consensus for the definition of post-mastectomy pain syndrome to provide a standardized clinical and research approach - a review of the literature and discussion. *Can J Surg* (2016) 59:342–50. doi: 10.1503/cjs.000716

42. Miller E. The world health organization analgesic ladder. *J Midwifery Women's Health* (2004) 49:542–45. doi: 10.1016/j.jmwh.2004.08.021

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 03 August 2022
DOI 10.3389/fonc.2022.918349

OPEN ACCESS

EDITED BY
Maria Rosaria De Miglio,
University of Sassari, Italy

REVIEWED BY
Sarah Lewis,
The University of Sydney, Australia
Claudia Mello-Thoms,
The University of Iowa, United States

*CORRESPONDENCE
Guang-Xun Lin
linguangxun@hotmail.com
Ping-ming Fan
18907577180@163.com
Xu-Chen Cao
caoxuchen0328@163.com

†These authors have contributed equally to this work

‡These authors have contributed equally to this work as corresponding co-authors

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 12 April 2022
ACCEPTED 11 July 2022
PUBLISHED 03 August 2022

CITATION
Lyu P-f, Li J-t, Deng T, Lin G-X, Fan P-m and Cao X-C (2022) Research trends and hotspots of breast cancer management during the COVID-19 pandemic: A bibliometric analysis. *Front. Oncol.* 12:918349. doi: 10.3389/fonc.2022.918349

Research trends and hotspots of breast cancer management during the COVID-19 pandemic: A bibliometric analysis

Peng-fei Lyu[1,2†], Jing-tai Li[2†], Tang Deng[3†], Guang-Xun Lin[4,5*‡], Ping-ming Fan[2*‡] and Xu-Chen Cao[1*‡]

[1]The First Department of Breast Cancer, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Key Laboratory of Breast Cancer Prevention and Therapy, Ministry of Education, Tianjin's Clinical Research Center for Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin Medical University, Tianjin, China, [2]Department of Breast Surgery, The First Affiliated Hospital of Hainan Medical University, Haikou, China, [3]Department of Interventional Radiology and Vascular Surgery, The First Affiliated Hospital of Hainan Medical University, Haikou, China, [4]Department of Orthopedics, The First Affiliated Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China, [5]The Third Clinical Medical College, Fujian Medical University, Fuzhou, China

Background: The coronavirus disease 2019 (COVID-19) pandemic is disrupting routine medical care of cancer patients, including those who have cancer or are undergoing cancer screening. In this study, breast cancer management during the COVID-19 pandemic (BCMP) is reviewed, and the research trends of BCMP are evaluated by quantitative and qualitative evaluation.

Methods: In this study, published studies relating to BCMP from 1 January 2020 to 1 April 2022 were searched from the Web of Science database (WoS). Bibliometric indicators consisted of publications, research hotspots, keywords, authors, journals, institutions, nations, and h-index.

Results: A total of 182 articles investigating BCMP were searched. The United States of America and the University of Rome Tor Vergata were the nation and the institution with the most publications on BCMP. The first three periodicals with leading published BCMP studies were *Breast Cancer Research and Treatment, Breast*, and *In Vivo*. Buonomo OC was the most prolific author in this field, publishing nine articles (9/182, 4.94%). The co-keywords analysis of BCMP suggests that the top hotspots and trends in research are screening, surgery, rehabilitation, emotion, diagnosis, treatment, and vaccine management of breast cancer during the pandemic. The hotspot words were divided into six clusters, namely, screening for breast cancer patients in the pandemic, breast cancer surgery in the pandemic, recovery of breast cancer patients in the pandemic, motion effect of the outbreak on breast cancer patients, diagnosis and treatment of breast cancer patients in the pandemic, and vaccination management for breast cancer patients during a pandemic.

Conclusion: BCMP has received attention from scholars in many nations over the last 3 years. This study revealed significant contributions to BCMP research by nations, institutions, scholars, and journals. The stratified clustering study provided the current status and future trends of BCMP to help physicians with the diagnosis and treatment of breast cancer through the pandemic, and provide a reference for in-depth clinical studies on BCMP.

KEYWORDS

COVID-19, breast cancer, management, research hotspots, bibliometric analysis

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2, has significantly affected >220 million individuals worldwide (1). The susceptibility to the adverse effects caused by COVID-19 has received huge amounts of global attention, due to the potentially increased vulnerability of COVID-19-induced mortality (2). During the pandemic, the management of cancer patients has changed significantly, which consists of delayed breast cancer screening, untimely treatment and follow-up, and breast cancer recovery after infection with COVID-19 (3–8). The true effect of the COVID-19 pandemic on patient outcomes remains unknown, though most of the changes were reasonable responses to the current healthcare emergency (3). Breast cancer is one of the most common malignancies in women worldwide, and breast cancer has surpassed lung cancer as the most commonly diagnosed cancer, with an estimated 2.3 million new cases within a year (9, 10). Accordingly, there is an urgent need to find research on breast cancer management in pandemics for breast cancer patients. Bibliometric is a method of quantitative analysis, which employs co-keyword and co-citation analysis of previous studies to facilitate the identification of popular themes and emerging trends in all study fields (11, 12). Thus, many scholars have performed bibliometric analysis on diseases (13–16), using CiteSpace, VOSviewer, and Bibliographic Items Co-occurrence Matrix Builder (BICOMB) for analysis and visualization (17–19). Nevertheless, there have been no bibliometric studies about BCMP throughout the COVID-19 pandemic. Thus, through our study, research hotspots and future directions in this field were highlighted, providing a reference for in-depth clinical practice related to BCMP.

Methods

Systematic search strategy

This study was not approved by an institutional committee since the relevant public data were retrospectively reviewed. Articles were searched from the Web of Science (WoS) database. The literature between 1 January 2020 and 30 April 2022 was reviewed. The time frame of the study was from the outbreak of the COVID-19 to the present. Search phrases included (TI=("neoplasm of the breast" OR "breast neoplasm" OR "carcinoma breast" OR "carcinoma of the breast" OR "breast cancer" OR "cancer of the breast" OR "breast cancer")) AND TI=("SARS-COV2" OR "Severe Acute Respiratory Syndrome Coronavirus-2" OR "SARS coronavirus 2" OR "2019 novel coronavirus" OR 2019-nCoV OR SARS-CoV-2 OR "coronavirus disease 2019" OR "coronavirus 2019" OR "COVID 19") AND PY= (2012–2021)) AND DT=(Article)). Original articles were only incorporated; letters, editorial material, and reviews were excluded. A total of 182 articles were correlated with our topics. Two researchers verified that these publications matched the themes of this study. Any differences of opinion were discussed until a consensus was reached.

Data analysis

CiteSpace, R language, and VOSviewer were utilized for creating data tables and visual knowledge graphs. CiteSpace is largely based on co-citation analysis and pathfinder network scaling to investigate the articles on a particular subject, which allows users to find the vital development and knowledge turning point in the discipline history (20). VOSviewer is a tool to create maps based on network, bibliographic, or text data (21). BCMP was analyzed with the use of the R-based Biblioshiny app, thus creating a web interface for bibliometrics (https://bibliometrix.org/) (22). Results are based on the qualitative and quantitative investigation according to the numbers of publications, nations, h-index [a valid and reliable indicator for academic assessment (23)], keywords, hotpots, co-occurrence status, citations, authors, journals, and institutions. The degree of communication in this field was partly based on surveys of co-authors. The links between the visualization knowledge maps among nodes showed the cooperative ties. The size of the circle represents the amount of relevant domain volume.

Bibliographic Item Co-Occurrence Matrix Builder (BICOMB) is available freely online (23, 24). Next, with the use of the software "gCLUTO", version 1.0 (Graphical CLUstering TOolkit, a graphical front-end in terms of the CLUTO data clustering library, proposed by Rasmussen, http://glaros.dtc.umn.edu/gkhome/cluto/gcluto/download), a binary matrix was built in accordance with BICOMB based on commonplace significant MeSH terms representing the rows and with source articles representing the columns in terms of further biclustering (24). Parameters of biclustering in gCLUTO were set according to those appropriate for biclustering analysis based on articles (25). Repeated bisection was selected for the clustering method, cosine for the similarity function, and I^2 for the clustering criterion function. To distinguish the optimal number of clusters, the biclustering with different cluster numbers was rerun (26). With the use of matrix visualization as well as mountain visualization, we presented the biclustering results achieved by the matrix of extensive major keywords-source articles (27). The basic framework of research hotspots of BCMP was generated and studied based on the semantic relationship among hotspot words and the content of the typical paper in the respective cluster.

Results

Current status

After screening, 182 articles on the topic of BCMP during the COVID-19 pandemic were acquired from the WOS database in less than 3 years, particularly in 2021 with 104 articles accounting for 57.1% of the total literature, thus significantly contributing to this study.

Analysis of nations and institutions

A total of 60 nations contributed to breast cancer management during the COVID-19 pandemic in the study period. The United States of America had the largest number of articles (54 of 182 [29.67%]), followed by Italy (34 articles [18.68%]), P.R. China (17 articles [9.34%]), Turkey (14 articles [7.69%]), and England (13 articles [7.14%]) (Figure 1). Italy achieved the maximum h-indexes (10), followed by the United States of America, China, Turkey, and England (Figure 1). The collaboration world map shows the number of publications, with darker colors representing more papers; the number of connecting lines represents the amount of cooperation between nations (Figure 2). The United States of America, China, and the UK, with the United States of America at the core, are working and communicating tightly in terms of BCMP. Figure 3 presents the prolific institutions in BMCP. Policlinico Tor Vergata University published 30 articles, Huazhong University of Science and Technology published 20 articles, and University Medical Center Utrecht published 17 articles. Policlinico Tor Vergata University is the most relevant institution linked to BCMP because it has the largest number of documents (Figure 3). Figure 4 presents a map of the institution's collaborative network related to BCMP. The same color means that the institution is from the same nation. Larger circles mean more articles were published. More connecting lines means more collaboration. The connecting line means cooperation, showing a centralized distribution and good collaboration among the above institutions. As depicted in Figure 4, Huazhong University of Science and Technology, University Medical Center Utrecht, and Policlinico Tor Vergata University cooperated closely in the field of BCMP.

Analysis of journals

Articles regarding BCMP were published in 101 science journals. The top five journals consisted of *Breast Cancer Research and Treatment*, with 11 papers (6.04%); *Breast*, with 7 (3.84%); the *In Vivo*, with 7 (3.84%); *Cancer*, with 5 (2.74%); and the *European Journal of Breast Health*, with 5 (2.74%) (3.75%; Figure 5). The average impact factor of the journals was about 4, with most belonging to Q2.

The top 5 countries contributed to research publications in the field

Rank	Country	Number	Percentage	H-index	Citations
1	USA	54	29.67%	8	143
2	ITALY	34	18.681%	10	375
3	CHINA	17	9.341%	5	101
4	TURKEY	14	7.692%	3	25
5	ENGLAND	13	7.143%	5	117

FIGURE 1
The top five countries that contributed to research publications in the field.

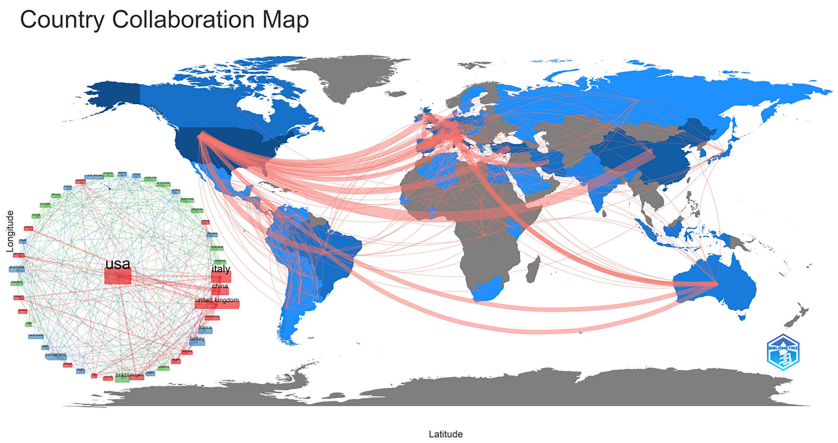


FIGURE 2
The country collaborative map in the field of BCMP.

Analysis of authors and references

Buonomo OC was the most prolific author in this field, publishing nine articles (9/182, 4.94%), followed by Vanni G, Matarazzo M, and Pellicciaro M, who published eight articles (4.40%) and were cited over 30 times. Interestingly, all of them achieved the same h-index (h-index = 6) and came from the same institution (University of Rome Tor Vergata) and nation (Italy). The top 10 authors have a steady output and total citation in the 2 years. A list of the top authors' productions over time is shown in Figure 6. The size of the dark blue circles represents the number of publications by the author; the size of the light blue circles represents how many times the article has been cited. Thus, Figure 6 shows the evolution of publications and citations over time for highly productive authors. Authors who have published prolific articles are also highly cited authors.

The top 10 papers have been cited 465 times (Supplementary Table S1). The greatest and smallest number of citations for a particular article were 110 and 24. Four articles were from Italy, three from the UK, and one from China, France, and the United States of America.

Analysis of keywords

With the use of high-frequency keywords to identify the hotspots of research, these and other vital issues can be effectively determined. A total of 380 keywords were extracted based on BICOMB from the 182 publications. The frequency of 2

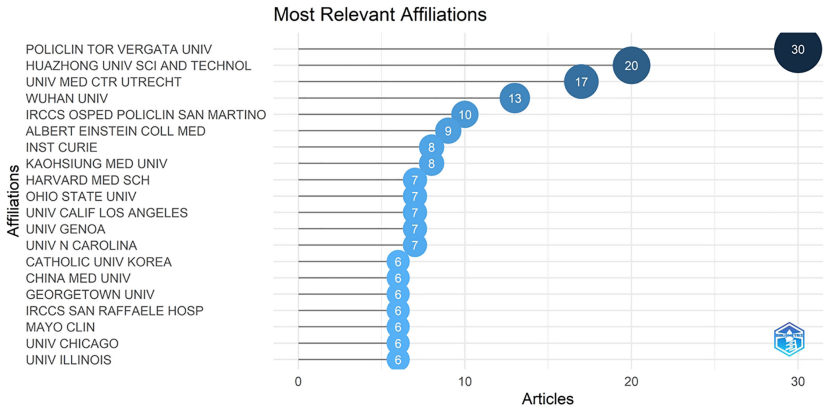


FIGURE 3
The most relevant affiliations linked to articles of BCMP (top 20).

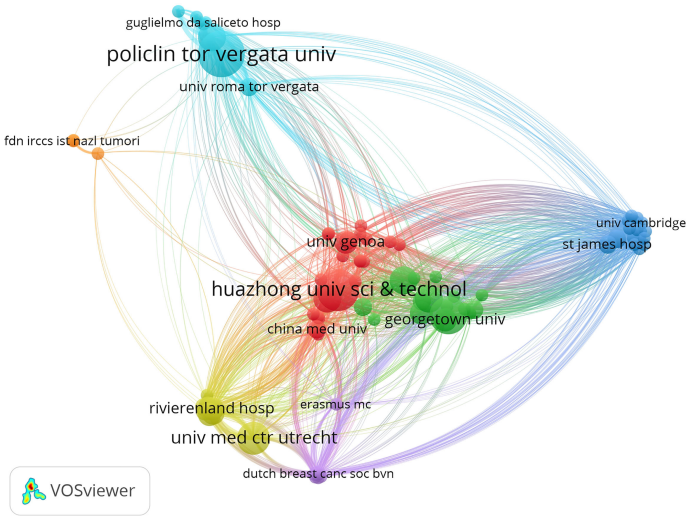


FIGURE 4
The map of the institution's collaborative network.

was defined as high-frequency keywords, and 60 keyword matrixes were classified. The 10 most frequent keywords consisted of COVID-19, breast cancer, quality of life, women, impact, therapy, diagnosis, survival, health, and surgery. The keywords co-occurrence map shows that larger nodes had larger keyword weights, and the linkage of keywords represents simultaneous appearance in one document (Figure 7). The different colors in the co-occurrence chart can be observed, the dark color represents the keywords appearing earlier, and the light color represents the keywords appearing recently (Figure 7). The development direction of BCMP was analyzed according to the thematic map (Figure 8). The abscissa is the correlation degree of centrality, and the ordinate is the development degree of hotspot keywords. It is suggested that the basic themes are emotional influence, quality of life, and treatment. The mainstream themes are chemotherapy, neoadjuvant therapy, endocrine therapy, radiotherapy, breast-conserving surgery, and follow-up. The decline theme is questionnaire, telemedicine, and mammography.

Cluster analysis of research hotspots

Subsequently, similar categories of keywords are assigned to the same cluster using gCLUTO for biclustering analysis. The above clusters revealed the vital research field and critical research content. The substantial number represents the cluster number within the visualized mountain map (Figure 9). The mountain volume was directly proportional to the keyword number in the cluster. In addition, a proportional relationship was found between height and within-group similarity. The red spikes represent good intraclass similarity, the yellow and green spikes represent

The top five journals in number of articles published

Rank	Journal	Count	Proportion	IF (2020)	Category (2020)
1	BREAST CANCER RESEARCH AND TREATMENT	11	6.04%	4.872	Q2
2	BREAST	7	3.85%	4.38	Q2
3	IN VIVO	7	3.85%	2.15	Q4
4	CANCER	5	2.75%	6.86	Q1
5	FRONTIERS IN PSYCHOLOGY	5	2.75%	2.99	Q2

FIGURE 5
The top five journals in number of articles published.

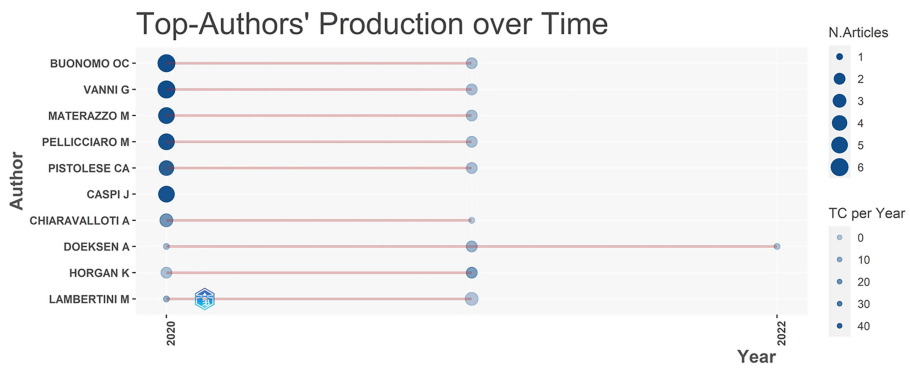


FIGURE 6
The top author's production over time.

average intraclass similarity, and the blue proxy variant shows low intraclass similarity. The distance between the peaks can be used to evaluate the similarity between clusters. The six small mountains were relatively independent and distributed, suggesting a significant clustering effect. Figure 10 presents the visualized heat map linked to the keyword matrix. Rows represent published literature and columns represent cluster numbers. The colors stand for values within the initial data matrix. In general, color depth stands for the values in the initial data matrix. The white area stands for a value approaching zero. The deepened red area can represent a significant value. The major keywords for the six clusters are presented in Supplementary Table S2. The greater the internal similarity, the better the clustering; the smaller the external similarity, the better the clustering. Six research clusters in the field of BCMP were demonstrated by identifying the semantic connection in words with a high frequency and their source articles:

Cluster 0: Screening for breast cancer patients in the pandemic

Cluster 1: Breast cancer surgery in the pandemic

Cluster 2: Recovery of breast cancer patients in the pandemic

Cluster 3: Emotion effect of the outbreak on breast cancer patients

Cluster 4: Diagnosis and treatment of breast cancer patients in the pandemic

Cluster 5: Clinical vaccination management for breast cancer patients during the pandemic

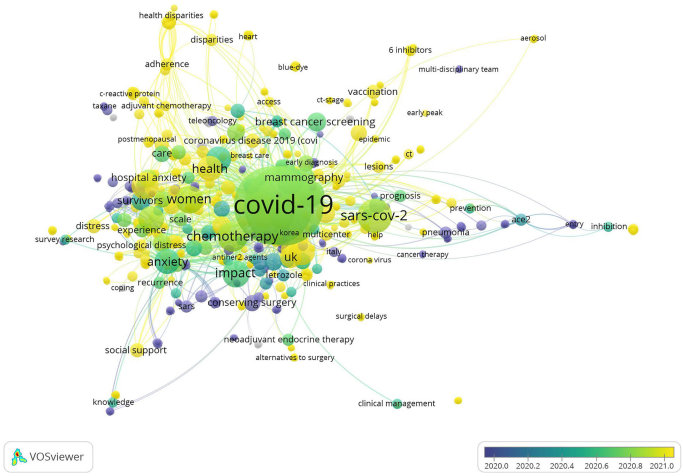


FIGURE 7
The co-occurrence map of keywords.

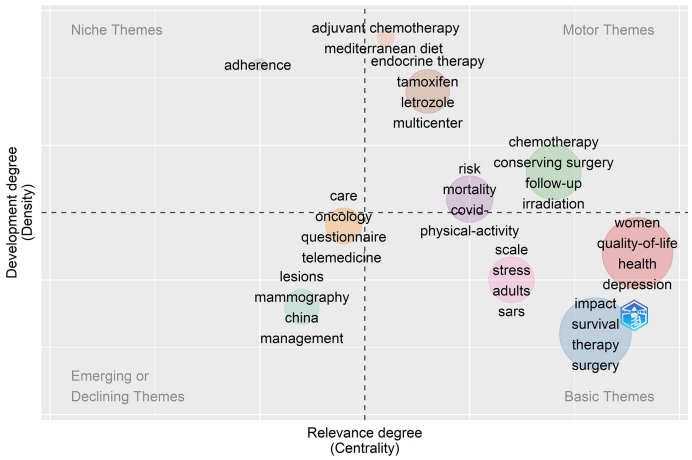


FIGURE 8
The thematic map of keywords plus.

Discussion

The COVID-19 pandemic has caused major disruption to healthcare systems and professionals around the globe (28). Numerous experts provide recommendations to prepare for the effect of the COVID-19 pandemic on breast cancer patients and propose suggestions in terms of the method of triaging, prioritizing and organizing medical treatment, radiation, surgeries, and diagnoses (28–30).

The BCMP publications between 2020 and 2022 were investigated with the use of information visualization methods. A total of 421 BCMP-related articles were identified. Furthermore, 182 original articles were finally studied by de-duplicating verification, excluding reviews, conference articles, and letters.

The highest number of articles was from the United States of America. Although Italy has not published the most articles, it achieved the largest h-index. The reason may be that the

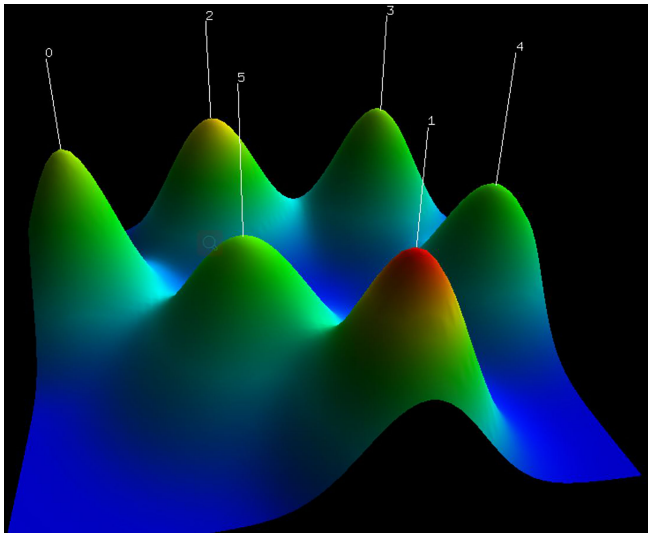


FIGURE 9
The visualized mountain map of the keywords: Cluster 0: Screening for breast cancer patients during the pandemic; Cluster 1: Breast cancer surgery in the pandemic; Cluster 2: Recovery of breast cancer patients during the pandemic; Cluster 3: Motion impact of the outbreak on breast cancer patients; Cluster 4: Diagnosis and treatment of breast cancer patients during the pandemic; Cluster 5: Clinical vaccine management of breast cancer patients during a pandemic.

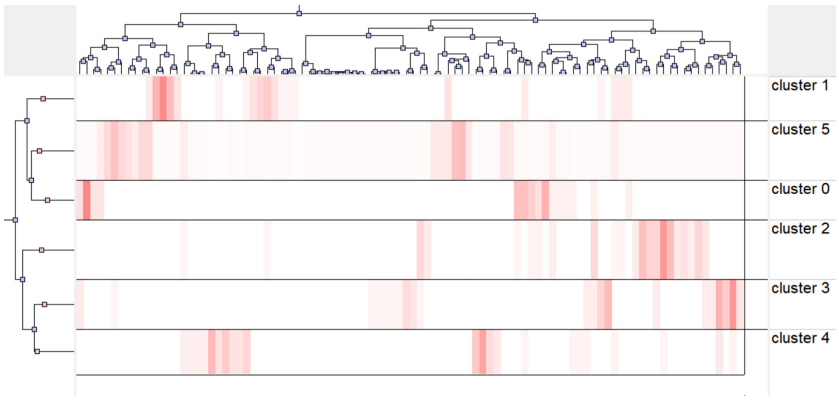


FIGURE 10
The visualized heat map linked to data matrix.

pandemic situation in Italy was serious in the early stage of the pandemic, and more scholars have studied this field. University of Rome Tor Vergata published 30 papers, and Huazhong Universality of Science Technology published 20 articles. There is a certain amount of cooperation between the above nations or institutions. This analysis revealed that a considerable number of scholars and institutions have been concerned with BCMP over the past 3 years and explored corresponding solutions.

During the pandemic, doctors may be more concerned about breast cancer diagnosis, treatment, and quality of life. Since the pandemic can lead to quarantines and lockdowns, the above can cause delays in breast cancer-related diagnosis and treatment (31–33).

We analyzed the development direction of BCMP through the thematic map, which was derived from keywords plus, thus providing a more comprehensive view of trends in the field. The mainstream themes include chemotherapy, neoadjuvant therapy, endocrine therapy, radiotherapy, breast-conserving surgery, and follow-up, which are likely to be further developed in the future as it has a high level of development and relevance.

Cluster 0: Screening for breast cancer patients in the pandemic

Pandemic-associated deficits in the number of breast examinations have been declining with time. The interrupted time series investigation demonstrated smaller frequencies of breast biopsy, diagnostic mammography, as well as screening mammography after the outbreak (34). The need for a modern, flexible national health system for making up for new challenges generated by further emerging pandemics has increased due to the COVID-19 pandemic (32, 35).

Furthermore, there may be a viable approach (36) that divides breast cancer screening into four types, namely, non-COVID-19 patients, confirmed COVID-19 in asymptomatic screening patients, suspected COVID-19 with symptomatic or confirmed breast cancer, and confirmed COVID-19 with symptomatic or confirmed breast cancer. Through the above approach, none of the medical staff or paramedics involved in the screening were infected (36).

Cluster 1: Breast cancer surgery in the pandemic

Breast cancer surgery can be safely carried out and integrated with a stringent protocol for reducing COVID-19 exposure and transmission, despite the pressures associated with the COVID-19 pandemic (37). During the pandemic, scholars from Turkey considered administering neoadjuvant systemic therapy in patients with luminal A-like, HER2-positive, small-size triple-negative, and node-negative tumors until the conditions were improved by surgical treatment (38). A study from South Korea suggested that the prognosis of patients with delayed surgeries did not seem to change compared with patients who proceeded with their surgeries (39). Some doctors from China suggested that for early-stage breast cancer, especially stage I, surgical treatment should be performed within 30 days if conditions permit (40). To minimize the delay of treatment during the pandemic, Vanni et al. suggested that multi-disciplinary treatment (MDT) should triage patients and schedule surgical procedures to optimize the allocation of the limited resources to urgent cases (41). Nevertheless, some scholars suggested that complex reconstruction surgeries should be delayed in areas where the pandemic is not well controlled (28, 42, 43) due to the extended hospital stay for complex reconstructive surgery and the possible complications.

Cluster 2: Recovery of breast cancer patients in the pandemic

A paper suggested that the treatment of ACEIs (angiotensin-converting enzyme ACE inhibitors) in Luminal A breast cancer might facilitate tumor progression (44). Jiang et al. suggested that breast tumor tissues can be further reduced at ACE2 expression level (angiotensin-converting enzyme 2) after SARS-CoV-2 infection, which further deteriorates immune infiltration and worsens the prognosis of luminal B breast cancer after SARS-CoV-2 infection (45).

Moreover, Mella-Abarca suggested that telerehabilitation may take on a great significance in people with breast cancer during the pandemic (46). It comprises a phone call, an individual video call using a mobile device (computer or smartphone), or a group video call, which is dependent on the convenience and availability of the individual's devices (e.g., implementation of the model, prevention of lymphedema, lymphedema, and pre-surgical evaluation for breast cancer) (46).

During the COVID-19 pandemic, Okechukwu et al. suggested that cancer patients should exercise at home on a tele-supervised home-based exercise oncology platform tailored by a physician and certified clinical exercise physiologist based on their preferences, contraindications, exercise tolerance, current clinical status, medical history, and cardiorespiratory fitness/functional capacity, instead of exercising within an indoor public fitness facility or outdoor spaces to curb the risk of COVID-19 infection and cardiovascular events (47).

Cluster 3: Emotion effect arising from the outbreak on breast cancer patients

During the pandemic, many breast cancer patients experienced many stressors related to more significant anxiety, depression, fear of cancer recurrence (FCR), and insomnia (48). Simultaneously, the quality of life of breast cancer patients was adversely affected (49). It is imperative to have conversations (phone or video) with breast cancer survivors about mental health and provide accessible services. Moreover, Papautsky et al. suggested that cancer patients should be trained with stress management strategies to acquire skills to manage their stress and prevent the adverse consequences of stress (50, 51).

Cluster 4: Diagnosis and treatment of breast cancer patients in the pandemic

Some physicians suggested classifying people at risk of breast cancer and trying to diagnose them as early as possible, while those at low risk should be observed and followed up at home (52). The use of chemotherapeutic agents with low side effects is recommended for patients with postoperative adjuvant chemotherapy (53, 54).

Endocrine treatments [tamoxifen, aromatase inhibitors, and luteinizing hormone-releasing hormone (LHRH) agonist] were continued during the COVID-19 pandemic since they do not affect the immune system (55). In terms of radiotherapy, Leonardi et al. reported that there was no significant difference in the time interval between treatments and radiotherapy for high-risk patients (56).

Cluster 5: Clinical vaccine management of breast cancer patients during the pandemic

Vaccination is an essential step in the fight against this devastating pandemic and is relatively safe for breast cancer patients. Can people using CDK 4/6 inhibitors be vaccinated, and what is the effect? The answer is that vaccination is available. Patients with breast cancer who underwent the treatment of CDK4/6 inhibitors developed SARS-CoV-2 NAbs in response to the first dose of COVID-19 vaccines, similar to the general population (57, 58). It is also worth noting that overdiagnosis should be avoided in breast cancer patients who develop lymphadenopathy (LAP) after vaccination. LAP related to COVID-19 vaccine tended to show increased cortical thickness without cortical irregularity, showing some suspicious features more often than others and persisting longer than anticipated (59). Accordingly, the recommendation for breast cancer patients about to undergo surgery is that the vaccination is given before or 1 week after surgery (60). The above findings from clinical studies suggest that vaccine-related adverse events are low and most of them have a short duration in cancer patients, that no serious adverse events directly related to the vaccine have been observed, and that the benefits of the vaccine may far outweigh the vaccine-related harms (61).

Limitations

Although bibliometric analysis and visualization methods were initially employed for the evaluation of the quality and quantity of research BCMP in this study, it also had some limitations. First, the bibliometric analysis only included a single database for search. Second, we only searched the titles, and there may be distribution articles missing. Third, burst keywords analysis cannot be performed due to the publication of the literature from 1 January 2020 to 1 April 2022. Despite the above limitations, our analysis can provide a reference for the research characteristics of BCMP.

Conclusion

Bibliometric techniques were employed for examining publications, research hotspots, and trends in breast cancer management during the pandemic. The findings of this study reveal that the United States of America, Italy, and China have made substantial contributions to the number of publications, institutions, magazines, and citations, which has facilitated the development of BCMP. The Buonomo-centered team, University of Rome Tor Vergata, and the *Breast Cancer Research and Treatment* journal were the most prolific in the field. Furthermore, hotspots and trends in research are screening, surgery, rehabilitation, emotion, diagnostic treatment, and vaccine management of breast cancer during the pandemic. As more insights are gained into COVID-19, breast cancer management is ever-changing and requires ongoing research and conclusion.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

Writing original draft: P-fL, J-tL, and TD. Validation: J-tL and TD. Investigation: P-mF and G-XL. Methodology: J-tL and TD. Software: P-fL. Supervision: X-CC. Project administration: P-fL. Data interpretation: Ping-ming Fan. Review and editing: G-XL, X-CC, and P-mF. All authors contributed to the article and approved the submitted version.

Acknowledgments

The author G-XL wishes to acknowledge the financial support of the "Xiamen Health High-Level Talent Training Program".

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.918349/full#supplementary-material

References

1. Seneviratne SL, Wijerathne W, Yasawardene P, Somawardana B. Covid-19 in cancer patients. *Trans Roy Soc Trop Med Hyg* (2022) 1–31. doi: 10.1093/trstmh/trac015

2. Taghizadeh-Hesary F, Porouhan P, Soroosh D, PeyroShabany B, Shahidsales S, Keykhosravi B, et al. Covid-19 in cancer and non-cancer patients. *Int J Cancer Manag* (2021) 14(4):6. doi: 10.5812/ijcm.110907

3. Costa DA, Nobre JGG, Fernandes JP, Batista MV, Simas A, Sales C, et al. Impact of the covid-19 pandemic on breast cancer management in Portugal: A cross-sectional survey-based study of medical oncologists. *Oncol Ther* (2022) 16:225–40. doi: 10.1007/s40487-022-00191-7

4. Gercek Oter EG, Ozkan S, Cinar H. The effectiveness of using telemedicine to follow-up breast cancer during the covid-19 pandemic: A scoping review. *Turk Onkol Derg* (2022) 37(1):93–9. doi: 10.5505/tjo.2021.3356

5. Hanna D, Halliday E, Needleman S. Preparing breast cancer patients for radiotherapy treatment in the covid-19 era. *Clin Oncol* (2022) 34(4):E179–E. doi: 10.1016/j.clon.2021.12.032

6. Knowlton CA. Breast cancer management during the covid-19 pandemic: The radiation oncology perspective. *Curr Breast Cancer Rep* (2022) 14(1):8–16. doi: 10.1007/s12609-022-00441-7

7. Loubani K, Schreuer N, Kizony R. Telerehabilitation for managing daily participation among breast cancer survivors during covid-19: A feasibility study. *J Clin Med* (2022) 11(4):17. doi: 10.3390/jcm11041022

8. Reese JB, El-Jawahri A, Sorice K, Cruz C, Bober SL, Daly MB, et al. Investigating the impact of the covid-19 pandemic on breast cancer clinicians' communication about sexual health. *Support Care Cancer* (2022)10:5801–10. doi: 10.1007/s00520-022-07003-8

9. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA-Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

10. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA-Cancer J Clin* (2018) 68(6):394–424. doi: 10.3322/caac.21492

11. Ahmad P, Asif JA, Alam MK, Slots J. A bibliometric analysis of periodontology 2000. *Periodontol 2000* (2020) 82(1):286–97. doi: 10.1111/prd.12328

12. Kumar S, Mohammad H, Vora H, Kar K. Reporting quality of randomized controlled trials of periodontal diseases in journal abstracts-a cross-sectional survey and bibliometric analysis. *J Evid-Based Dent Pract* (2018) 18(2):130–+. doi: 10.1016/j.jebdp.2017.08.005

13. Glynn RW, Scutaru C, Kerin MJ, Sweeney KJ. Breast cancer research output, 1945-2008: A bibliometric and density-equalizing analysis. *Breast Cancer Res* (2010) 12(6):9. doi: 10.1186/bcr2795

14. He LG, Fang H, Chen C, Wu YQ, Wang YY, Ge HW, et al. Metastatic castration-resistant prostate cancer: Academic insights and perspectives through bibliometric analysis. *Med (Baltimore)* (2020) 99(15):14. doi: 10.1097/md.0000000000019760

15. Miao Y, Liu R, Pu YP, Yin LH. Trends in esophageal and esophagogastric junction cancer research from 2007 to 2016: A bibliometric analysis. *Med (Baltimore)* (2017) 96(20):12. doi: 10.1097/md.0000000000006924

16. Powell A, Hughes DL, Wheat JR, Lewis WG. The 100 most influential manuscripts in gastric cancer: A bibliometric analysis. *Int J Surg* (2016) 28:83–90. doi: 10.1016/j.ijsu.2016.02.028

17. Chen CM. Searching for intellectual turning points: Progressive knowledge domain visualization. *Proc Natl Acad Sci U.S.A.* (2004) 101:5303–10. doi: 10.1073/pnas.0307513100

18. Chen CM. Citespace ii: Detecting and visualizing emerging trends and transient patterns in scientific literature. *J Am Soc Inf Sci Technol* (2006) 57(3):359–77. doi: 10.1002/asi.20317

19. Hao KJ, Jia X, Dai WT, Huo ZM, Zhang HQ, Liu JW, et al. Mapping intellectual structures and research hotspots of triple negative breast cancer: A bibliometric analysis. *Front Oncol* (2022) 11:689553. doi: 10.3389/fonc.2021.689553

20. Chen CM, Hu ZG, Liu SB, Tseng H. Emerging trends in regenerative medicine: A scientometric analysis in citespace. *Expert Opin Biol Ther* (2012) 12 (5):593–608. doi: 10.1517/14712598.2012.674507

21. van Eck NJ, Waltman L. Software survey: Vosviewer, a computer program for bibliometric mapping. *Scientometrics* (2010) 84(2):523–38. doi: 10.1007/s11192-009-0146-3

22. Rusydiana AS. Bibliometric analysis of journals, authors, and topics related to covid-19 and Islamic finance listed in the dimensions database by biblioshiny. *Sci Ed* (2021) 8(1):72–8. doi: 10.6087/kcse.232

23. Hirsch JE. Does the h index have predictive power? *Proc Natl Acad Sci U.S.A.* (2007) 104(49):19193–8. doi: 10.1073/pnas.0707962104

24. Li F, Li M, Guan P, Ma S, Cui L. Mapping publication trends and identifying hot spots of research on Internet health information seeking behavior: A quantitative and Co-word biclustering analysis. *J Med Internet Res* (2015) 17 (3):14. doi: 10.2196/jmir.3326

25. Tao L, Zhou SM, Tao ZB, Wen KC, Da W, Meng Y, et al. The publication trends and hot spots of scoliosis research from 2009 to 2018: A 10-year bibliometric analysis. *Ann Transl Med* (2020) 8(6):15. doi: 10.21037/atm.2020.02.67

26. Zhou SM, Tao ZB, Zhu Y, Tao L. Mapping theme trends and recognizing hot spots in postmenopausal osteoporosis research: A bibliometric analysis. *PeerJ* (2019) 7:21. doi: 10.7717/peerj.8145

27. Zhai KL, Ma WF, Huang T. Hot spots and trends in knee revision research since the 21st century: A bibliometric analysis. *Ann Transl Med* (2021) 9(5):19. doi: 10.21037/atm-20-3969

28. Curigliano G, Cardoso MJ, Poortmans P, Gentilini O, Pravettoni G, Mazzocco K, et al. Recommendations for triage, prioritization and treatment of breast cancer patients during the covid-19 pandemic. *Breast* (2020) 52:8–16. doi: 10.1016/j.breast.2020.04.006

29. Dowsett M, Ellis MJ, Dixon JM, Gluz O, Robertson J, Kates R, et al. Evidence-based guidelines for managing patients with primary er+ Her2- breast cancer deferred from surgery due to the covid-19 pandemic. *NPJ Breast Cancer* (2020) 6(1):10. doi: 10.1038/s41523-020-0168-9

30. Gathani T, Clayton G, MacInnes E, Horgan K. The covid-19 pandemic and impact on breast cancer diagnoses: What happened in England in the first half of 2020. *Br J Cancer* (2021) 124(4):710–2. doi: 10.1038/s41416-020-01182-z

31. Kan WC, Chou W, Chien TW, Yeh YT, Chou PH. The most-cited authors who published papers in jmir mhealth and uhealth using the authorship-weighted scheme: Bibliometric analysis. *JMIR mHealth uHealth* (2020) 8(5):13. doi: 10.2196/11567

32. Vanni G, Pellicciaro M, Materazzo M, Bruno V, Oldani C, Pistolese CA, et al. Lockdown of breast cancer screening for covid-19: Possible scenario. *In Vivo* (2020) 34(5):3047–53. doi: 10.21873/invivo.12139

33. Vanni G, Tazzioli G, Pellicciaro M, Materazzo M, Paolo O, Cattadori F, et al. Delay in breast cancer treatments during the first covid-19 lockdown. a multicentric analysis of 432 patients. *Anticancer Res* (2020) 40(12):7119–25. doi: 10.21873/anticanres.14741

34. Nyante SJ, Benefield TS, Kuzmiak CM, Earnhardt K, Pritchard M, Henderson LM. Population-level impact of coronavirus disease 2019 on breast cancer screening and diagnostic procedures. *Cancer* (2021) 127(12):2111–21. doi: 10.1002/cncr.33460

35. Bernardi D, Asti E, Bonavina G, Luporini A, Clemente C, Bonavina L. Delayed presentation of inflammatory breast carcinoma during the covid-19 pandemic. *Eur Surg* (2022) 5:212–16. doi: 10.1007/s10353-021-00726-8

36. Maio F, Tari DU, Granata V, Fusco R, Grassi R, Petrillo A, et al. Breast cancer screening during covid-19 emergency: Patients and department management in a local experience. *J Pers Med* (2021) 11(5):10. doi: 10.3390/jpm11050380

37. MacInnes EG, Piper J, Tait C, Waterworth A, Achuthan R, Hogan B, et al. Breast cancer surgery during the covid-19 pandemic peak in the uk: Operative outcomes. *Cureus* (2020) 12(7):9. doi: 10.7759/cureus.9280

38. Sezer A, Cicin I, Cakmak GK, Gurdal SO, Basaran G, Oyan B, et al. Turkish National consensus on breast cancer management during temporary state of emergency due to covid-19 outbreak. *Turk J Surg* (2020) 36(2):147–63. doi: 10.5578/turkjsurg.4815

39. Lee J, Jung JH, Kim WW, Park CS, Park HY. Patterns of delaying surgery for breast cancer during the covid-19 outbreak in daegu, south Korea. *Front Surg* (2020) 7:576196. doi: 10.3389/fsurg.2020.576196

40. Wang W, Guo BL, Cui CG, Sun T, Liu SN. Management of early-stage breast cancer patients during the coronavirus disease 2019 (Covid-19) pandemic: The experience in China from a surgical standpoint. *J Cancer* (2021) 12(8):2190–8. doi: 10.7150/jca.50501

41. Vanni G, Materazzo M, Pellicciaro M, Ingallinella S, Rho M, Santori F, et al. Breast cancer and covid-19: The effect of fear on patients' decision-making process. *In Vivo* (2020) 34:1651–9. doi: 10.21873/invivo.11957

42. Dietz JR, Moran MS, Isakoff SJ, Kurtzman SH, Willey SC, Burstein HJ, et al. Recommendations for prioritization, treatment, and triage of breast cancer patients during the covid-19 pandemic. the covid-19 pandemic breast cancer consortium. *Breast Cancer Res Treat* (2020) 181(3):487–97. doi: 10.1007/s10549-020-05644-z

43. Courtney A, O'Connell R, Rattay T, Kim B, Cutress RI, Kirwan CC, et al. The b-Map-C study: Breast cancer management pathways during the covid-19 pandemic. study protocol. *Int J Surg Protocol* (2020) 24:1–5. doi: 10.1016/j.isjp.2020.07.003

44. Bhari VK, Kumar D, Kumar S, Mishra R. Sars-Cov-2 cell receptor gene Ace2 -mediated immunomodulation in breast cancer subtypes. *Biochem Biophys Rep* (2020) 24:8. doi: 10.1016/j.bbrep.2020.100844

45. Jiang YF, Chen L, Shen JS, Mei XF, Yao JL, Chen T, et al. The potential role of abnormal angiotensin-converting enzyme 2 expression correlated with immune infiltration after sars-Cov-2 infection in the prognosis of breast cancer. *Aging-US* (2021) 13(17):20886–95. doi: 10.18632/aging.203418

46. Mella-Abarca W, Barraza-Sanchez V, Ramirez-Parada K. Telerehabilitation for people with breast cancer through the covid-19 pandemic in Chile. *eCancerMedicalScience* (2020) 14:8. doi: 10.3332/ecancer.2020.1085

47. Okechukwu CE, Okechukwu CE, Deb AA, Agag A, Naushad N, Abbas S. Precautionary measures before tailoring and commencing a tele-supervised home-based exercise oncology program for older patients with cancer and post-treatment cancer survivors in the covid-19 era. *J Geriatr Oncol* (2022) 13(2):241–4. doi: 10.1016/j.jgo.2021.08.001

48. Massicotte V, Ivers H, Savard J. Covid-19 pandemic stressors and psychological symptoms in breast cancer patients. *Curr Oncol* (2021) 28(1):294–300. doi: 10.3390/curroncol28010034

49. Zhao FY, Henderson TO, Cipriano TM, Copley BL, Liu M, Burra R, et al. The impact of coronavirus disease 2019 on the quality of life and treatment disruption of patients with breast cancer in a multiethnic cohort. *Cancer* (2021) 127 (21):4072–80. doi: 10.1002/cncr.33798

50. Papautsky EL, Hamlish T. Emotional response of us breast cancer survivors during the covid-19 pandemic. *Cancer Invest* (2021) 39(1):3–8. doi: 10.1080/07357907.2020.1841220

51. Charsouei S, Esfahlani MZ, Dorosti A, Zamiri RE. Effects of covid-19 pandemic on perceived stress, quality of life, and coping strategies of women with breast cancer with spinal metastasis under chemotherapy. *Int J Womens Health Reprod Sci* (2021) 9(1):55–60. doi: 10.15296/ijwhr.2021.10

52. Vibert F, Martel C, Ionescu RA, Mathelin C, Ame S. A new modality for breast cancer diagnosis during the covid-19 pandemic: A case report. *Eur J Breast Health* (2022) 18(1):91–3. doi: 10.4274/ejbh.galenos.2021.2021-4-1

53. Gowda SM, Kabeer KK, Jafferbhoy S, Marla S, Soumian S, Misra V, et al. Breast cancer management guidelines during covid-19 pandemic. *Indian J Surg* (2020) 82(3):251–8. doi: 10.1007/s12262-020-02466-7

54. Horiguchi J, Nakashoji A, Kawahara N, Matsui A, Kinoshita T. Chemotherapy resumption in breast cancer patient after covid-19. *Surg Case Rep* (2021) 7(1):5. doi: 10.1186/s40792-021-01253-0

55. Yahyaoui Y, Ghodhbani Z, Hamdi A, Letaief F, Zenzri Y, Ben Said A, et al. Suggestion of tunisia's medical oncologist in the management of breast cancer during covid-19 pandemic. *J Oncol Pharm Pract* (2020) 26(7):1732–4. doi: 10.1177/1078155220948943

56. Leonardi MC, Montagna E, Galimberti VE, Zaffaroni M, Rojas DP, Dicuonzo S, et al. Breast adjuvant radiotherapy amid the covid-19 crisis in a hub cancer center, Lombardy, Italy. *Breast Care* (2021) 16(5):500–6. doi: 10.1159/000513227

57. Zagouri F, Terpos E, Fiste O, Liontos M, Briasoulis A, Katsiana I, et al. Sars-Cov-2 neutralizing antibodies after first vaccination dose in breast cancer patients receiving Cdk4/6 inhibitors. *Breast* (2021) 60:58–61. doi: 10.1016/j.breast.2021.08.017

58. Barba M, Krasniqi E, Pizzuti L, Mazzotta M, Marinelli D, Giuliano G, et al. Covid-19 risk in breast cancer patients receiving Cdk4/6 inhibitors: Literature data and a monocentric experience. *Breast J* (2021) 27(4):359–62. doi: 10.1111/tbj.14204

59. Lim J, Lee SA, Khil EK, Byeon SJ, Kang HJ, Choi JA. Covid-19 vaccine-related axillary lymphadenopathy in breast cancer patients: Case series with a review of literature. *Semin Oncol* (2021) 48(4-6):283–91. doi: 10.1053/j.seminoncol.2021.10.002

60. Ko G, Hota S, Cil TD. Covid-19 vaccination and breast cancer surgery timing. *Breast Cancer Res Treat* (2021) 188(3):825–6. doi: 10.1007/s10549-021-06293-6

61. Forster M, Wuerstlein R, Koenig A, Amann N, Beyer S, Kaltofen T, et al. Covid-19 vaccination in patients with breast cancer and gynecological malignancies: A German perspective. *Breast* (2021) 60:214–22. doi: 10.1016/j.breast.2021.10.012

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 15 August 2022
DOI 10.3389/fonc.2022.968306

OPEN ACCESS

EDITED BY
Maria Rosaria De Miglio,
University of Sassari, Italy

REVIEWED BY
Arunasalam Dharmarajan,
Sri Ramachandra Institute of Higher Education and Research, India
Badrul Hisham Yahaya,
Universiti Sains Malaysia (USM), Malaysia

*CORRESPONDENCE
Linhai Zhu
yzzlhai@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 13 June 2022
ACCEPTED 26 July 2022
PUBLISHED 15 August 2022

CITATION
Xu H, Zhang F, Gao X, Zhou Q and Zhu L (2022) Fate decisions of breast cancer stem cells in cancer progression.
Front. Oncol. 12:968306.
doi: 10.3389/fonc.2022.968306

Fate decisions of breast cancer stem cells in cancer progression

Hui Xu†, Fengxia Zhang†, Xiaokang Gao, Qiwang Zhou and Linhai Zhu*

Department of General Surgery, The Affiliated Hospital of Yangzhou University, Yangzhou, China

Breast cancer has a marked recurrence and metastatic trait and is one of the most prevalent malignancies affecting women's health worldwide. Tumor initiation and progression begin after the cell goes from a quiescent to an activated state and requires different mechanisms to act in concert to regulate t a specific set of spectral genes for expression. Cancer stem cells (CSCs) have been proven to initiate and drive tumorigenesis due to their capability of self-renew and differentiate. In addition, CSCs are believed to be capable of causing resistance to anti-tumor drugs, recurrence and metastasis. Therefore, exploring the origin, regulatory mechanisms and ultimate fate decision of CSCs in breast cancer outcomes has far-reaching clinical implications for the development of breast cancer stem cell (BCSC)-targeted therapeutic strategies. In this review, we will highlight the contribution of BCSCs to breast cancer and explore the internal and external factors that regulate the fate of BCSCs.

KEYWORDS

breast cancer stem cells, breast cancer, heterogeneity, tumor microenvironment, transcription factors, non-coding RNAs

Introduction

Breast cancer is the second major risk of cancer death in women (1). At present, surgical resection is the preferred treatment for breast cancer, including breast-conserving surgery (BCS) and mastectomy, and a series of comprehensive treatment measures such as chemotherapy, radiao-therapy, hormone therapy and other novel therapies are combined according to clinical-pathology. Despite increasingly accessible systems for early diagnosis and therapy of breast cancer, it remains the most prevalent of female malignancies in terms of mortality. Recurrence and metastasis are the main reason for the increase in mortality (2–4). Most breast cancer patients express receptors for estrogen (ER) and progesterone (PR) and therefore respond to hormone therapy or aromatase inhibitors. However, triple negative breast cancer (TNBC) lacks the expression of ER, PR and human epidermal growth factor receptor-2 (HER-2) (5). Breast cancer contains a heterogeneous cell population and is divided into for major molecular

subtypes according to genetic expression, including luminal A, luminal B, HER2-enriched, and triple-negative (6, 7). Certain subtypes are prone to drug resistance, resulting in limited treatment efficacy, which poses a significant challenge to clinical cure and survival of breast cancer patients.

BCSCs are a class of cells with the ability to continuously self-renew, proliferate indefinitely and differentiate in multiple directions, and possess multiple drug-resistant molecules that are the main cause of drug resistance in breast cancer (8–10). There are two hypotheses on the origin of BCSCs: one is that BCSCs originate from adult stem cells and can acquire malignant behaviors by changing their genetic characteristics; the other is that BCSCs are transformed by early progenitor cells that have acquired the ability to self-renew (11, 12). The concept of BCSCs has been further developed to be involved in mediating tumor heterogeneity, with the ability to clonally regenerate tumors after seemingly successful treatment, and is of profound importance in understanding and treating hierarchically organized breast cancer (13). Therefore, further understanding of the fate decisions of BCSCs, identifying significant roles in tumor recurrence, metastasis and drug resistance, and developing therapeutic strategies to target BCSCs are of great clinical significance for the treatment of breast cancer. Hence, we outline the hierarchy of BCSCs in the origin of breast cancer and their role in tumor heterogeneity, recurrence, metastasis and drug resistance, in conjunction with a discussion of the potential of BCSCs as therapeutic targets to provide clinicians with new strategies to improve breast cancer treatment.

Unraveling the routes of mammary stem cell differentiation

A highly dynamic organ that produces and secretes milk to nourish offspring, the mammary gland undergoes multiple phases of remodeling throughout a female's life and consists of two main parts: the parenchyma and surrounding stroma. The parenchyma contains mainly epithelial cells, glandular cells and myoepithelial cells: the epithelial cells are located in the inner layer of the milk ducts; the glandular cells form the alveoli, whose main function during lactation is to secrete milk; and the myoepithelial cells form the basement membrane, which usually surrounds or separates the epithelial cells from the glandular cells (14, 15). The proliferation and differentiation of the mammary gland is regulated by hormones and growth factors, for example estradiol, progesterone and prolactin. According to the characteristics of mammary gland development, it can be roughly divided into six developmental stages: embryonic stage, birth to early sexual maturity, sexual maturity, pregnancy, lactation and involution, as well as each estrous/menstrual cycle, both local and systemic stimuli can set off the mammary cell expansions and/or differentiation (16).

The mammary gland shows such obvious periodicity because a hierarchical array of mammary stem cells (MaSCs) and progenitor cells (PCs) are located in the organ, which maintain the homeostasis under physiological conditions (17). The differentiation of MaSCs is a two-step journey, consisting of cell lineage determination (from MaSCs to PCs of a specific lineage) and maturation (from PCs to particular cell types). These cells can yield all the mature cell types in the mammary gland, including ductal, alveolar and myoepithelial, and the primary outgrowths contain daughter cells that have the same regenerative capacity as the original stem cells (18). Thus, these cells have the dual hallmarks of stem cells, multidirectional differentiation and the ability to self-renew. Stem cell fate decisions, which begin after the cells differentiate from a quiescent to an activated state, require different mechanisms to coordinate and regulate the expression of a specific set of lineage genes. The presence of stem cells is necessary for the regeneration of the mammary gland and is important for studying the mechanisms of organogenesis and cell differentiation, but abnormal differentiation and proliferation of stem cells can lead to occurrence of tumors.

Stem cells as the cellular origin of breast cancer

Unmasking the origins of breast cancer to be still a challenging and creative topic in the field of oncology research. The cellular origin of cancer continues to be an important scientific question. Two major models have been developed to describe the cellular origin of cancer. In the somatic mutation model, the stepwise accumulation of a series of independent mutations in differentiated cells promote the capability to gradually reprogram and obtain malignant genotypes (19, 20), while the second hypothesis involves mutations in stem or progenitor cells (21). It is inevitable that these two models will co-exist. About 5-10% of breast cancers are inherited susceptibility due to germline mutations, such as BRCA1 and BRCA2 (22–24). Using single-cell assays, in BRCA1 mutation breast cancer, basal-like breast cancer (ER^-) and luminal breast cancer (ER^{high}) respectively derive from luminal progenitors and mature luminal cells respectively (25). These discoveries indicate that breast cancers may be initiated by mutations in differentiated cells. Interestingly, it is evident that cancer cells and stem cells share many characteristics, including high proliferative capacity, longevity, pliancy and the activity of molecular pathways that regulate stem cells (26). Corinne A Boulanger1 and Gilbert H Smith's inventive research in breast cancer was the first to demonstrate that the mammary epithelial stem cells were indeed responsible for the evolution of carcinogenesis in mature mammary gland and formed tumor stem cell populations (27). Increased expression of stemness-

associated genes, such as pseudokinase Tribble 3 (TRIB3) (28), NOTCH1 (29) and SOX9 (30), is positively correlated with the development of breast cancer. Patients with TNBC tend to have a comparatively worse outcome than other subtypes, due to their inherently invasive trait and the lack of molecular targets for treatment (31). TNBC is often, but not always, a basal-like subtype and expresses basal like markers (K5, K14, ITGA6, P-cadherin and Id4) with the character of stemness (32, 33). Definitely, high expression of stem cell-related gene traits in the BCSC subpopulation were a potential predictor of worse prognosis (34, 35). Interestingly, it is hypothesized that the apparent heterogeneity within breast cancer tumors reflects the different mammary epithelial cells as the cellular origin and drivers of malignant transformation (36, 37). A comparison between specific molecular features of normal breast epithelial subpopulations and different breast cancer subtypes revealed that the tumor subtypes appeared to have similar differentiation characteristics to normal breast cells. The basal-like subtype expresses intracavitary progenitor cell markers. This appears to correlate with the basal MaSC molecular subtype and therefore intuitively indicates that MaSC are a potential cellular source of basal-like breast cancer. Correspondingly, the HER2, luminal A, and luminal B subtypes express luminal lineage markers (18) (Figure 1). Collectively, these discoveries provide several insights into the origin of breast cancer cells: 1. Is oncogene-induced transdifferentiation of mammary gland cell sufficient to explain the plasticity and heterogeneity observed in breast cancer? 2. Does the continuous differentiation of normal stem cells to replenish the pool of progenitor cells during the maintenance of mammary gland homeostasis contribute to tumorigenesis?

Stem cell hierarchies in breast cancer

During the last few decades, numerous studies have demonstrated that both tissue stem cells and CSCs can survive for long periods of time and have a great proliferative capacity, which means not only that they can accumulate many mutations, but also that they share the same capacity for reversibly entering a quiescent cell-cell state, multidirectional differentiation, an overlapping immunophenotype and gene regulatory networks (26). Through xenotransplant experiments, Al-Hajj together with colleagues presented directly the first investigated evidence for the presence of so-called BCSCs ($CD44^{+}CD24^{-/low}Lineage^{-}$), which are located at the top of breast cancer with a hierarchical structure (38). Subsequently, Christophe Ginestier and colleagues raveled out that aldehyde dehydrogenase (ALDH) can act as a potential marker for BCSCs, these cells with the widest lineage differentiation potential and the greatest capacity for growth (39). Surprisingly, BCSCs ($CD44^{+}CD24^{-}$) are predominantly quiescent and localized at the front of the tumor invasion, whereas epithelial-like BCSCs expressing ALDH are more proliferative and more centrally located (40). High-throughput sequencing technologies developed in recent decades have

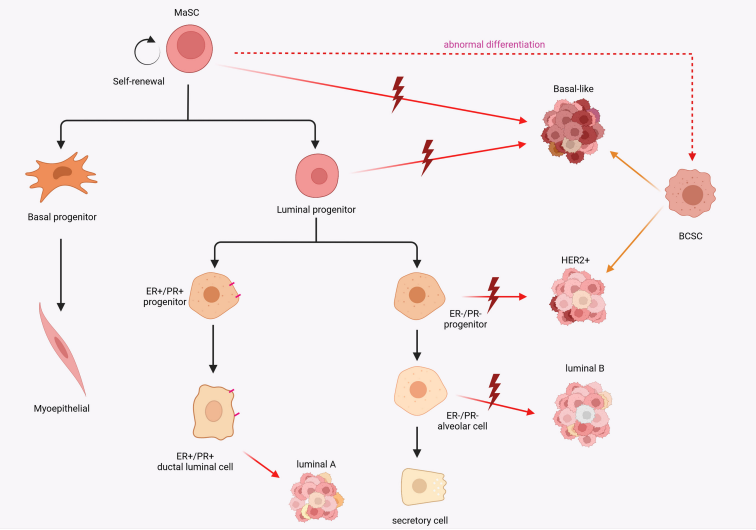


FIGURE 1
Schematic diagram of the potential relevance of breast epithelial cell hierarchy and breast cancer stem cell origin to breast cancer subtypes. MaSCs expose to mutations that cause abnormal differentiation and transformation into cancer stem cells. A comparison between specific molecular features of normal breast epithelial subpopulations and different breast cancer subtypes revealed that the tumor subtypes appeared to have similar differentiation characteristics to normal breast cells.

enabled us to accumulate a wealth of relevant data on BCSC hierarchies and fate decisions. At the single-cell level, BCSCs showed high tumorigenesis and expressed stemness and EMT-related genes, in particular ZEB2, SOX2, ID1 and TWIST1 (41). Along with the ground, acquiring the properties of EMT allows the cells to be reprogrammed to a more stem-like state (42). Importantly, BCSCs, located at the top of the cancer hierarchy, are considered to be in line with their healthy MaSCs, and they can exacerbate breast cancer. More studies have confirmed that BCSCs expressing relevant cell surface markers have biological properties similar to MaSCs, including ALDH1$^+$ (39), CK5$^+$ (43), CD49f$^+$ (44), ITGA6$^+$ (45). These discoveries provide insightful evidence not only of the clinical relevance of BCSCs in breast cancer, but also indicate that breast cancer should be uniquely therapeutic according to their gene profile (Figure 2).

Noticeably, not all cancers are considered to be stringently hierarchical in organization. It is generally believed that the CSC lies at the top of the hierarchy, whereas in reality the CSC hypothesis is more complex than a simple linear model. Several discoveries have shown that some non-CSCs can lead to dedifferentiation through genetic mutation, and exhibit plasticity by reversibly transitioning between a stem and non-stem state (46). Importantly, the hierarchical heterogeneity is beyond genetic mutations and covers non-genetic characteristics with regard to epigenetic programs, immune characteristics, inflammatory states and microenvironmental composition. Lineage plasticity is important for the development of aggressive BLBC, transcription factor SOX9 can regulate cell phenotypic plasticity and breast cancer progression (30). Similarly, EMT-mediated phenotypic plasticity has important clinical implications for breast cancer progression and drug resistance (47). Similar to normal breast cells, BCSCs are able to respond to external or internal stimuli, debugging their phenotype and behavior *via* EMT, reversible quiescence and senescence or metabolic plasticity to counteract the stress of treatment. All of these characteristics contribute to the resistance to treatment of CSCs. For example, BCSCs have built-in mechanisms to promote phospholipid metabolism and the generation of free fatty acids, which activate the relevant signals and maintain stemness, thus contributing to chemo-resistance as cisplatin, doxorubicin, or tamoxifen. In this case, phospholipase A2 inhibitors, such as Giripladib, are required in combination to effectively eliminate BCSCs and inhibit tumorigenesis (48). Alexander Swarbrick and his colleagues demonstrated that stromal cues form CAFs, including FGF5 and fibrillar collagen, are capable of inducing and maintain a stem-like phenotype in TNBC cells by providing a supportive niche (49). Corporately, these evidences suggest that BCSCs are not in a specific-widespread phenotype but stay at a certain plasticity. In conclusion, the model of tumor origin and evolution is not limited to a single hierarchical level, but also needs to take fully into account the polyclonal heterogeneity that characterizes the successive interactions between different cell populations. Certainly, they are not mutually exclusive and there may be transitions between BCSC and BCSC-like states, and the concept of BCSC populations needs to be treated more dialectically, that is, there may be multiple BCSC populations of different subtypes.

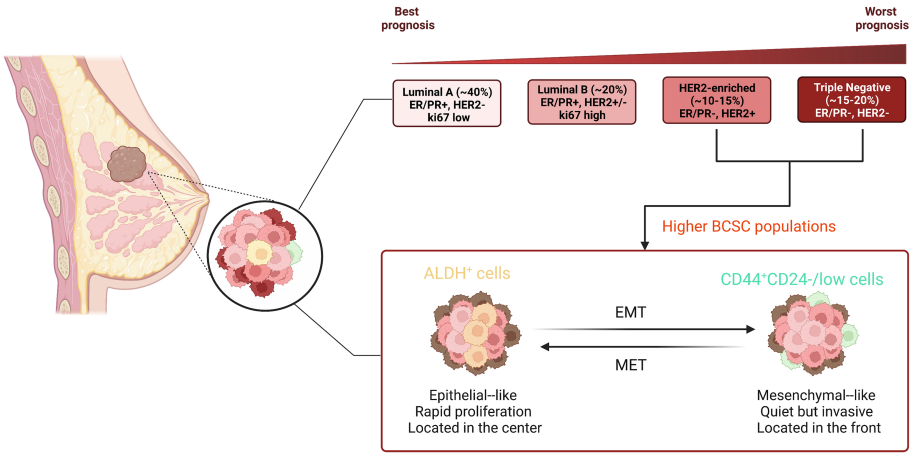


FIGURE 2
Different subtypes of breast cancer and distinct state of BCSCs. Breast cancer contains a heterogeneous cell population and is divided into four major molecular subtypes according to genetic expression, including luminal A, luminal B, HER2-enriched, and triple-negative. The typical molecular expression in each subtype is shown in the figure. There are two major distinct phenotypes of BCSCs: CD44$^+$CD24$^{-/low}$ and ALDH$^+$. BCSCs (CD44$^+$CD24$^-$) are mesenchymal-like and predominantly quiescent and localized at the front of the tumor invasion, whereas epithelial-like BCSCs expressing ALDH are more proliferative and more centrally located.

BCSCs as participant of breast cancer heterogeneity

Breast cancers show marked heterogeneity due to genomic, transcriptomic and microenvironmental differences, resulting in different phenotypes and variability in biological behavior (50). Due to inter-tumor heterogeneity, they can be classified into different types based on their morphology, molecular expression or genomic copy number patterns. There are currently two models that address the issue of heterogeneous origins: the clonal evolution model and the CSC model (37). These two hypotheses are not independent, but rather a coexisting, dynamic process that provides a theoretical basis for explaining inter-tumor heterogeneity and intrinsic differences in the regenerative capacity of breast cancer (51). The clonal evolutionary model assumes that any undifferentiated and differentiated cell is capable of accumulating mutations that lead to the creation of clonal populations of cells within a tumor, while individual tumor cells in a monoclonal clone share a degree of identical genetic variation, and different subpopulations of tumor cells have the ability to mutate individually during tumor evolution, thereby mediating the creation of tumor heterogeneity. The CSC model holds that a tumor actually consists of a cluster of stem cells, as well as cells that are unevenly differentiated, and can explain breast cancer tumorigenesis (52, 53). Typically, CSCs in tumors are genetically unstable, with multiple isoforms, and CSCs that survive adaptively in a clonal pool following altered microenvironmental niches and targeted therapeutic approaches, mediating the intar- and/or inter-tumor hierarchy and promoting malignant progression. In conclusion, further refinement and emphasis on the evolutionary and adaptive CSC dynamic concept is complementary to explain the possible causes of tumorigenesis, recurrence and metastasis. Therefore, eliminating the most diverse types of tumor cells, including BCSCs, is the most fundamental strategy for curing breast cancer.

BCSC identity markers

The development of BCSC-specific biomarkers for breast cancer has expanded the understanding of heterogeneity and has been further validated in both *in vivo* and *in vitro* breast cancer models. These breast cancer stem cells represent only a small fraction of the cells within the tumor and are extracted by flow cytometry technique capable of identifying certain patterns of surface markers (54, 55). A growing number of studies have revealed and characterized BCSC markers, and these markers have been shown to identify different stem cell populations well. As mentioned above, $CD44^+CD24^-$ and $ALDH^+$ are common molecular markers for BCSCs. Equally important, due to the highly heterogeneous character of breast cancer, in which more different phenotypes of BCSCs may exist, the discovery and identification of their biological functions could achieve a substantially more constructive reaction to anti-cancer therapy in the design of new drugs targeting BCSCs (Table 1).

Regulatory mechanisms of BCSCs

The establishment of the BCSC theory provides the theoretical basis for explaining the hierarchy and heterogeneity of breast cancer. These fickle BCSC populations initiate and fuel tumor growth and are intimately associated with intrinsic

TABLE 1 Principal BCSC identity markers.

Phenotypes	Sample sources	IsolationIdentification	Ref.
$CD44^+/CD24^{-/low}$	Human primary breast tumor Pleural Effusion Injections	FACS	(38)
$ALDH^+$	Human breast tumors	FACS ALDH1 IF	(39)
$CD133^+$	$BRCA1^{\Delta exon11}p53^{+/-}$ mouse mammary tumors	FACS CD133 IF	(56)
$CD24^+CD29^+$ and $CD24^+CD49f^+$	BRCA1-mutant mouse mammary tumors	FACS Tumorsphere	(57)
$CD44^+CD24^-ESA^+$	Human SUM159, SUM1315 and MAD-MB-231 cell lines	FACS BrdU label	(58)
$CD49f^+EpCAM^+$	BRCA1-mutant human mammary tissues	FACS Microarray hybridizations	(59)
$GD2^+$	Human breast tumor tissue and SUM-159, HS578T, MDA-MB-231 and MDA-MB-468 cell line	FACS Microarray analysis	(60)
$CD90^{hi}$	Human MAD-MB-231 cell line	FACS CD90 IF	(61)
$CD133^{high}CXCR4^{high}$ -$ALDH1^{high}$	Human breast tumor tissue with chemo-treated patients	Sphere-formation	(62)

treatment-resistant. BCSCs possess significant stemness and plasticity, and their fate decisions that extensive and complex regulatory mechanisms are required to coordinate and regulate the expression of specific lineages of genes, starting after the cell differentiates from a quiescent to an activated state. Here we focus on the contribution of transcriptional regulation, signaling pathway, epigenetic regulation, and post-transcriptional modifications that occur during this process.

Transcription factors

Transcription factors (TFs), also known as trans-acting factors, are functional protein molecules that specifically bind to DNA and regulate gene transcription. Most TFs bind to DNA before forming dimers or multimers through protein-protein interactions. In addition to TFs that bind DNA directly, there are regulatory proteins that do not bind DNA directly, but rather bind DNA indirectly through protein-protein interactions, regulating gene transcription and thus forming expression regulatory complexes. The gene expression that defines the phenotype is highly coordinated. As a result, regulatory programs meticulously curated by crucial TFs have been posited to have a central function in the determination of cell fate.

Evidently, intratumoral hypoxia is a common manifestation in advanced cancers. In hypoxic breast cancer cells, HIFs activate the transcription of target genes that play important roles in tumor progression, metabolic reprogramming, motility and chemoresistance (63). Numerous studies have shown that the response of BCSCs to hypoxia requires HIFs to regulate and maintain the direct or indirect transcriptional regulation of BCSC stemness-related factors including NANOG, SOX2, and KLF4 (64, 65). In addition, HIF-1α maintains the onset of hypoxia-induced EMT and regulates the plasticity of BCSC (66, 67). Recently, researches showed that HIF-dependent ALKBH5 and S100A10 expression mediates the enrichment of BCSCs in the hypoxic tumor microenvironment (68, 69). Similarly, HIF-1 can directly activate calreticulin (CALR) transcription and facilitate breast cancer progression by promoting the BCSC phenotype in hypoxic (70). Collectively, these discoveries exhibit that hypoxia increases the percentage of BCSCs and governs their phenotypic transformation in a HIF-dependent manner.

Metastasis is the cause of up to 90% of cancer-related deaths, yet it continues to be the least known integral part of cancer pathogenesis. The most common sites of metastasis from breast cancer are bone, lung, brain and liver. Truncated glioma-associated oncogene homolog 1 (TGLI1) was found to transcriptionally activate the expression of CD44 and OCT4, contributing to BCSC renewal and thus promoting brain metastasis (71). Mechanistically, malignant progression in breast cancer is accompanied by an increase in the proportion of these BCSCs within the tumor and activation of the EMT (72, 73). EMT is a complex transdifferentiation program characterized by the loss of epithelial-specific features accompanied by the acquisition of mesenchymal phenotypes that fuels non-transformed cells and tumor cells to acquire stemness (74, 75). The loss of epithelial-specific features means that the tumor is more aggressive and has a poorer prognosis. Intrinsically, EMT-associated TFs (EMT-TFs) were crucial regulatory mechanism for tumor progression and metastasis including, Snail 2, Twist 1, Slug, SOX2/9 and Zeb1/2.

Non-coding RNAs

In contrast to well-known molecular signaling pathways, the involvement of non-coding RNAs (ncRNAs) in CSC lineage commitment has only just been discovered. Based on their biological functions, ncRNAs are divided into two major categories: housekeeping ncRNAs and regulatory ncRNAs. Regulatory ncRNAs can be divided into short chain ncRNAs and long chain ncRNAs according to the sequence length. ncRNAs with short chains include microRNA (miRNA), small interfering RNA (siRNA), piRNA and transcription initiation RNA (tiRNA) have the characteristics of small molecule and high sequence conservation. Whole-genome sequencing revealed that ncRNAs comprise 98% of the human gene transcriptome and consist mainly of miRNAs and LncRNAs that do not have protein-coding functions (76). A variety of miRNAs and LncRNAs are responsible for the modulation of BCSCs.

microRNAs

miRNAs are commonly expressed in organisms that are approximately 18-25 nucleotides in length and can complement the 3'-UTR of mRNA, leading to the degradation and/or translational repression of target genes (77). miRNAs act as regulators in stem cell proliferation, differentiation, apoptosis, and metabolism (78, 79). In this way, miRNAs act as a switch of gene networks, either as an oncogene or as a tumor suppressor gene, and these miRNAs have quickly become an important class of regulatory genes controlling developmental and disease processes. In contrast to transcription factors and molecular signaling pathways, miRNAs involved in stem cell lineage determination have only just started to be studied. An increasing number of miRNAs have been found to be implicated in BCSCs to regulate fate decisions.

Interestingly, miRNAs with micro size but macro function are known to have profound effects on maintaining and regulating the behavior of BCSCs by specifically targeting relevant TFs and oncogenic signaling pathways and play an important role in breast cancer initiation and prognosis. miRNAs serve as oncogenes as well as tumor suppressors. Based on the current

findings, we will focus on describing the regulatory role and underlying mechanisms of miRNAs management of BSCS self-renewal, differentiation, metastasis, EMT, drug resistance and recurrence as potential links to breast cancer pathogenesis. Analysis of 11 surgically resected breast cancer patient samples revealed differentially expressed miRNAs in human BCSCs versus nontumorigenic cells (NTG cells) (80). Three clusters of miRNAs, including miRNA-200c-141, miR-200b-200a-429 and miR-183-96-182 cluster, were consistently downregulated in human BCSCs (80). The miR-200 family maintains the stemness of BCSCs and is able to target the EMT-associated transcription factor ZEB1, thereby up-regulating E-cadherin, the expression of which is reduced and its inhibitory effect on EMT is diminished (80–83). Furthermore, other miRNAs, including let-7, miR-27b and miR-185-3p, were differentially expressed in BCSCs and NTG cells (84–86). It was recently shown that in BCSCs E2F1 binds to the Nanog gene to promote its transcription and that miR-185-3p can target E2F1 leading to a reduction in its expression, thereby inhibiting the stemness of BCSCs (86). Similarly, miR-378a-3p and miR-378d can activate the WNT and NOTCH pathways through targeted inhibition of DKK3 and NUMB, leading to doxorubicin (DOX) and paclitaxel (PTX) resistance (87). Taken together, these discoveries show that miRNAs are instrumental in determining the fate of BCSCs by targeting key coding TFs and related signaling pathways.

Long noncoding RNAs

LncRNAs are ncRNAs with transcripts longer than 200 nucleotides and little or no protein-coding function. They regulate gene expression and are involved in biological processes such as apoptosis, metastasis, stemness maintenance, proliferation, differentiation, metabolism and drug resistance. LncRNAs can repress or activate gene expression through a variety of mechanisms and exhibit specific expression patterns in different cell and tissue types, respond to different stimuli, and regulate cell fate (88). In the last decade, researchers have shown great interest in the role of LncRNAs in CSC lineage Commitment and differentiation.

LncRNAs influence cell growth, apoptosis and tumor metastasis by participating in epigenetic, transcriptional or post-transcriptional gene regulation. Brown and colleagues summarized the LncRNAs in BCSCs and revealed that a series of BCSC-associated LncRNAs were enriched in TNBC (89). Notably, LncRNAs show differential expression in BCSCs versus non-BCSCs. LncRNA lnc030, which is highly expressed in BCSCs, is able to stabilize squalene epoxidase (SQLE) mRNA cooperating with poly(rC) binding protein 2 (PCBP2) and promote cholesterol synthesis, thereby activating PI3K/Akt to amplify the stemness properties of BCSCs (90). Likewise, high expression of LncRNA-ROPM can increase the stability of PLA2G16 mRNA, thereby promoting phospholipid metabolism and activating PI3K/AKT signaling (48). In addition, other LncRNAs that are upregulated in BCSCs, such as LncRNA-ROR, LncRNA-HOTAIR, LncRNA-HAL, LncRNA-Hh (91) and LncRNA-PVT1 (92), are able to induce EMT, consequently increasing the percentage of BCSC population and stemness. As LncRNAs research progresses, more and more LncRNAs will be demonstrated in the regulation of BCSCs. LncRNAs is a novel regulator of BCSCs by regulating mRNAs, miRNAs and other LncRNAs and will improve the understanding of new molecular regulation of BCSCs.

Tumor microenvironment

The tumor microenvironment (TME) plays a pivotal function in several steps of tumorigenesis and progression, including drug resistance, immune escape and distant metastasis. The microenvironment regulates the biological behavior of BCSCs through direct contact or ECM and paracrine factors (93). The microenvironment provides fuel and a proper niche for BCSCs, highly regulates their fate, protects them from genotoxicity and improves their tolerance to treatment. Reciprocally, BCSCs are able to influence the TME while adapting to changes in the TME. The TME mainly consists of surrounding normal tissue cells, tumor stroma and microvessels. For example, tumor cells can release immune inhibitory cytokines to evade detection by immune cells in TEM, resulting in immune escape (94). Concurrently, the TME provides the driving force for BCSC plasticity, inducing angiogenesis and recruitment of immune and stromal cells, which in turn accelerates tumor invasion and metastasis.

Stromal cells, such as cancer-associated fibroblasts (CAFs), are verified that affect BCSC activity through the cell-cell interactions, the secretion growth factors, cytokines, chemokines, and the remodeling of the ECM (95). These secreted factors are involved in a variety of regulatory roles for cells in TME and tumor cells. In particular, CAFs, a major component of the stroma, have been shown to support CSC function by secreting cytokines such as IL-6, IL-8 and IL-1β, activating signaling pathways, and promoting BCSC stemness and plasticity (96). The origin of CAFs is now thought to be multiple, including transference of resident fibroblasts (97), transdifferentiation of perivascular cells (97), differentiation of mesenchymal stem cells (MSCs) and EMT. CAFs haven been found to be able to secrete periostin, which in turn recruits Wnt ligands, activates intracellular Wnt signaling in BCSCs, remodels the ECM, establishes a nascent stromal niche and creates the conditions for metastatic colonization of BCSCs (98). Similarly, CAFs also secrete FGF5, which promotes fibronectin collagen formation and remodels the ECM, resulting in the induction of a reversible BCSC phenotype preferentially at the tumor-stromal interface (49).

In addition, CAFs are involved in regulating the biological behavior of BCSCs through their association with other signaling pathways. Activation of WNT/β-catenin and HGF/Met signaling in the mammary gland tumors accelerates the secretion of the Hedgehog ligand SHH in BCSCs, which regulates CAFs *via* a paracrine pathway, and in turn CAFs further secrete factors (99). Accordingly, the Hedgehog inhibitor vismodegib was able to reduce the activity of fibroblasts and breast cancer-forming cells, mechanistically indicating that Hedgehog signaling to CAFs is a potential mediator of CSC plasticity and an intriguing new therapeutic target in breast cancer (49). MSCs and CAFs express high levels of PEAK1 protein in a PEAK1-dependent manner, which activates p-AKT, enhancing tumorigenesis (100). In addition, when MSCs were co-cultured with breast cancer cells, they were able to induce aberrant expression of microRNAs, such as mir-199a upregulation, providing breast cancer cells with enhanced BCSC properties (101). Collectively, these findings identify a potential mechanism of crosstalk between stromal cells and BCSCs and aberrant signaling pathway perturbations, and therefore the development of targeted inhibitors may offer a novel therapy strategy for the management of breast cancer.

Macrophages are a group of plastic and heterogeneous cells that are involved in the innate immune response as another major component of the TME and are capable of regulating the formation and maintenance of BCSCs through the modulating the M1/M2 phenotype. It has been shown that tumor-associated macrophages (TAMs) can activate Src and NF-κB *via* EphA4, which in turn induce the secretion of a variety of cytokines such as IL-6, IL-8 and GM-CSF, thereby establishing a BCSC niche (102). Consistently, in breast cancer, the reduction of macrophages reduced the number of BCSC population (103). In addition, TME-derived endothelial cells provided Jag1 to neighboring BCSCs, increasing the upregulation of zeb1, which in turn increased VEGFA production by ectopic zeb1, inducing endothelial cells to express jag1 in a paracrine manner (29). Similarly, the cell-cell interaction of BCSCs with CD8+ T lymphocytes in TME can establish immune tolerance, mainly due to the ability of BCSCs highly expressing PD-L1 to bind to the PD-1 receptor on the surface of T cells, which in turn exerts an inhibitory effect and leads to T cell exhaustion (104). In addition, ECM, a major component of TME, is a niche that determines the behavior of BCSCs, such as hydroxylated collagen, hyaluronic acid, integrates the intra-/extra- cellular environment signals and activates multiple signaling pathways leading to BCSC metastatic growth (105, 106).

In a nutshell, the TME provides a niche for BCSCs and governs their biological behavior. Importantly, the TME varies markedly between patients, so an exhaustive understanding of the interactivity of the components of the TME on tumor progression is paramount. It has been revealed that TME is potentially of a complex character (107). In parallel, the heterogeneity of TME has been shown to be a potential prognostic factor in identifying different subtypes of breast cancer (108). Building on this, further refinement of breast cancer types and understanding of the specificity of BCSCs offers the potential to accurately predict tumor prognosis and develop new personalized treatment strategies (Figure 3).

Signaling pathways

BCSCs are usually quiescent and are able to transforming their phenotype through EMT, metabolic plasticity, and microenvironment, resulting in limited specific markers. Therefore, more researches have focused on defining the mechanisms of relevant signaling pathways regulating the tumor initiating ability of CSCs. Exhilaratingly, insightful investigations have verified that many key signaling pathways are implicated in modulating the lineage commitment and biological processes of BCSCs.

Wnt

The Wnt family consists of a large number of secreted glycoproteins with both paracrine and autocrine functions (109). Wnt is participating in many important biological processes (109–111). Wnt ligands bind to the seven transmembrane structural domains of the frizzled receptor, FZD) and LRP5/6 co-receptors and stabilizes β-catenin by preventing its phosphorylation (112). The Wnt pathway plays a key role in BCSC fate.

Abnormal WNT/β-catenin signals are more prevalent in breast cancer, and clinical evidence indicates that increased WNT/β-catenin signals are correlated with higher tumor grade and poorer prognosis (98, 113). In BCSCs, WNT/β-catenin is relevant to stemness, plasticity and microsphere formation (114). Canonical and noncanonical Wnt signaling pathways by promoting the expression CD44 and ALDH1, which in turn increase the stemness of BCSCs. Canonical Wnt signaling through β-catenin stabilization and subsequent nuclear translocation leads to transcriptional activation of β-catenin-TCF/LEF target genes. Inhibition of β-catenin reduces BCSC population, tumor size and resistance to doxorubicin (Dox) in TNBC cells (115). Non-canonical WNT/Ca^{2+} signaling regulates the biological behavior of BCSCs through the activation of RTKs such as ROR1/2 and PI3K/AKT. Just as, Wnt plays an important role in BCSC, so targeting the canonical and/or noncanonical Wnt signaling pathway may be an effective marker for eliminating BCSCs. Recent studies showed that DKK1 inhibited lung metastasis by inhibiting PTGS2-induced macrophage and neutrophil recruitment and thereby antagonizing non-classical WNT/PCP-RAC1-JNK signaling. Conversely, DKK1 promotes bone metastasis by regulating canonical Wnt signaling of osteoblasts (114). These results

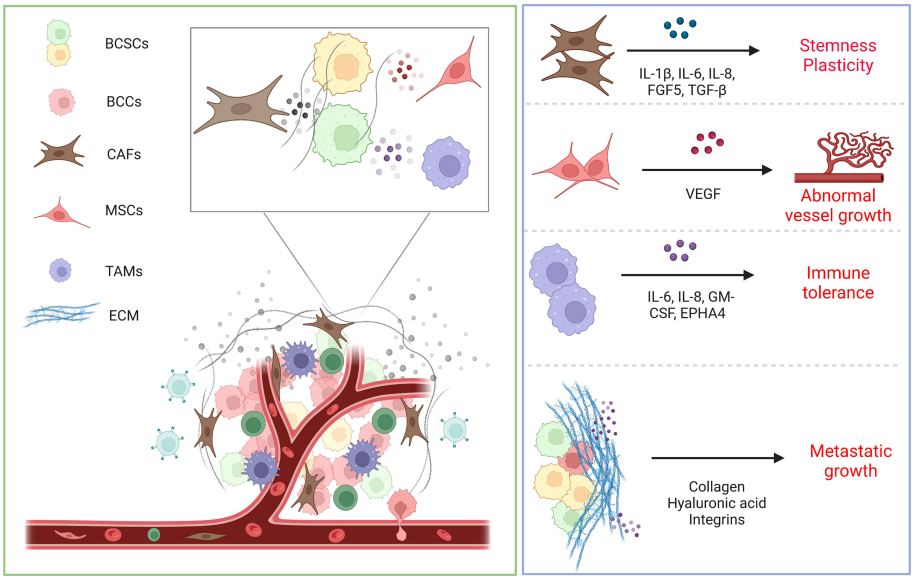


FIGURE 3
Schematic representation of interactions between TME and BCSCs. The microenvironment regulates the biological behavior of BCSCs through direct contact or ECM and paracrine factors. CAFs secrete cytokines such as IL-6, IL-8 and IL-1β to promote BCSC stemness and plasticity. MSCs secrete VEGF to feed BCSCs, leading to abnormal vessel growth. Macrophages likewise secrete various cytokines that establish the BCSC niche and lead to immune tolerance. ECM offers protection to BCSCs from treatment pressure and safeguards their metastatic growth.

reveal that amplified Wnt signaling is instrumental in the self-renewal, apoptosis inhibition and metastasis of BCSCs, and therefore inhibition of wnt is essential for the elimination of BCSCs (Table 2). A growing number of preclinical researches are treating breast cancer by targeting inhibition of Wnt signaling in BCSCs including OMP-18R5 (Vantictumab) (123), NSC668036 (124) and Pyrvinium pamoate (PP) (122).

Notch

The Notch signaling pathway enables ligand-receptor interactions through direct cell-to-cell contact. In mammals, the Notch signaling pathway involves the Notch receptor (Notch1-4) and Notch ligand, which is divided into two classes (Jagged1-2 and Delta-like 1,3,4) that differ due to the presence of a cysteine-rich structural domain in the Jagged ligand (125). Notch receptors are activated by ligands on their neighboring cells, which trigger signals regulating various cellular differentiation processes.

Notch signaling plays a variety of roles in cancer, including oncogenesis, carcinogenesis or both. Concurrently, notch pathway is associated with many aspects of cancer biology, including metabolism, metastasis, drug resistance and the maintenance of CSCs. Multiple discoveries have confirmed that Notch signaling is associated with CSC activity in various

TABLE 2 Antagonist of WNT signaling and their effects on BCSCs.

Antagonist	Target	Functional effects	Ref.
PF-06647020	PTK7-ADC	Tumor regressions and outperforming standard-of-care chemotherapy in PDX model	(116)
OMP-18R5 (Vantictumab)	FZD1/2/5/7/8	Synergistic activity with standard-of-care chemotherapeutic agents	(117)
XAV93	Tankyrase 1/2	Combination paclitaxel for TNBC and external carcinogen-induced breast cancer	(118)
LGK974	PORCN	Inhibition of MMTV-Wnt1-driven mechanistic breast cancer models in mice and rats	(119)
Celecoxib	Wnt/β-catenin	Inhibition of the Wnt/β-catenin pathway to eradicate BCSCs	(120)
Sulforaphane	Wnt/β-catenin	Inhibition of BCSCs and the Wnt/β-catenin self-renewal pathways	(121)
Pyrvinium pamoate	Unknown	Inhibition of stemness regulator expression and tumor regressions in NOD/SCID mice	(122)
IONP	Wnt/β-catenin	Inhibition the expression of Wnt/β-catenin, CD44 and uPAR	(115)

forms of breast cancer. A meta-analysis of tumor molecular landscapes and several pathological studies have shown that Notch1 activity is associated with the risk of recurrence in ER^+ breast cancer (126). Endocrine resistant BCSCs, most of which are Notch4-dependent, are a major factor in tumor recurrence and death (127). Interestingly, unlike Notch4, which is predominantly located in the basal cell population, Notch1 is predominantly expressed in the luminal cells of normal breast epithelium, indirectly suggesting that both may play this specific role in different subpopulations of BCSCs (127, 128). In patients with trastuzumab-resistant and $HER2^+$ breast cancer, Notch1 expression was associated with poorer prognosis (129). Under the circumstances, abrogation of Notch1 expression resulted in a significant reduction of cancer proliferation *in vivo* (130). In particular, Notch3 was capable to act as a mediator of PD-L1 overexpression in BCSCs, activating mTOR and maintaining the self-renewal and invasive capacity of BCSCs (131). What's more, it has been reported that Notch3 does effectively downregulate Notch1 signaling by repressing the expression of the downstream genes Hes1 and Hes5 (132). Interestingly, In ER^- human breast cancer samples, survival advantage of $Notch2^{High}$ over $Notch2^{Low}$ patients in primary and bone metastatic breast cancer (133). Taken together, these observations suggest a common theme: deciphering the variation in the expression of Notch family members in different breast cancer types is necessary to develop effective treatments for the eradication of BCSCs.

Eph

Eph receptors are the largest family of RTKs in mammals and are activated by membrane-linked Ephrin ligands (134–136). The Eph receptor and its Ephrin ligand have been implicated as cell-cell communication complexes that influence the behavior of epithelial cells (137). The function of the Eph/Ephrin in the initiation of breast cancer has been analyzed in detail. In the Eph/ephrin system, chromosomal abnormalities, gene methylation, and alterations in transcription regulators induce dysregulation of the Eph/ephrin expression and tumorigenesis (136). It was demonstrated that EPHB6, an intrinsically catalytically inactive member of the Eph group, partially inhibits EMT, synergistically activates RAS-ERK signaling and promotes the expression of OCT4 in BCSCs, thus exhibiting higher stemness (138). PF-06647263 was a humanized monoclonal antibody that selected Ephrin-A4 as a pharmacological target to inhibit the activity of Ephrin-A4, which was highly expressed in BCSCs, in order to alleviate the clinical symptoms of TNBC (139). Importantly, understanding the complexity of the Eph/Ephrin system will help to elucidate the mechanisms of breast cancer.

Hedgehogs

Hedgehogs signaling includes SHH, IHH and DHH. The precursors can be cleaved to produce an active 19kd N-terminal fragment which binds to the membrane protein Patched gene (Ptc) and Smoothened gene (Smo). As Hedgehog genes are linked, Smo is released, leading to the activation of transcription factors (Gli1-3). In BCSCs, tetraspanin-8 (TSPAN8) was significantly upregulated, recruiting the deubiquitinating enzyme ATXN3 to inhibit the degradation of the SHH/PTCH1 complex, leading to SMO translocation to cilia, causing resistance to chemotherapeutic agents in CSCs and enhancing tumorigenesis in mice (140). Dehydrocholesterol reductase (DHCR24), a key enzyme in cholesterol synthesis, could promote breast cancer development by enhancing the Hedgehog and BCSC populations (141). While, Neuropilin-2 (NRP2) had the ability to activate Gli-1 and α6β1 integrins to induce BCSC initiation (142). Further in depth, Gli-1 and α6β1 integrins mediated the self-renewal and progression of BCSC by promoting angiogenesis and triggering focal adhesion kinase (FAK) signaling, respectively (143, 144). Consequently, targeting the SHH, α6β1, TSPAN8, and FAK can represent an attractive strategy for breast cancer treatment. Curcumin, a polyphenolic compound from the rhizome of Curcuma longa, has been reported to inhibit the proliferation and metastasis of TNBC cells, EMT and BCSC characteristics *via* the Hedgehog/Gli1 pathway (145). Similarly, genistein reduced the population of BCSCs by inhibiting Hedgehog (146). In summary, the search for integrated interventions in Hedgehog signaling and targeted inhibition of BCSC biological behavior could provide a new direction for breast cancer treatment.

PI3K/AKT

PI3K is an intracellular phosphatidylinositol kinase (147). AKT is composed of three main isoforms (AKT1-3), which are key effectors of PI3K and can be directly activated by PI3K (148). PI3K/AKT is involved in regulating BCSC self-renewal, EMT and invasion (149, 150). PI3K/AKT also induced the of activation WNT signaling, which in turn increased the stemness and metastasis of BCSCs. HER2 dysregulation leads to aberrant activation of (PI3K)-Akt and/or WNT signaling and enhanced activity of the BCSC population, resulting in trastuzumab treatment resistance (151). Reciprocally, the role of the HER2 signaling in BCSCs can be enhanced by the PI3K/Akt pathway (152). Therefore, an open-label phase II study demonstrated that trastuzumab and lapatinib, which targeted HER2, inhibited the expression of FOXO, STAT5 and PI3K/AKT and suppress BCSC subpopulations (153).

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase consisting mainly of two distinct protein

complexes, mTORC1 and mTORC2, which are key target genes downstream of AKT (154). Activation of PI3K promotes activation of mTORC1 and mTORC2, while the mTOR activity is frequently upregulated in human cancers (155). What's more, the mTOR pathway is generally considered to be over-activated in CSCs. The inhibitory effect of some mTOR inhibitors on CSCs has been demonstrated (156). Rapamycin, everolimus and PF-04691502 inhibit tamoxifen-induced activation of BCSCs (157). Inhibition of mTOR restores AKT/mTOR-induced resistance to radiotherapy in BCSCs (158). Although mTOR has a role in suppressing BCSCs, a study showed that treatment of TNBC cells with mTOR inhibitors upregulated FGF1-FGFR-Notch1 signaling, leading to an increase in BCSC population (159). In this case, combined blockade of FGFR or Notch1 may prevent resistance to mTORC1/2 inhibitors by eliminating BCSCs (160). Mechanistically, adaptation or resistance to mTOR inhibition in BCSCs is manifested mainly by transcriptional reprogramming of the EVI1 and SOX9 to upregulate REHB and RAPTOR and metastasis-associated mediators (FSCN1 and SPARC) (161). Corporately, a link between PI3K-Akt-mTOR and BCSCs is evident.

Intertwining of signaling pathways in BCSCs

As described previously, these intricate signal transduction pathways are not linear. The crosstalk among multiple pathways is also common in breast cancer, for instance, a discovery has revealed that Syndecan-1 promoted the activation of IL-6/STAT3 and EGFR *via* Notch to regulate inflammation and phenotype of BCSCs (162). The Hippo transducer TAZ confers BCSC-related features, including self-renew and tumor-initiation capacities, through MET (42). FAK can regulate YAP/TAZ activation (163). Aberrant regulation of signaling pathways, such as ERα, Notch and Hedgehog, can lead to abnormal activation of Hippo, resulting in BCSC fate perturbations (164–166). The cumulative effect of aberrant regulation of these pathways in breast cancer maintains and enhances the characteristics of BCSCs, ultimately culminating in malignant tumor progression. Consequently, a thorough insight into the perturbations of different pathways in individual patients is necessary to optimize personalized therapeutic strategies. Importantly, fully assessing the characteristics and subpopulation distribution of BCSCs and developing novel vehicles to eliminate them (Figure 4).

Therapeutic strategies to target BCSCs: an adventurous voyage

From a clinical perspective, deciphering the relevance of BCSCs in therapy resistance, including chemotherapy, radiotherapy, immunotherapy and endocrinotherapy, is one of the major challenges in the clinical translation of anti-CSC therapies. Actually, CSCs are involved in tumor recurrence, metastasis and drug resistance, therefore targeting CSCs may be helpful and complementary to the treatment of breast cancer, combating concerns about safety and treatment failure.

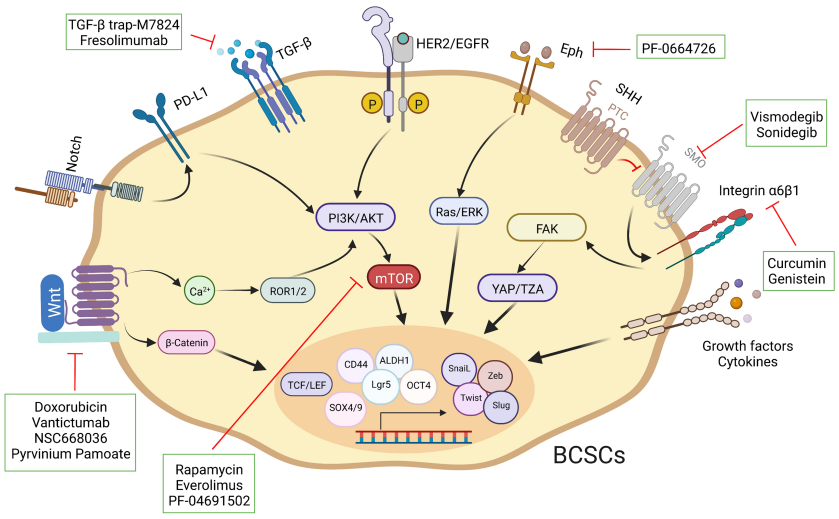


FIGURE 4
Schematic representation of different strategies used to target BCSCs. Specific pathways have been implicated in the fate of BCSCs. A select set of inhibitors have been developed to inhibit specific pathways.

Excitingly, researchers have recently explored targeted therapeutic strategies for BCSC quiescence, maintenance pathways and specific markers.

The availability of BCSC-specific markers has facilitated researchers to effectively identify them, and commonly markers used to isolate BCSC include $CD44^+CD24^-$, CD133 and ALDH1. The high expression of these phenotypically and functionally significant markers in BCSCs compared to normal tissue could allow novel drugs to identify and block relevant BCSC signaling pathways, making them more susceptible to elimination by therapeutic strategies. CD44 is a cell surface receptor that binds to its ligand hyaluronic acid (HA) and that activates a variety of intracellular signals, and the interaction between them is used as a drug target. A study has demonstrated that lapatinib nanocrystals coated with HA have better therapeutic efficacy than uncoated HA in TNBC (167). Comparably, CD133, a membrane glycoprotein, has a demonstrated association with tumor resistance and recurrence. Polymeric nanoparticles loaded with paclitaxel targeting CD133 can markedly reinforce $CD133^+$ cell internalization while significantly suppressing tumor regrowth in a xenograft model (168). Going further, conjugation anti-CD133 mAbs with saporin causes $CD133^+$ BCSC proliferation arrest followed by cell death (169). However, unlike the traditional membrane proteins, ALDH1 is an enzyme with an activity that is intimately associated with the ability of BCSCs to self-renewal. Therefore, targeting ALDH1 is an effective therapeutic agent to eliminate BCSCs.

In fact, the surface phenotype of BCSCs is constantly in flux during cancer progression, differentiating or evolving into different cancer cells and thus obtaining distinct phenotypic recurrences. As a consequence, this will be the most prominent challenge in the design of targeted BCSC therapeutic interventions. Mechanistically, BCSCs undergo cell fate shifts in response to therapeutic pressure or metastasis, leading to malignant progression, which is mainly driven by their inherent genomic and epigenetic instability. Consequently, the strategy applied in clinical trials should take adequate consideration of the comprehensive range of elements leading to selective cell fate decisions, including the tumor microenvironment, intratumor heterogeneous cells and signaling cascades. The TME supports the self-renewal and differentiation of BCSCs, providing a niche to regulate their cell fate in the form of secreted factors and intercellular communication. The xenograft NOD/SCID mice model demonstrated that by affecting the expression of IL-6 in the local microenvironment of BCSCs, it was possible to regain ER expression and subsequently $CD133^{hi}$ cells were able to respond to hormone therapy (170). Inevitably, the damage to a single local microenvironment established through these animal models alone cannot fully replicate the reality of human breast cancer progression, but these adventurous research methods provide an important theoretical and temporal basis for extending preclinical studies. With technological advances, methods such as primary cell culture, organoid culture and microfluidic 3D biomimetic model allow for an improved mimicking of the normal tissue microenvironment, thus providing a new voyage to target the variable traits of BCSCs (171, 172).

Of vital note, signaling pathways are one of the key factors regulating the maintenance and evolution of BCSCs, and therefore targeting these key signals has proven to be an invaluable vehicle for the elimination of BCSCs. The major signaling pathways include Wnt, Notch, Eph, Hedgehogs and PI3K/AKT, which often interact with together in breast cancer stem cells during the development of breast cancer. Equally excitingly, with intensive research into cellular immunity, an additional option for oncology treatments has been developed with novel anti-BCSC immunotherapies such as immunologic checkpoint blocking or CAR-T cell therapies. PD-L1 is detected in 20% of TNBC (173). Deletion of RBMS1 expression by specific shRNA activates PD-L1 immune checkpoint receptor blockade to promote anti-tumor immunity in TNBC (174). In a phase I clinical study of 54 TNBC patients, Atezolizumab showed an objective response rate of 19% as an inhibitor of PD-L1 (175). For CAR-T cell therapy, TEM8 (176) and NKG2D (177) have been used for BCSC-targeted immunotherapy. Collaboratively, these discoveries shed new perspective on the preparation of clinically feasible therapeutic strategies for targeting BCSCs (Table 3).

TABLE 3 Targeting BCSCs with different agents in clinical trials.

Agents	Target	Sample size	Phase	Status	NCT Number
Bevacizumab	ALDH1	75	II	Completed	NCT01190345
MK-0752	Notch	30	I/II	Completed	NCT00645333
LDE225	Hh	30	I	Completed	NCT01954355
AZD8055	PI3K	64	I	Completed	NCT00731263
OMP-54F28	Wnt/β-catenin	26	I	Completed	NCT01608867
Reparixin	CXCR-1	33	I	Completed	NCT02001974
LY2157299	TGFBR1	12	I	Completed	NCT01722825
Lutetium Lu 177 Dotatate	SSTR2	10	II	Not yet recruiting	NCT04529044
GSK3326595	PRMT5	60	II	Not yet recruiting	NCT04676516

Conclusion and prospect

To date, we recognize that BCSCs are a small population of cancer cells with self-renewal and differentiation potential that are involved in mediating tumor heterogeneity, recurrence, metastasis and treatment resistance. There is current research indicating that BCSCs are an attractive target for tackling resistance and recurrence in the clinical therapy of breast cancer. Fortunately, BCSCs have the expression of their own specific markers that can provide post-therapeutic local biopsies with timely information on treatable targets for the remaining tumor tissue on the basis of variable biomarkers, thus allowing the selection of targets for the use of personalized and precise second-line therapy (178, 179). Especially, it is the introduction of the breast cancer stem cell concept, which focuses on biomarkers of BCSCs in the post-treatment period, that offers a new alternative to combating tumor recurrence. However, further attention needs to be given to the fact that normal stem cells in the tissue may also express the overlapping biomarkers and signaling pathways as BCSCs. This therefore requires that the possible side effects of targeting BCSCs for the treatment of breast cancer be fully considered, which in turn requires the rigorous elaboration of identity markers and signaling patterns that are specific or even unique to the targeted BCSCs. Meanwhile, BCSCs tend to have quiescent properties during response therapy, so therapeutic strategies to inhibit tumor progression do not fully prove to be due to the efficacy of targeted inhibition of BCSCs. In addition, BCSCs exist in a specific niche surrounded by heterogeneous cells such as TAMs, MSCs and CAFs that maintain their long-term survival. However, most current researches deficient a microenvironment have used isolated BCSCs and the relationship between BCSCs and their niches is currently ambiguous. Finally, it is undeniable that the immunodeficient animal models lacking adaptive immunity used in the current studies on BCSCs are not capable of recapitulating the biological complexity of tumors in the clinic (180). Collectively, there are still many obstacles to cross in achieving efficient and safe elimination of BCSCs.

In conclusion, the discovery of BCSCs has well revealed that individual cancer cells from the same tumor exhibit essential heterogeneity in terms of mutations, transcriptional programs, immune characteristics and functional properties. Indeed, BCSCs exist in a dynamic state, with multiple pools in individual tumors, so combining multiple treatment strategies to eradicate the pools of therapy-resistant BCSCs on the top of the heterogeneity is clinically important for preventing cancer recurrence. Deeply, cellular plasticity that mediates stemness, fueling cancer heterogeneity and responding to therapeutic pressure, further leading to the limitations of anti-CSC therapeutic strategies. Importantly, the BCSC concept not only has broad and profound implications for our understanding of cancer origins and progression, but also has significant clinical value for the design of more effective and personalized treatment options in the future. Therefore, a combination of conventional cytotoxic drugs, immunotherapy agents, endocrine therapy and eradication of BCSC therapy is a future direction of great significance for improving the clinical prognosis of breast cancer.

Author contributions

HX and LZ contributed to the conception of the study. HX and FZ were responsible for the collection and assembly of data. XG and QZ were responsible for literature search. All authors were involved in the writing and final approval of the manuscript.

Acknowledgments

We are grateful to the BioRender.com platform for providing online drawing tools (Agreement number of Figures 1-4: SG24652NWG, AP241A6DG7, JE241A6DHK, KU241A6DJM).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654

2. Liang Y, Zhang H, Song X, Yang Q. Metastatic heterogeneity of breast cancer: Molecular mechanism and potential therapeutic targets. *Semin Cancer Biol* (2020) 60:14–27. doi: 10.1016/j.semcancer.2019.08.012

3. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. *Nature* (2012) 490(7418):61–70. doi: 10.1038/nature11412

4. Early Breast Cancer Trialists' Collaborative G. Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: Meta-analysis

of individual patient data from ten randomised trials. *Lancet Oncol* (2018) 19 (1):27–39. doi: 10.1016/S1470-2045(17)30777-5

5. Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, et al. Breast cancer. *Nat Rev Dis Primers* (2019) 5(1):66. doi: 10.1038/s41572-019-0111-2

6. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* (2000) 406(6797):747–52. doi: 10.1038/35021093

7. Sorlie T, Wang Y, Xiao C, Johnsen H, Naume B, Samaha RR, et al. Distinct molecular mechanisms underlying clinically relevant subtypes of breast cancer: Gene expression analyses across three different platforms. *BMC Genomics* (2006) 7:127. doi: 10.1186/1471-2164-7-127

8. Bai X, Ni J, Beretov J, Graham P, Li Y. Cancer stem cell in breast cancer therapeutic resistance. *Cancer Treat Rev* (2018) 69:152–63. doi: 10.1016/j.ctrv.2018.07.004

9. Dandawate PR, Subramaniam D, Jensen RA, Anant S. Targeting cancer stem cells and signaling pathways by phytochemicals: Novel approach for breast cancer therapy. *Semin Cancer Biol* (2016) 40-41:192–208. doi: 10.1016/j.semcancer.2016.09.001

10. Carnero A, Garcia-Mayea Y, Mir C, Lorente J, Rubio IT, ME LL. The cancer stem-cell signaling network and resistance to therapy. *Cancer Treat Rev* (2016) 49:25–36. doi: 10.1016/j.ctrv.2016.07.001

11. Polyak K. Breast cancer: Origins and evolution. *J Clin Invest* (2007) 117 (11):3155–63. doi: 10.1172/JCI33295

12. Skibinski A, Kuperwasser C. The origin of breast tumor heterogeneity. *Oncogene* (2015) 34(42):5309–16. doi: 10.1038/onc.2014.475

13. Trumpp A, Haas S. Cancer stem cells: The adventurous journey from hematopoietic to leukemic stem cells. *Cell* (2022) 185(8):1266–70. doi: 10.1016/j.cell.2022.03.025

14. Inman JL, Robertson C, Mott JD, Bissell MJ. Mammary gland development: Cell fate specification, stem cells and the microenvironment. *Development* (2015) 142(6):1028–42. doi: 10.1242/dev.087643

15. Sakakura T, Suzuki Y, Shiurba R. Mammary stroma in development and carcinogenesis. *J Mammary Gland Biol Neoplasia* (2013) 18(2):189–97. doi: 10.1007/s10911-013-9281-9

16. Srivastava V, Huycke TR, Phong KT, Gartner ZJ. Organoid models for mammary gland dynamics and breast cancer. *Curr Opin Cell Biol* (2020) 66:51–8. doi: 10.1016/j.ceb.2020.05.003

17. Rios AC, Fu NY, Lindeman GJ, Visvader JE. *In situ* identification of bipotent stem cells in the mammary gland. *Nature* (2014) 506(7488):322–7. doi: 10.1038/nature12948

18. Fu NY, Nolan E, Lindeman GJ, Visvader JE. Stem cells and the differentiation hierarchy in mammary gland development. *Physiol Rev* (2020) 100(2):489–523. doi: 10.1152/physrev.00040.2018

19. Puisieux A, Pommier RM, Morel AP, Lavial F. Cellular pliancy and the multistep process of tumorigenesis. *Cancer Cell* (2018) 33(2):164–72. doi: 10.1016/j.ccell.2018.01.007

20. Martincorena I, Raine KM, Gerstung M, Dawson KJ, Haase K, Van Loo P, et al. Universal patterns of selection in cancer and somatic tissues. *Cell* (2017) 171 (5):1029–41.e21. doi: 10.1016/j.cell.2017.09.042

21. Fu N, Lindeman GJ, Visvader JE. The mammary stem cell hierarchy. *Curr Top Dev Biol* (2014) 107:133–60. doi: 10.1016/B978-0-12-416022-4.00005-6

22. Ripperger T, Gadzicki D, Meindl A, Schlegelberger B. Breast cancer susceptibility: Current knowledge and implications for genetic counselling. *Eur J Hum Genet* (2009) 17(6):722–31. doi: 10.1038/ejhg.2008.212

23. Fackenthal JD, Olopade OI. Breast cancer risk associated with Brca1 and Brca2 in diverse populations. *Nat Rev Cancer* (2007) 7(12):937–48. doi: 10.1038/nrc2054

24. Couch FJ, Nathanson KL, Offit K. Two decades after brca: Setting paradigms in personalized cancer care and prevention. *Science* (2014) 343(6178):1466–70. doi: 10.1126/science.1251827

25. Hu L, Su L, Cheng H, Mo C, Ouyang T, Li J, et al. Single-cell rna sequencing reveals the cellular origin and evolution of breast cancer in Brca1 mutation carriers. *Cancer Res* (2021) 81(10):2600–11. doi: 10.1158/0008-5472.CAN-20-2123

26. Guo W, Keckesova Z, Donaher JL, Shibue T, Tischler V, Reinhardt F, et al. Slug and Sox9 cooperatively determine the mammary stem cell state. *Cell* (2012) 148(5):1015–28. doi: 10.1016/j.cell.2012.02.008

27. Boulanger CA, Smith GH. Reducing mammary cancer risk through premature stem cell senescence. *Oncogene* (2001) 20(18):2264–72. doi: 10.1038/sj.onc.1204312

28. Yu JM, Sun W, Wang ZH, Liang X, Hua F, Li K, et al. Trib3 supports breast cancer stemness by suppressing Foxo1 degradation and enhancing Sox2 transcription. *Nat Commun* (2019) 10(1):5720. doi: 10.1038/s41467-019-13700-6

29. Jiang H, Zhou C, Zhang Z, Wang Q, Wei H, Shi W, et al. Jagged1-Notch1-Deployed tumor perivascular niche promotes breast cancer stem cell phenotype through Zeb1. *Nat Commun* (2020) 11(1):5129. doi: 10.1038/s41467-020-18860-4

30. Christin JR, Wang C, Chung CY, Liu Y, Dravis C, Tang W, et al. Stem cell determinant Sox9 promotes lineage plasticity and progression in basal-like breast cancer. *Cell Rep* (2020) 31(10):107742. doi: 10.1016/j.celrep.2020.107742

31. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. *N Engl J Med* (2010) 363(20):1938–48. doi: 10.1056/NEJMra1001389

32. Vieira AF, Ricardo S, Ablett MP, Dionisio MR, Mendes N, Albergaria A, et al. P-cadherin is coexpressed with Cd44 and Cd49f and mediates stem cell properties in basal-like breast cancer. *Stem Cells* (2012) 30(5):854–64. doi: 10.1002/stem.1075

33. Prat A, Adamo B, Cheang MC, Anders CK, Carey LA, Perou CM. Molecular characterization of basal-like and non-Basal-Like triple-negative breast cancer. *Oncologist* (2013) 18(2):123–33. doi: 10.1634/theoncologist.2012-0397

34. Vijay GV, Zhao N, Den Hollander P, Toneff MJ, Joseph R, Pietila M, et al. Gsk3beta regulates epithelial-mesenchymal transition and cancer stem cell properties in triple-negative breast cancer. *Breast Cancer Res* (2019) 21(1):37. doi: 10.1186/s13058-019-1125-0

35. Wang X, Sun Y, Wong J, Conklin DS. Ppargamma maintains Erbb2-positive breast cancer stem cells. *Oncogene* (2013) 32(49):5512–21. doi: 10.1038/onc.2013.217

36. Asselin-Labat ML, Vaillant F, Sheridan JM, Pal B, Wu D, Simpson ER, et al. Control of mammary stem cell function by steroid hormone signalling. *Nature* (2010) 465(7299):798–802. doi: 10.1038/nature09027

37. Visvader JE. Cells of origin in cancer. *Nature* (2011) 469(7330):314–22. doi: 10.1038/nature09781

38. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci United States America* (2003) 100(7):3983–8. doi: 10.1073/pnas.0530291100

39. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. Aldh1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* (2007) 1(5):555–67. doi: 10.1016/j.stem.2007.08.014

40. Liu S, Cong Y, Wang D, Sun Y, Deng L, Liu Y, et al. Breast cancer stem cells transition between epithelial and mesenchymal states reflective of their normal counterparts. *Stem Cell Rep* (2014) 2(1):78–91. doi: 10.1016/j.stemcr.2013.11.009

41. Colacino JA, Azizi E, Brooks MD, Harouaka R, Fouladdel S, McDermott SP, et al. Heterogeneity of human breast stem and progenitor cells as revealed by transcriptional profiling. *Stem Cell Rep* (2018) 10(5):1596–609. doi: 10.1016/j.stemcr.2018.03.001

42. Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, et al. The hippo transducer taz confers cancer stem cell-related traits on breast cancer cells. *Cell* (2011) 147(4):759–72. doi: 10.1016/j.cell.2011.09.048

43. Kabos P, Haughian JM, Wang X, Dye WW, Finlayson C, Elias A, et al. Cytokeratin 5 positive cells represent a steroid receptor negative and therapy resistant subpopulation in luminal breast cancers. *Breast Cancer Res Treat* (2011) 128(1):45–55. doi: 10.1007/s10549-010-1078-6

44. Ye F, Zhong X, Qiu Y, Yang L, Wei B, Zhang Z, et al. Cd49f can act as a biomarker for local or distant recurrence in breast cancer. *J Breast Cancer* (2017) 20 (2):142–9. doi: 10.4048/jbc.2017.20.2.142

45. Cariati M, Naderi A, Brown JP, Smalley MJ, Pinder SE, Caldas C, et al. Alpha-6 integrin is necessary for the tumourigenicity of a stem cell-like subpopulation within the Mcf7 breast cancer cell line. *Int J Cancer* (2008) 122 (2):298–304. doi: 10.1002/ijc.23103

46. Chaffer CL, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, et al. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc Natl Acad Sci United States America* (2011) 108(19):7950–5. doi: 10.1073/pnas.1102454108

47. Navas T, Kinders RJ, Lawrence SM, Ferry-Galow KV, Borgel S, Hollingshead MG, et al. Clinical evolution of epithelial-mesenchymal transition in human carcinomas. *Cancer Res* (2020) 80(2):304–18. doi: 10.1158/0008-5472.CAN-18-3539

48. Liu S, Sun Y, Hou Y, Yang L, Wan X, Qin Y, et al. A novel lncrna ropm-mediated lipid metabolism governs breast cancer stem cell properties. *J Hematol Oncol* (2021) 14(1):178. doi: 10.1186/s13045-021-01194-z

49. Cazet AS, Hui MN, Elsworth BL, Wu SZ, Roden D, Chan CL, et al. Targeting stromal remodeling and cancer stem cell plasticity overcomes chemoresistance in triple negative breast cancer. *Nat Commun* (2018) 9(1):2897. doi: 10.1038/s41467-018-05220-6

50. Russnes HG, Navin N, Hicks J, Borresen-Dale AL. Insight into the heterogeneity of breast cancer through next-generation sequencing. *J Clin Invest* (2011) 121(10):3810–8. doi: 10.1172/JCI57088

51. Kai K, Arima Y, Kamiya T, Saya H. Breast cancer stem cells. *Breast Cancer* (2010) 17(2):80–5. doi: 10.1007/s12282-009-0176-y

52. Pontier SM, Muller WJ. Integrins in mammary-Stem-Cell biology and breast-cancer progression–a role in cancer stem cells? *J Cell Sci* (2009) 122(Pt 2):207–14. doi: 10.1242/jcs.040394

53. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* (2001) 414(6859):105–11. doi: 10.1038/35102167

54. Boman BM, Wicha MS. Cancer stem cells: A step toward the cure. *J Clin Oncol* (2008) 26(17):2795–9. doi: 10.1200/JCO.2008.17.7436

55. Velasco-Velazquez MA, Popov VM, Lisanti MP, Pestell RG. The role of breast cancer stem cells in metastasis and therapeutic implications. *Am J Pathol* (2011) 179(1):2–11. doi: 10.1016/j.ajpath.2011.03.005

56. Wright MH, Calcagno AM, Salcido CD, Carlson MD, Ambudkar SV, Varticovski L. Brca1 breast tumors contain distinct Cd44+/Cd24- and Cd133+ cells with cancer stem cell characteristics. *Breast Cancer Res* (2008) 10(1):R10. doi: 10.1186/bcr1855

57. Vassilopoulos A, Wang RH, Petrovas C, Ambrozak D, Koup R, Deng CX. Identification and characterization of cancer initiating cells from Brca1 related mammary tumors using markers for normal mammary stem cells. *Int J Biol Sci* (2008) 4(3):133–42. doi: 10.7150/ijbs.4.133

58. Fillmore CM, Kuperwasser C. Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy. *Breast Cancer Res* (2008) 10(2):R25. doi: 10.1186/bcr1982

59. Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in Brca1 mutation carriers. *Nat Med* (2009) 15(8):907–13. doi: 10.1038/nm.2000

60. Battula VL, Shi Y, Evans KW, Wang RY, Spaeth EL, Jacamo RO, et al. Ganglioside Gd2 identifies breast cancer stem cells and promotes tumorigenesis. *J Clin Invest* (2012) 122(6):2066–78. doi: 10.1172/JCI59735

61. Lu H, Clauser KR, Tam WL, Frose J, Ye X, Eaton EN, et al. Addendum: A breast cancer stem cell niche supported by juxtacrine signalling from monocytes and macrophages. *Nat Cell Biol* (2015) 17(12):1607. doi: 10.1038/ncb3281

62. Saha SK, Choi HY, Kim BW, Dayem AA, Yang GM, Kim KS, et al. Krt19 directly interacts with beta-Catenin/Rac1 complex to regulate numb-dependent notch signaling pathway and breast cancer properties. *Oncogene* (2017) 36(3):332–49. doi: 10.1038/onc.2016.221

63. Semenza GL. The hypoxic tumor microenvironment: A driving force for breast cancer progression. *Biochim Biophys Acta* (2016) 1863(3):382–91. doi: 10.1016/j.bbamcr.2015.05.036

64. Zhang H, Lu H, Xiang L, Bullen JW, Zhang C, Samanta D, et al. Hif-1 regulates Cd47 expression in breast cancer cells to promote evasion of phagocytosis and maintenance of cancer stem cells. *Proc Natl Acad Sci United States America* (2015) 112(45):E6215–23. doi: 10.1073/pnas.1520032112

65. Samanta D, Gilkes DM, Chaturvedi P, Xiang L, Semenza GL. Hypoxia-inducible factors are required for chemotherapy resistance of breast cancer stem cells. *Proc Natl Acad Sci United States America* (2014) 111(50):E5429–38. doi: 10.1073/pnas.1421438111

66. Yoo YG, Christensen J, Gu J, Huang LE. Hif-1alpha mediates tumor hypoxia to confer a perpetual mesenchymal phenotype for malignant progression. *Sci Signal* (2011) 4(178):pt4. doi: 10.1126/scisignal.2002072

67. Scheel C, Eaton EN, Li SH, Chaffer CL, Reinhardt F, Kah KJ, et al. Paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast. *Cell* (2011) 145(6):926–40. doi: 10.1016/j.cell.2011.04.029

68. Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, Chen I, et al. Hypoxia induces the breast cancer stem cell phenotype by hif-dependent and Alkbh5-mediated M(6)a-demethylation of nanog mrna. *Proc Natl Acad Sci United States America* (2016) 113(14):E2047–56. doi: 10.1073/pnas.1602883113

69. Lu H, Xie Y, Tran L, Lan J, Yang Y, Murugan NL, et al. Chemotherapy-induced S100a10 recruits Kdm6a to facilitate Oct4-mediated breast cancer stemness. *J Clin Invest* (2020) 130(9):4607–23. doi: 10.1172/JCI138577

70. Liu X, Xie P, Hao N, Zhang M, Liu Y, Liu P, et al. Hif-1-Regulated expression of calreticulin promotes breast tumorigenesis and progression through Wnt/Beta-catenin pathway activation. *Proc Natl Acad Sci USA* (2021) 118(44):e2109144118. doi: 10.1073/pnas.2109144118

71. Sirkisoon SR, Carpenter RL, Rimkus T, Doheny D, Zhu D, Aguayo NR, et al. Tgli1 transcription factor mediates breast cancer brain metastasis *Via* activating metastasis-initiating cancer stem cells and astrocytes in the tumor microenvironment. *Oncogene* (2020) 39(1):64–78. doi: 10.1038/s41388-019-0959-3

72. Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, et al. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. *Cell* (2010) 140(1):62–73. doi: 10.1016/j.cell.2009.12.007

73. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science* (2011) 331(6024):1559–64. doi: 10.1126/science.1203543

74. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* (2009) 139(5):871–90. doi: 10.1016/j.cell.2009.11.007

75. Ye X, Tam WL, Shibue T, Kaygusuz Y, Reinhardt F, Ng Eaton E, et al. Distinct emt programs control normal mammary stem cells and tumour-initiating cells. *Nature* (2015) 525(7568):256–60. doi: 10.1038/nature14897

76. Rinn J, Guttman M. Rna function. rna and dynamic nuclear organization. *Science* (2014) 345(6202):1240–1. doi: 10.1126/science.1252966

77. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, et al. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking mirna-1-2. *Cell* (2007) 129(2):303–17. doi: 10.1016/j.cell.2007.03.030

78. Rottiers V, Naar AM. Micrornas in metabolism and metabolic disorders. *Nat Rev Mol Cell Biol* (2012) 13(4):239–50. doi: 10.1038/nrm3313

79. Singh SK, Pal Bhadra M, Girschick HJ, Bhadra U. Micrornas–micro in size but macro in function. *FEBS J* (2008) 275(20):4929–44. doi: 10.1111/j.1742-4658.2008.06624.x

80. Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D, et al. Downregulation of mirna-200c links breast cancer stem cells with normal stem cells. *Cell* (2009) 138(3):592–603. doi: 10.1016/j.cell.2009.07.011

81. Christoffersen NR, Silahtaroglu A, Orom UA, Kauppinen S, Lund AH. Mir-200b mediates post-transcriptional repression of Zfhx1b. *RNA* (2007) 13(8):1172–8. doi: 10.1261/rna.586807

82. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, et al. The mir-200 family and mir-205 regulate epithelial to mesenchymal transition by targeting Zeb1 and Sip1. *Nat Cell Biol* (2008) 10(5):593–601. doi: 10.1038/ncb1722

83. Park SM, Gaur AB, Lengyel E, Peter ME. The mir-200 family determines the epithelial phenotype of cancer cells by targeting the e-cadherin repressors Zeb1 and Zeb2. *Genes Dev* (2008) 22(7):894–907. doi: 10.1101/gad.1640608

84. Liu C, Tang DG. Microrna regulation of cancer stem cells. *Cancer Res* (2011) 71(18):5950–4. doi: 10.1158/0008-5472.CAN-11-1035

85. Takahashi RU, Miyazaki H, Takeshita F, Yamamoto Y, Minoura K, Ono M, et al. Loss of microrna-27b contributes to breast cancer stem cell generation by activating Enpp1. *Nat Commun* (2015) 6:7318. doi: 10.1038/ncomms8318

86. Lu G, Li Y, Ma Y, Lu J, Chen Y, Jiang Q, et al. Long noncoding rna Linc00511 contributes to breast cancer tumourigenesis and stemness by inducing the mir-185-3p/E2f1/Nanog axis. *J Exp Clin Cancer Res* (2018) 37(1):289. doi: 10.1186/s13046-018-0945-6

87. Yang Q, Zhao S, Shi Z, Cao L, Liu J, Pan T, et al. Chemotherapy-elicited exosomal mir-378a-3p and mir-378d promote breast cancer stemness and chemoresistance *Via* the activation of Ezh2/Stat3 signaling. *J Exp Clin Cancer Res* (2021) 40(1):120. doi: 10.1186/s13046-021-01901-1

88. Conte I, Banfi S, Bovolenta P. Non-coding rnas in the development of sensory organs and related diseases. *Cell Mol Life Sci* (2013) 70(21):4141–55. doi: 10.1007/s00018-013-1335-z

89. Brown JM, Wasson MD, Marcato P. The missing lnc: The potential of targeting triple-negative breast cancer and cancer stem cells by inhibiting long non-coding rnas. *Cells* (2020) 9(3):763. doi: 10.3390/cells9030763

90. Qin Y, Hou Y, Liu S, Zhu P, Wan X, Zhao M, et al. A novel long non-coding rna Lnc030 maintains breast cancer stem cell stemness by stabilizing sqle mrna and increasing cholesterol synthesis. *Adv Sci (Weinh)* (2021) 8(2):2002232. doi: 10.1002/advs.202002232

91. Zhou M, Hou Y, Yang G, Zhang H, Tu G, Du YE, et al. Lncrna-hh strengthen cancer stem cells generation in twist-positive breast cancer *Via* activation of hedgehog signaling pathway. *Stem Cells* (2016) 34(1):55–66. doi: 10.1002/stem.2219

92. Cho SW, Xu J, Sun R, Mumbach MR, Carter AC, Chen YG, et al. Promoter of lncrna gene Pvt1 is a tumor-suppressor DNA boundary element. *Cell* (2018) 173(6):1398–412.e22. doi: 10.1016/j.cell.2018.03.068

93. Santamaria-Martinez A, Huelsken J. The niche under siege: Novel targets for metastasis therapy. *J Intern Med* (2013) 274(2):127–36. doi: 10.1111/joim.12024

94. Joyce JA, Fearon DT. T Cell exclusion, immune privilege, and the tumor microenvironment. *Science* (2015) 348(6230):74–80. doi: 10.1126/science.aaa6204

95. Ohlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med* (2017) 214(3):579–96. doi: 10.1084/jem.20162024

96. Su S, Chen J, Yao H, Liu J, Yu S, Lao L, et al. Cd10(+)Gpr77(+) cancer-associated fibroblasts promote cancer formation and chemoresistance by sustaining cancer stemness. *Cell* (2018) 172(4):841–56.e16. doi: 10.1016/j.cell.2018.01.009

97. Bartoschek M, Oskolkov N, Bocci M, Lovrot J, Larsson C, Sommarin M, et al. Spatially and functionally distinct subclasses of breast cancer-associated fibroblasts revealed by single cell rna sequencing. *Nat Commun* (2018) 9(1):5150. doi: 10.1038/s41467-018-07582-3

98. Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Delaloye JF, et al. Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature* (2011) 481(7379):85–9. doi: 10.1038/nature10694

99. Valenti G, Quinn HM, Heynen G, Lan L, Holland JD, Vogel R, et al. Cancer stem cells regulate cancer-associated fibroblasts *Via* activation of hedgehog signaling in mammary gland tumors. *Cancer Res* (2017) 77(8):2134–47. doi: 10.1158/0008-5472.CAN-15-3490

100. Hamalian S, Guth R, Runa F, Sanchez F, Vickers E, Agajanian M, et al. A Snai2-Peak1-Inhba stromal axis drives progression and lapatinib resistance in Her2-positive breast cancer by supporting subpopulations of tumor cells positive for antiapoptotic and stress signaling markers. *Oncogene* (2021) 40(33):5224–35. doi: 10.1038/s41388-021-01906-2

101. Cuiffo BG, Campagne A, Bell GW, Lembo A, Orso F, Lien EC, et al. Msc-regulated micrornas converge on the transcription factor Foxp2 and promote breast cancer metastasis. *Cell Stem Cell* (2014) 15(6):762–74. doi: 10.1016/j.stem.2014.10.001

102. Lu H, Clauser KR, Tam WL, Frose J, Ye X, Eaton EN, et al. A breast cancer stem cell niche supported by juxtacrine signalling from monocytes and macrophages. *Nat Cell Biol* (2014) 16(11):1105–17. doi: 10.1038/ncb3041

103. Yang J, Liao D, Chen C, Liu Y, Chuang TH, Xiang R, et al. Tumor-associated macrophages regulate murine breast cancer stem cells through a novel paracrine Egfr/Stat3/Sox-2 signaling pathway. *Stem Cells* (2013) 31(2):248–58. doi: 10.1002/stem.1281

104. Boyle ST, Kochetkova M. Breast cancer stem cells and the immune system: Promotion, evasion and therapy. *J Mammary Gland Biol Neoplasia* (2014) 19 (2):203–11. doi: 10.1007/s10911-014-9323-y

105. Elia I, Rossi M, Stegen S, Broekaert D, Doglioni G, van Gorsel M, et al. Breast cancer cells rely on environmental pyruvate to shape the metastatic niche. *Nature* (2019) 568(7750):117–21. doi: 10.1038/s41586-019-0977-x

106. Brown RL, Reinke LM, Damerow MS, Perez D, Chodosh LA, Yang J, et al. Cd44 splice isoform switching in human and mouse epithelium is essential for epithelial-mesenchymal transition and breast cancer progression. *J Clin Invest* (2011) 121(3):1064–74. doi: 10.1172/JCI44540

107. Azizi E, Carr AJ, Plitas G, Cornish AE, Konopacki C, Prabhakaran S, et al. Single-cell map of diverse immune phenotypes in the breast tumor microenvironment. *Cell* (2018) 174(5):1293–308 e36. doi: 10.1016/j.cell.2018.05.060

108. Tekpli X, Lien T, Rossevold AH, Nebdal D, Borgen E, Ohnstad HO, et al. An independent poor-prognosis subtype of breast cancer defined by a distinct tumor immune microenvironment. *Nat Commun* (2019) 10(1):5499. doi: 10.1038/s41467-019-13329-5

109. Clevers H, Loh KM, Nusse R. Stem cell signaling. an integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science* (2014) 346(6205):1248012. doi: 10.1126/science.1248012

110. Sherwood V. Wnt signaling: An emerging mediator of cancer cell metabolism? *Mol Cell Biol* (2015) 35(1):2–10. doi: 10.1128/MCB.00992-14

111. Kahn M. Wnt signaling in stem cells and cancer stem cells: A tale of two coactivators. *Prog Mol Biol Transl Sci* (2018) 153:209–44. doi: 10.1016/bs.pmbts.2017.11.007

112. Muruganandan S, Roman AA, Sinal CJ. Adipocyte differentiation of bone marrow-derived mesenchymal stem cells: Cross talk with the osteoblastogenic program. *Cell Mol Life Sci* (2009) 66(2):236–53. doi: 10.1007/s00018-008-8429-z

113. Malladi S, Macalinao DG, Jin X, He L, Basnet H, Zou Y, et al. Metastatic latency and immune evasion through autocrine inhibition of wnt. *Cell* (2016) 165 (1):45–60. doi: 10.1016/j.cell.2016.02.025

114. Zhuang X, Zhang H, Li X, Li X, Cong M, Peng F, et al. Differential effects on lung and bone metastasis of breast cancer by wnt signalling inhibitor Dkk1. *Nat Cell Biol* (2017) 19(10):1274–85. doi: 10.1038/ncb3613

115. Miller-Kleinhenz J, Guo X, Qian W, Zhou H, Bozeman EN, Zhu L, et al. Dual-targeting wnt and upa receptors using peptide conjugated ultra-small nanoparticle drug carriers inhibited cancer stem-cell phenotype in chemo-resistant breast cancer. *Biomaterials* (2018) 152:47–62. doi: 10.1016/j.biomaterials.2017.10.035

116. Katoh M. Antibody-drug conjugate targeting protein tyrosine kinase 7, a receptor tyrosine kinase-like molecule involved in wnt and vascular endothelial growth factor signaling: Effects on cancer stem cells, tumor microenvironment and whole-body homeostasis. *Ann Transl Med* (2017) 5(23):462. doi: 10.21037/atm.2017.09.11

117. Gurney A, Axelrod F, Bond CJ, Cain J, Chartier C, Donigan L, et al. Wnt pathway inhibition *Via* the targeting of frizzled receptors results in decreased growth and tumorigenicity of human tumors. *Proc Natl Acad Sci United States America* (2012) 109(29):11717–22. doi: 10.1073/pnas.1120068109

118. Shetti D, Zhang B, Fan C, Mo C, Lee BH, Wei K. Low dose of paclitaxel combined with Xav939 attenuates metastasis, angiogenesis and growth in breast cancer by suppressing wnt signaling. *Cells* (2019) 8(8):892. doi: 10.3390/cells8080892

119. Liu J, Pan S, Hsieh MH, Ng N, Sun F, Wang T, et al. Targeting wnt-driven cancer through the inhibition of porcupine by Lgk974. *Proc Natl Acad Sci United States America* (2013) 110(50):20224–9. doi: 10.1073/pnas.1314239110

120. Huang C, Chen Y, Liu H, Yang J, Song X, Zhao J, et al. Celecoxib targets breast cancer stem cells by inhibiting the synthesis of prostaglandin E2 and down-regulating the wnt pathway activity. *Oncotarget* (2017) 8(70):115254–69. doi: 10.18632/oncotarget.23250

121. Li Y, Zhang T, Korkaya H, Liu S, Lee HF, Newman B, et al. Sulforaphane, a dietary component of Broccoli/Broccoli sprouts, inhibits breast cancer stem cells. *Clin Cancer Res* (2010) 16(9):2580–90. doi: 10.1158/1078-0432.CCR-09-2937

122. Xu L, Zhang L, Hu C, Liang S, Fei X, Yan N, et al. Wnt pathway inhibitor pyrvinium pamoate inhibits the self-renewal and metastasis of breast cancer stem cells. *Int J Oncol* (2016) 48(3):1175–86. doi: 10.3892/ijo.2016.3337

123. Zhang Y, Wang X. Targeting the Wnt/Beta-catenin signaling pathway in cancer. *J Hematol Oncol* (2020) 13(1):165. doi: 10.1186/s13045-020-00990-3

124. Abetov D, Mustapova Z, Saliev T, Bulanin D, Batyrbekov K, Gilman CP. Novel small molecule inhibitors of cancer stem cell signaling pathways. *Stem Cell Rev Rep* (2015) 11(6):909–18. doi: 10.1007/s12015-015-9612-x

125. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: Cell fate control and signal integration in development. *Science* (1999) 284(5415):770–6. doi: 10.1126/science.284.5415.770

126. Mollen EWJ, Ient J, Tjan-Heijnen VCG, Boersma LJ, Miele L, Smidt ML, et al. Moving breast cancer therapy up a notch. *Front Oncol* (2018) 8:518. doi: 10.3389/fonc.2018.00518

127. Harrison H, Farnie G, Howell SJ, Rock RE, Stylianou S, Brennan KR, et al. Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. *Cancer Res* (2010) 70(2):709–18. doi: 10.1158/0008-5472.CAN-09-1681

128. Raouf A, Zhao Y, To K, Stingl J, Delaney A, Barbara M, et al. Transcriptome analysis of the normal human mammary cell commitment and differentiation process. *Cell Stem Cell* (2008) 3(1):109–18. doi: 10.1016/j.stem.2008.05.018

129. Shah D, Wyatt D, Baker AT, Simms P, Peiffer DS, Fernandez M, et al. Inhibition of Her2 increases Jagged1-dependent breast cancer stem cells: Role for membrane Jagged1. *Clin Cancer Res* (2018) 24(18):4566–78. doi: 10.1158/1078-0432.CCR-17-1952

130. Baker A, Wyatt D, Bocchetta M, Li J, Filipovic A, Green A, et al. Notch-1-Pten-Erk1/2 signaling axis promotes Her2+ breast cancer cell proliferation and stem cell survival. *Oncogene* (2018) 37(33):4489–504. doi: 10.1038/s41388-018-0251-y

131. Mansour FA, Al-Mazrou A, Al-Mohanna F, Al-Alwan M, Ghebeh H. Pd-L1 is overexpressed on breast cancer stem cells through Notch3/Mtor axis. *Oncoimmunology* (2020) 9(1):1729299. doi: 10.1080/2162402X.2020.1729299

132. Zhou W, Wang G, Guo S. Regulation of angiogenesis *Via* notch signaling in breast cancer and cancer stem cells. *Biochim Biophys Acta* (2013) 1836(2):304–20. doi: 10.1016/j.bbcan.2013.10.003

133. Capulli M, Hristova D, Valbret Z, Carys K, Arjan R, Maurizi A, et al. Notch2 pathway mediates breast cancer cellular dormancy and mobilisation in bone and contributes to haematopoietic stem cell mimicry. *Br J Cancer* (2019) 121 (2):157–71. doi: 10.1038/s41416-019-0501-y

134. Pasquale EB. Eph receptor signalling casts a wide net on cell behaviour. *Nat Rev Mol Cell Biol* (2005) 6(6):462–75. doi: 10.1038/nrm1662

135. Lackmann M, Boyd AW. Eph, a protein family coming of age: More confusion, insight, or complexity? *Sci Signal* (2008) 1(15):re2. doi: 10.1126/stke.115re2

136. Pasquale EB. Eph receptors and ephrins in cancer: Bidirectional signalling and beyond. *Nat Rev Cancer* (2010) 10(3):165–80. doi: 10.1038/nrc2806

137. Perez White BE, Getsios S. Eph receptor and ephrin function in breast, gut, and skin epithelia. *Cell Adh Migr* (2014) 8(4):327–38. doi: 10.4161/19336918.2014.970012

138. Toosi BM, El Zawily A, Truitt L, Shannon M, Allonby O, Babu M, et al. Ephb6 augments both development and drug sensitivity of triple-negative breast cancer tumours. *Oncogene* (2018) 37(30):4073–93. doi: 10.1038/s41388-018-0228-x

139. Damelin M, Bankovich A, Park A, Aguilar J, Anderson W, Santaguida M, et al. Anti-Efna4 calicheamicin conjugates effectively target triple-negative breast and ovarian tumor-initiating cells to result in sustained tumor regressions. *Clin Cancer Res* (2015) 21(18):4165–73. doi: 10.1158/1078-0432.CCR-15-0695

140. Zhu R, Gires O, Zhu L, Liu J, Li J, Yang H, et al. Tspan8 promotes cancer cell stemness *Via* activation of sonic hedgehog signaling. *Nat Commun* (2019) 10 (1):2863. doi: 10.1038/s41467-019-10739-3

141. Qiu T, Cao J, Chen W, Wang J, Wang Y, Zhao L, et al. 24-dehydrocholesterol reductase promotes the growth of breast cancer stem-like

cells through the hedgehog pathway. *Cancer Sci* (2020) 111(10):3653–64. doi: 10.1111/cas.14587

142. Goel HL, Pursell B, Chang C, Shaw LM, Mao J, Simin K, et al. Gli1 regulates a novel neuropilin-2/Alpha6beta1 integrin based autocrine pathway that contributes to breast cancer initiation. *EMBO Mol Med* (2013) 5(4):488–508. doi: 10.1002/emmm.201202078

143. Ge X, Lyu P, Gu Y, Li L, Li J, Wang Y, et al. Sonic hedgehog stimulates glycolysis and proliferation of breast cancer cells: Modulation of Pfkfb3 activation. *Biochem Biophys Res Commun* (2015) 464(3):862–8. doi: 10.1016/j.bbrc.2015.07.052

144. Habib JG, O'Shaughnessy JA. The hedgehog pathway in triple-negative breast cancer. *Cancer Med* (2016) 5(10):2989–3006. doi: 10.1002/cam4.833

145. Li M, Guo T, Lin J, Huang X, Ke Q, Wu Y, et al. Curcumin inhibits the invasion and metastasis of triple negative breast cancer *Via* Hedgehog/Gli1 signaling pathway. *J Ethnopharmacol* (2022) 283:114689. doi: 10.1016/j.jep.2021.114689

146. Fan P, Fan S, Wang H, Mao J, Shi Y, Ibrahim MM, et al. Genistein decreases the breast cancer stem-like cell population through hedgehog pathway. *Stem Cell Res Ther* (2013) 4(6):146. doi: 10.1186/scrt357

147. Tasian SK, Teachey DT, Rheingold SR. Targeting the Pi3k/Mtor pathway in pediatric hematologic malignancies. *Front Oncol* (2014) 4:108. doi: 10.3389/fonc.2014.00108

148. Wang Q, Chen X, Hay N. Akt as a target for cancer therapy: More is not always better (Lessons from studies in mice). *Br J Cancer* (2017) 117(2):159–63. doi: 10.1038/bjc.2017.153

149. Gao X, Qin T, Mao J, Zhang J, Fan S, Lu Y, et al. Ptenp1/Mir-20a/Pten axis contributes to breast cancer progression by regulating pten *Via* Pi3k/Akt pathway. *J Exp Clin Cancer Res* (2019) 38(1):256. doi: 10.1186/s13046-019-1260-6

150. Bai J, Chen WB, Zhang XY, Kang XN, Jin LJ, Zhang H, et al. Hif-2alpha regulates Cd44 to promote cancer stem cell activation in triple-negative breast cancer *Via* Pi3k/Akt/Mtor signaling. *World J Stem Cells* (2020) 12(1):87–99. doi: 10.4252/wjsc.v12.i1.87

151. Choi HJ, Jin S, Cho H, Won HY, An HW, Jeong GY, et al. Cdk12 drives breast tumor initiation and trastuzumab resistance *Via* wnt and Irs1-Erbb-Pi3k signaling. *EMBO Rep* (2019) 20(10):e48058. doi: 10.15252/embr.201948058

152. Alanazi IO, Khan Z. Understanding egfr signaling in breast cancer and breast cancer stem cells: Overexpression and therapeutic implications. *Asian Pac J Cancer Prev* (2016) 17(2):445–53. doi: 10.7314/apjcp.2016.17.2.445

153. Holmes FA, Espina V, Liotta LA, Nagarwala YM, Danso M, McIntyre KJ, et al. Pathologic complete response after preoperative anti-Her2 therapy correlates with alterations in pten, foxo, phosphorylated Stat5, and autophagy protein signaling. *BMC Res Notes* (2013) 6:507. doi: 10.1186/1756-0500-6-507

154. Alzahrani AS. Pi3k/Akt/Mtor inhibitors in cancer: At the bench and bedside. *Semin Cancer Biol* (2019) 59:125–32. doi: 10.1016/j.semcancer.2019.07.009

155. Hua H, Kong Q, Zhang H, Wang J, Luo T, Jiang Y. Targeting mtor for cancer therapy. *J Hematol Oncol* (2019) 12(1):71. doi: 10.1186/s13045-019-0754-1

156. Francipane MG, Lagasse E. Therapeutic potential of mtor inhibitors for targeting cancer stem cells. *Br J Clin Pharmacol* (2016) 82(5):1180–8. doi: 10.1111/bcp.12844

157. Karthik GM, Ma R, Lovrot J, Kis LL, Lindh C, Blomquist L, et al. Mtor inhibitors counteract tamoxifen-induced activation of breast cancer stem cells. *Cancer Lett* (2015) 367(1):76–87. doi: 10.1016/j.canlet.2015.07.017

158. Lai Y, Yu X, Lin X, He S. Inhibition of mtor sensitizes breast cancer stem cells to radiation-induced repression of self-renewal through the regulation of mnsod and akt. *Int J Mol Med* (2016) 37(2):369–77. doi: 10.3892/ijmm.2015.2441

159. Bhola NE, Jansen VM, Koch JP, Li H, Formisano L, Williams JA, et al. Treatment of triple-negative breast cancer with Torc1/2 inhibitors sustains a drug-resistant and notch-dependent cancer stem cell population. *Cancer Res* (2016) 76(2):440–52. doi: 10.1158/0008-5472.CAN-15-1640-T

160. Hoxhaj G, Hughes-Hallett J, Timson RC, Ilagan E, Yuan M, Asara JM, et al. The Mtorc1 signaling network senses changes in cellular purine nucleotide levels. *Cell Rep* (2017) 21(5):1331–46. doi: 10.1016/j.celrep.2017.10.029

161. Mateo F, Arenas EJ, Aguilar H, Serra-Musach J, de Garibay GR, Boni J, et al. Stem cell-like transcriptional reprogramming mediates metastatic resistance to mtor inhibition. *Oncogene* (2017) 36(19):2737–49. doi: 10.1038/onc.2016.427

162. Ibrahim SA, Gadalla R, El-Ghonaimy EA, Samir O, Mohamed HT, Hassan H, et al. Syndecan-1 is a novel molecular marker for triple negative inflammatory breast cancer and modulates the cancer stem cell phenotype *Via* the il-6/Stat3, notch and egfr signaling pathways. *Mol Cancer* (2017) 16(1):57. doi: 10.1186/s12943-017-0621-z

163. Wang S, Englund E, Kjellman P, Li Z, Ahnlide JK, Rodriguez-Cupello C, et al. Ccm3 is a gatekeeper in focal adhesions regulating mechanotransduction and Yap/Taz signalling. *Nat Cell Biol* (2021) 23(7):758–70. doi: 10.1038/s41556-021-00702-0

164. Britschgi A, Duss S, Kim S, Couto JP, Brinkhaus H, Koren S, et al. The hippo kinases Lats1 and 2 control human breast cell fate *Via* crosstalk with eralpha. *Nature* (2017) 541(7638):541–5. doi: 10.1038/nature20829

165. Lim SK, Lu SY, Kang SA, Tan HJ, Li Z, Adrian Wee ZN, et al. Wnt signaling promotes breast cancer by blocking itch-mediated degradation of Yap/Taz transcriptional coactivator Wbp2. *Cancer Res* (2016) 76(21):6278–89. doi: 10.1158/0008-5472.CAN-15-3537

166. Ko YC, Choi HS, Liu R, Lee DS. Physalin a, 13,14-Seco-16, 24-Cyclo-Steroid, inhibits stemness of breast cancer cells by regulation of hedgehog signaling pathway and yes-associated protein 1 (Yap1). *Int J Mol Sci* (2021) 22(16):8718. doi: 10.3390/ijms22168718

167. Agrawal S, Dwivedi M, Ahmad H, Chadchan SB, Arya A, Sikandar R, et al. Cd44 targeting hyaluronic acid coated lapatinib nanocrystals foster the efficacy against triple-negative breast cancer. *Nanomedicine* (2018) 14(2):327–37. doi: 10.1016/j.nano.2017.10.010

168. Swaminathan SK, Roger E, Toti U, Niu L, Ohlfest JR, Panyam J. Cd133-targeted paclitaxel delivery inhibits local tumor recurrence in a mouse model of breast cancer. *J Control Release* (2013) 171(3):280–7. doi: 10.1016/j.jconrel.2013.07.014

169. Bostad M, Olsen CE, Peng Q, Berg K, Hogset A, Selbo PK. Light-controlled endosomal escape of the novel Cd133-targeting immunotoxin Ac133-saporin by photochemical internalization - a minimally invasive cancer stem cell-targeting strategy. *J Control Release* (2015) 206:37–48. doi: 10.1016/j.jconrel.2015.03.008

170. Sansone P, Ceccarelli C, Berishaj M, Chang Q, Rajasekhar VK, Perna F, et al. Self-renewal of Cd133(Hi) cells by Il6/Notch3 signalling regulates endocrine resistance in metastatic breast cancer. *Nat Commun* (2016) 7:10442. doi: 10.1038/ncomms10442

171. Dekkers JF, van Vliet EJ, Sachs N, Rosenbluth JM, Kopper O, Rebel HG, et al. Long-term culture, genetic manipulation and xenotransplantation of human normal and breast cancer organoids. *Nat Protoc* (2021) 16(4):1936–65. doi: 10.1038/s41596-020-00474-1

172. Berger Fridman I, Kostas J, Gregus M, Ray S, Sullivan MR, Ivanov AR, et al. High-throughput microfluidic 3d biomimetic model enabling quantitative description of the human breast tumor microenvironment. *Acta Biomater* (2021) 132:473–88. doi: 10.1016/j.actbio.2021.06.025

173. Sabatier R, Finetti P, Mamessier E, Adelaide J, Chaffanet M, Ali HR, et al. Prognostic and predictive value of Pdl1 expression in breast cancer. *Oncotarget* (2015) 6(7):5449–64. doi: 10.18632/oncotarget.3216

174. Zhang J, Zhang G, Zhang W, Bai L, Wang L, Li T, et al. Loss of Rbms1 promotes anti-tumor immunity through enabling pd-L1 checkpoint blockade in triple-negative breast cancer. *Cell Death Differ* (2022) 9(24):19. doi: 10.1038/s41418-022-01012-0

175. Gibson J. Anti-Pd-L1 for metastatic triple-negative breast cancer. *Lancet Oncol* (2015) 16(6):e264. doi: 10.1016/S1470-2045(15)70208-1

176. Byrd TT, Fousek K, Pignata A, Szot C, Samaha H, Seaman S, et al. Tem8/Antxr1-specific car T cells as a targeted therapy for triple-negative breast cancer. *Cancer Res* (2018) 78(2):489–500. doi: 10.1158/0008-5472.CAN-16-1911

177. Han Y, Xie W, Song DG, Powell DJJr. Control of triple-negative breast cancer using ex vivo self-enriched, costimulated Nkg2d car T cells. *J Hematol Oncol* (2018) 11(1):92. doi: 10.1186/s13045-018-0635-z

178. Phi LTH, Sari IN, Yang YG, Lee SH, Jun N, Kim KS, et al. Cancer stem cells (Cscs) in drug resistance and their therapeutic implications in cancer treatment. *Stem Cells Int* (2018) 2018:5416923. doi: 10.1155/2018/5416923

179. Shibue T, Weinberg RA. Emt, cscs, and drug resistance: The mechanistic link and clinical implications. *Nat Rev Clin Oncol* (2017) 14(10):611–29. doi: 10.1038/nrclinonc.2017.44

180. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: Progress, promise and challenges. *Nat Rev Immunol* (2012) 12(11):786–98. doi: 10.1038/nri3311

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 16 August 2022
DOI 10.3389/fonc.2022.929037

OPEN ACCESS

EDITED BY
Maria Rosaria De Miglio,
University of Sassari, Italy

REVIEWED BY
Rebeca Debora Martinez-Contreras,
Meritorious Autonomous University of Puebla, Mexico
Haihong Shen,
Gwangju Institute of Science and Technology, South Korea

*CORRESPONDENCE
Wenlin Chen
chenwenlin1@hotmail.com
Fei Ge
ajqnadjd@hotmail.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 26 April 2022
ACCEPTED 25 July 2022
PUBLISHED 16 August 2022

CITATION
Lu X, Zhong J, Liu L, Zhang W, Zhao S, Chen L, Wei Y, Zhang H, Wu J, Chen W and Ge F (2022) The function and regulatory mechanism of RNA-binding proteins in breast cancer and their future clinical treatment prospects. *Front. Oncol.* 12:929037. doi: 10.3389/fonc.2022.929037

The function and regulatory mechanism of RNA-binding proteins in breast cancer and their future clinical treatment prospects

Xingjia Lu[1,2†], Jian Zhong[3,4†], Linlin Liu[5], Wenzhu Zhang[1,2], Shengdi Zhao[1,2], Liang Chen[1], Yuxian Wei[6], Hong Zhang[1,2], Jingxuan Wu[1,2], Wenlin Chen[7*] and Fei Ge[1*]

[1]Department of Breast Surgery, First Affiliated Hospital of Kunming Medical University, Kunming, China, [2]Kunming Medical University, No. 1 School of Clinical Medicine, Kunming, China, [3]Department of Reproductive Medicine, Affiliated Jinling Hospital, Nanjing Medical University, Nanjing, China, [4]Department of Gynecology, Women's Hospital of Nanjing Medical University, Nanjing, China, [5]School of Forensic Medicine, Kunming Medical University, Kunming, China, [6]Department of Endocrine Breast Surgery, First Affiliated Hospital of Chongqing Medical University, Chongqing, China, [7]Third Department of Breast Surgery, The Third Affiliated Hospital of Kunming Medical University, Kunming, China

Breast cancer is the most common female malignancy, but the mechanisms regulating gene expression leading to its development are complex. In recent years, as epigenetic research has intensified, RNA-binding proteins (RBPs) have been identified as a class of posttranscriptional regulators that can participate in regulating gene expression through the regulation of RNA stabilization and degradation, intracellular localization, alternative splicing and alternative polyadenylation, and translational control. RBPs play an important role in the development of normal mammary glands and breast cancer. Functional inactivation or abnormal expression of RBPs may be closely associated with breast cancer development. In this review, we focus on the function and regulatory mechanisms of RBPs in breast cancer, as well as the advantages and challenges of RBPs as potential diagnostic and therapeutic targets in breast cancer, and discuss the potential of RBPs in clinical treatment.

KEYWORDS

breast cancer, RNA-binding protein, HuR, LIN28, Sam68, CPEB4

Introduction

Breast cancer is a highly prevalent malignancy worldwide and is the most common cause of cancer death in women in particular (1).In recent years, the incidence of breast cancer has increased at a rate of 0.5% per year. The reason for this increase is the continued decline in fertility and weight gain, so the global incidence of female breast cancer is predicted to be as high as 3.2 million cases per year by the year 2050 (2, 3). In terms of historical classification, to a large extent, breast carcinogenesis is based on the oncogenic activity of estrogen receptor α (ERα) as well as other hormone receptors, progesterone receptor (PR) and human epidermal growth factor receptor 2(HER2/ERBB2). Based on the expression of these proteins, breast cancers are classically classified into five subtypes: luminal A (ER+, PR+, HER2-), luminal B (ER+, PR-, HER2+), HER2-positive (ER-, PR-, HER2+), basal-like and triple-negative breast cancers (ER-, PR- and HER2-), while the last two subtypes are similar but distinct from invasive breast cancer (4).Currently, the treatment strategies for breast cancer are determined mainly based on tumor size, morphology, metastasis and expression of ER, PR, Ki67 and HER2, including surgery, radiotherapy, endocrine therapy and chemotherapy, which have led to a great delay in tumor progression and further improvement in patient survival (5).However, these therapeutic strategies have not been clinically effective, so there is an urgent need to explore additional molecular regulatory mechanisms of breast cancer to develop new diagnostic and therapeutic targets.

RNA-binding proteins (RBPs) bind to various types of RNAs through RNA-binding domains (RBDs), resulting in stable secondary and tertiary structures of RNA. The K-homology structural domain (KH), RNA recognition motif (RRM), zinc finger structural domain (ZNF), PUM structural domain (PUM), and double-stranded RNA binding structural domain (DSRBD) are the classical RBDs (6, 7). Specifically, RBPs can recognize and interact with RNA recognition motifs (RRMs) and/or binding motifs of RNA structures to form ribonucleoprotein (RNP) complexes to regulate RNAs through, for example, microRNA (MiRNA) processing, RNA stability, alternative premRNA splicing, mRNA decay, translocation, posttranslational nucleotide modifications, and RNA localization (Figure 1) (8, 9).Therefore, RBPs play a key role in the regulation of gene expression at the posttranscriptional level. Dysregulated gene expression of some RBPs may lead to the development of various diseases, including cancer (10).With the in-depth study of gene regulation in breast cancer, it has been found that some RBPs in breast cancer are functionally inactivated or have altered expression. Therefore, it is urgent to explore the function and mechanism of RBPs in breast cancer development.

In this review, we will discuss the function of RBPs in breast cancer cells and their regulatory mechanisms, as well as their potential targets for diagnosis and treatment, providing new therapeutic strategies for the future.

Mechanism of RBPs in breast cancer

In recent years, the specific expression and function of RBPs in breast cancer can be revealed using advanced bioinformatics tools, such as analysis of RNA-seq data based on The Cancer Genome Atlas (TCGA) data. The results of GO and KEGG analysis showed that these RBPs up- or down-regulated in breast cancer are mainly involved in RNA processing, splicing, localization and RNA silencing, and transcriptional regulation.In addition, there are RBPs associated with estrogen response, inflammatory mediators and translational regulation, which in turn are involved in the process of breast cancer development, invasion and metastasis. A recent study showed that 90 RBPs were upregulated and 115 RBPs were downregulated in breast cancer (11). Herein, we review the main regulatory mechanisms of RBPs in and how their dysregulation leads to the development of breast cancer (Table 1).

Dysregulation of miRNA processing of RBPs may contribute to breast cancer development

RBPs are key regulators that control the different stages of miRNA biogenesis and maturation, as well as their localization,

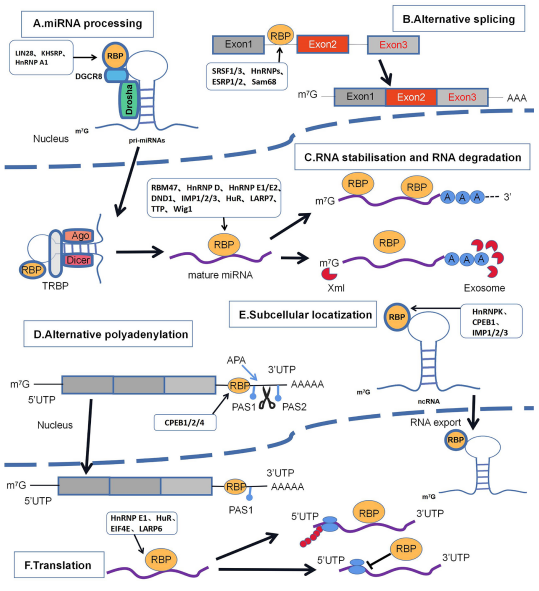


FIGURE 1
Major regulatory mechanisms of RBPs in breast cancer. including **(A)** miRNA processing; **(B)** selective splicing; **(C)** RNA stabilization and RNA degradation; **(D)** selective polyadenylation; **(E)** subcellular localization; **(F)** translation. The schematic diagram lists the RBPs involved in the regulatory mechanisms of breast cancer that appear in the article.

TABLE 1 Roles of RNA binding proteins (RBPs) in breast cancer.

RBP	Expression	Mechanisms	Targets	Traits	References
LIN28A/B	Upregulated	miRNA processing	let-7	Proliferation, invasion, metastasis, angiogenesis	(12–18)
KHSRP	Upregulated or downregulated	miRNA processing	miR-192-5p, let-7	EMT, invasion, metastasis	(19, 20)
HnRNP1	Upregulated	miRNA processing, Alternative splicing	miR-18a, let-7a, RON, caspase-2	Proliferation, EMT	(21–25)
HnRNPD(AUF1)	Upregulated	mRNA stability	c-Yes, Cyclin D1, MMP9, Myc	Proliferation, Senescence	(26, 27)
HnRNPE1/2 (PCBP1/2)	Upregulated or downregulated	mRNA stability, translation	p27, UFD1, NT5E, ILEI	Senescence, EMT, invasion, metastasis	(28–33)
HnRNP M	Upregulated	Alternative splicing	CD44	EMT, invasion, metastasis	(25, 34)
HnRNP I(PTB)	Upregulated	Alternative splicing	FGFR-1, USP5, PKM, Cyclin D3	Proliferation	(21, 35)
HnRNP H1	Upregulated or downregulated	Alternative splicing	MADD30, Bcl-xs △16HER2	Proliferation	(36)
HnRNP K	Upregulated	Subcellular localization	c-myc, lncRNA MLXIPL	Metastasis, proliferation	(37, 38)
SRSF1(SF2/ASF)	Upregulated	Alternative splicing	BIM, BIN1	Senescence, EMT, invasion, metastasis, proliferation, angiogenesis	(25, 39)
SRSF3 (SRp20)	Upregulated	Alternative splicing	FoxM1, GR	Proliferation, apoptosis, EMT, metastasis	(40, 41)
SRP 1/2	Upregulated	Alternative splicing	Rac1, CD44, E-cadherin	EMT, invasion, metastasis	(42, 43)
Sam68	Upregulated	Alternative splicing	CD44v5, Cyclin D1, Bcl-xs	Proliferation, EMT, invasion, metastasis	(44–46)
RBM47	Downregulated	mRNA stability	Dkk1	Metastasis	(47)
DND1	Downregulated	mRNA stability	BIM	Apoptosis	(48)
IGF2BP1 (IMP1/ ZBP1)	Upregulated	mRNA stability, Subcellular localization	β-catenin E-cadherin lncRNA UCA1	Proliferation, EMT, invasion, metastasis	(49–52)
IGFBP2(IMP2)	Upregulated	mRNA stability	E-cadherin, PR miR-200a	EMT, invasion, metastasis	(53)
IGF2BP3(IMP3)	Upregulated	mRNA stability	PR, miR-200a	Proliferation, EMT, invasion, metastasis	(53)
HuR	Upregulated or downregulated	mRNA stability, translation	p21, CDK1, CDK7, VEGF-A, MMP9, ER, IL-8, calmodulin HOX-A5, CD9, FOXO1, erbB2, CXCR-4, SiRT1, SOCS3, HIF-1-α, Wnt5a, TP63, BRCA1, IGF1R, miR-125b	Proliferation, apoptosis, angiogenesis,senescence, invasion, metastasis	(54–56)
LARP6	Upregulated	Translation	MMP-9, VEGF	Angiogenesis, EMT, Proliferation, invasion	(57, 58)
LARP7	Downregulated	mRNA stability	FOXC2,Slug,Twist1, ZEB2, 7SK snRNP	EMT, invasion, metastasis	(59, 60)
TTP	Upregulated or downregulated	mRNA stability	Cyclin B1,Cyclin D1, Bcl-2, VEGF	Angiogenesis, metastasis senescence, Proliferation	(61, 62)
Wig1 (ZMAT3)	Downregulated	mRNA stability	p53	Senescence	(63–65)
CPEB1	Upregulated	Alternative Polyadenylation, Subcellular localization	MMP9 ZO-1	Proliferation, invasion, angiogenesis, metastasis, EMT,	(66)
EIF4E	Upregulated	Translation	c-Myc, Cyclin D1	Apoptosis, angiogenesis, EMT, invasion, metastasis	(67–70)

degradation and activity, and they promote or inhibit miRNA processing mainly through their action on canonical proteins (such as DROSHA and Disher). In recent years, studies have shown that RBPs play an important role in miRNA processing and function; therefore, dysfunction and altered expression levels of RBPs are associated with miRNA processing disorders leading to the dysregulation of target mRNAs, which contribute to tumorigenesis and development of breast cancer (21, 71).

LIN28 (LIN28a and LIN28b) is known to be one of the RBPs with two RNA binding motifs: the cold shock structural domain (CSD) and the Cys-Cys-His-Cys(CCHC) zinc finger structural domain (12). These structural domains of LIN28 are required for direct interaction with the terminal loop (TL) of pre-let-7, thereby inhibiting the biogenesis of let-7 miRNAs (13). It has been reported that LIN28 is mainly localized in the cytoplasm, and LIN28 specifically binds to the terminal loop region of pre-let-7 miRNA, which isolates pri-miRNAs in the cytoplasm and acts as a distraction from the nuclear microprocessor complex, ultimately inhibiting miRNA processing (14). The family of let-7 microRNAs (miRNAs) is a key inhibitory target of LIN28 and exerts potent tumor suppression through posttranscriptional inhibition of multiple oncogenic messenger RNAs (mRNAs) (15). Research has shown that the most fundamental feature of LIN28 in breast cancer cells is its ability to promote and maintain slow proliferation. For example, LIN28 achieves direct or indirect regulation of let-7 by repressing let-7 to enable it to function as an oncogene, including the dysregulation of several genes that are components of the MYC, HMGA2, and PI3K-mTOR pathways (16). The reduction of let 7 mediated by LIN28 downregulates let-7 target genes, leading to abnormalities in the LIN28/let-7 pathway and contributing to tumor proliferation, invasion, metastasis, inflammation, and angiogenesis (17, 18).

KH-type splicing regulatory protein (KHSRP) is a single-stranded multifunctional RNA-binding protein that is involved in posttranscriptional aspects of RNA metabolism and plays an important role in the development of breast cancer (72, 73). KSRP, a component of the DROSHA and DICER complex, is able to regulate the biogenesis of a portion of miRNAs and is also a key regulator involved in miRNA precursor processing due to the high affinity of KSRP for the terminal loop (TL) of target miRNA precursors and promotes the maturation of miRNAs (19). KHSRP is a key factor in maintaining the epithelial phenotype, which facilitates mRNA decline and miRNA maturation. For example, KHSRP in NMuMg cells (a mouse immortalized mammary epithelial cell line) promotes maturation of precursor miR-192-5p, which upregulates EMT factor expression. In contrast, the expression of anti-miR-192-5p in NMuMg cells upregulates the expression of Zeb1, ZEB2, Snai1, Iglon5, and Mmp9 but does not affect the mRNA levels of FSTL1, even leading to the downregulation of the expression of EMT factors, such as Fn1, Col6a2, and Col12a1 (20).

Some specific RNA-binding proteins (RBPs) have emerged as important posttranscriptional regulators of miRNA processing, such as our discovery of heteronuclear ribonucleoprotein A1 (HnRNP A1), a cofactor containing two RRM structural domains that can make specific contacts through the terminal loop of RNA. Subsequently, processing of miRNA precursors can begin, such as the regulation of miRNA-18a (pri-mir-18a) processing, which mainly binds specifically to two UAG motifs of pri-miR-18a (one in the TL and one in the proximal stem region), forms a 1:1 complex with this miRNA, and relaxes the pri-miRNA stem, thus improving the cleavage efficiency of DROSHA (21, 22). When miR-18a expression is reduced, SREBP1 overexpression occurs, E-cadherin is suppressed, Snail/HDAC1/2 complex formation occurs, and EMT is ultimately induced in breast cancer cells (23). HnRNP A1 can also act as a negative regulator of let-7a processing, competing with the activator protein KHSRP for the pri-let-7a terminal loop, leading to a block in the interaction of KHSRP and thus increasing let-7a biogenesis, so HnRNP A1 and KHSRP have an antagonistic role in the posttranscriptional regulation of let-7 precursor processing (22, 24).

RBPs, as splicing factors, regulate alternative splicing to influence the related process of breast cancer

Alternative splicing is one of the most prevalent functions of RBPs in gene regulation. RNA splicing is a form of RNA processing in which newly generated precursor messenger RNA (premRNA) transcripts are converted into mature messenger RNA (mRNA) (74). Specifically, alternative splicing is the process of rearranging exon, partial exon, and/or partial intron combinations into mature RNAs by selecting different combinations of premRNAs from different regions to form different mature mRNAs, thus achieving genetic diversity (25, 75, 76). The splicing process is a sequential phosphodiester transfer reaction catalyzed by a large ribonucleoprotein complex composed of the small nuclear ribonucleoproteins (snRNPs) U1, U2, U4, U5 and U6 and splicing factors (which are RNA-binding proteins targeting specific RNA sequences or motifs) (25). Studies have shown that splicing factors play a dual role in activating or inhibiting splicing events, and once these RBPs bind to pre-RNAs, they can either facilitate or block the interaction between spliceosomes and premRNAs (25). Therefore, abnormalities in alternative splicing may systematically affect all cancer-related processes, such as epithelial-to-mesenchymal transition (EMT) (77).

Serine/arginine-rich splicing factors (SRS) belong to a family of serine-rich proteins, typically consisting of 12 members (SRSF1-12), that play a key role in controlling alternative splicing in cancer, for which aberrant expression of SRS, for example, leads to aberrant RNA splicing and ultimately affects tumor cell proliferation, migration and apoptosis (78). One study found that the SR proteins SRSF1, SRSF2, SRSF3, SRSF5

and SRSF6 are overexpressed in breast cancer (25). Overexpression of SRSF1 inhibits apoptosis and promotes the transformation of mammary epithelial cells by inducing alternative splicing of the antiapoptotic splice isoforms BIM and BIN1 and the expression of splice variants lacking the BH3 structural domain (39). SRSF3 is the smallest SR protein involved in the alternative splicing of FoxM1, producing FoxM1a, b and c1a isoforms (40). During alternative splicing, SRSR3 recognizes the CUC(U/G)UCY splice enhancer sequence, a process promoted by the N6-methyladenosine (m6A) reader protein YTH structural domain containing 1 (YTHDC1), which in turn prevents binding of SRSF10 mRNA and ultimately promotes exon inclusion of the target mRNA (79). It has been shown that SRSF3-induced expression promotes the splicing of glucocorticoid receptor (GR) to GRα, which upregulates activated C-kinase receptor 1 (RACK1) and leads to a significant increase in MDA-MB-231 cell migration. In contrast, silencing RACK1 or SRSF3 prevents this increase (41).

The splicing factor heterogeneous ribonucleoproteins (HnRNPs) are a family of RNA-binding proteins (RBPs) containing at least 20 members with a common structural domain that positively or negatively control splicing by binding to different regions of premRNA (80). In addition, SR proteins typically compete with splicing factors (HnRNPs) to block entry of spliceosome elements by binding to exon or intron splice silencing factors (ESSS or ISSS) and result in inhibition of splice site selection. SR proteins that act as antagonists of HnRNPs in a concentration-dependent manner can prevent exon skipping (81). The HnRNP family members HnRNPA1, HnRNPA2, HnRNPI, HnRNPM and HnRNPK have been reported to be highly expressed in breast cancer (25). In particular, HnRNPA1 not only reduces the formation of the EMT-driven isoform ΔRON by producing a tumorigenic splice variant of RON but also acts as an oncoprotein that promotes the inclusion of exon 9 of the tumor suppressor caspase-2, resulting in the production of the truncated antiapoptotic isoform caspase-2S (25). Binding to the GC-rich structural domain of CD44, HnRNPM promotes the skipping of exon 8, which ultimately promotes breast cancer metastasis by enhancing TGFβ signaling and thus activating the switch of alternative splicing that occurs during epithelial-mesenchymal transition (EMT) (25, 34). Known as polypyrimidine domain binding protein (PTB), HnRNPI functions as a splicing repressor, regulating cancer-associated alternative splicing events by interacting with pyrimidine-rich sequences, such as exon skipping or inclusion when PTB is knocked down (35).

Two splicing factors, HnRNP H1 and SRSF3, involved in the regulation of splicing in highly spliced regions were found to be present in HER2-overexpressing breast cancers by RNA interference experiments. However, the role of HnRNP H1 in cancer development is still complicated by its ability to upregulate anti-apoptotic heterodimers (MADD30) and pro-apoptotic spliceosomes (Bcl-xS), such as the increase in the oncogene Δ16HER2 variant observed following knockdown of HnRNP H1, suggesting that deletion of this splicing factor may lead to a more oncogenic phenotype (36).

ESRP1 and ESRP2 belong to the RNA-binding protein RBM family, also known as RBM35A and RBM35B, respectively, and are epithelial-specific splicing regulators that control the splicing process of epithelial-to-mesenchymal transition (EMT) in cancer. It has been found that knockdown of ESRP1 increases the expression of Rac1b isoforms by allowing alternative splicing of Rac1 mRNA to include variant exon 3b, while in ESRP1 knockdown cells, Rac1b regulates actin dynamics, increases cell motility and induces the formation of long filamentous pseudopods (42). It was shown that ESRP1 promotes lung cancer metastasis by regulating CD44 splicing in ER-negative 4T1 mouse mammary tumor cells. In addition, overexpression of ESRP1 and ESRP2 in basal-like breast cancer cells resulted in upregulation of E-cadherin expression, while in an ER-negative breast cancer model (MDA-MB-231 cells), low ESRP1 expression was associated with the development of EMT. In contrast, ESRP1 drove invasiveness in ER+ breast cancers independent of EMT, and thus, high ESRP1 expression but not ESRP2 was significantly associated with reduced overall survival in breast cancer patients as well as with poor prognosis in ER+ breast cancers, suggesting that the malignant phenotype of human breast cancer is associated with ESRP1 overexpression (42, 43).

Sam68 (68 kDa SRC-associated substrate during mitosis), which belongs to the STAR (signal transducer and RNA activator) RNA-binding protein family, is the first BRK phosphorylated substrate identified *in vivo* and promotes cell growth mainly by regulating alternative mRNA splicing. Sam68 regulates CD44v5, cyclinD1 and Bcl-xs mRNA splicing (44, 45). In living cells, Sam68, when phosphorylated by Src-like kinase, alters the splicing of Bcl-x and leads to the ratio change of the two splice variants it encodes, pro-apoptotic Bcl-x(S) and anti-apoptotic Bcl-x(L), which facilitates the accumulation of Bcl-x (L) and thus keeps cancer cells from undergoing apoptosis (82). Sam68 is significantly overexpressed in breast cancer cells and tissues and is associated with shorter survival rates; conversely, downregulation of endogenous Sam68 expression leads to suppression of proliferation and tumorigenicity of breast cancer cells (46).

RBPs maintain RNA stability by binding to the mRNA 3'UTR and thus affect breast cancer

One of the determinants of RNA stability is the 5'7-methylguanine nucleoside cap, which is bound together by cotranscription factors to prevent mRNA decline and facilitate translation initiation. Conversely, the well-known regulatory pathway of mRNA is the 3' end of polyadenosine. After

transcription, a group of terminal nucleotidyl transferases (Tents) called poly(A) polymerases (PAPs) add untemplated adenosine residues to the 3' end of the transcript to stabilize the mRNA by interacting with poly(A)-binding proteins (PAMPs) (83, 84).

RNA-binding motifs (RBMs) are novel RBPs with one or more RNA recognition motif (RRM) structural domains, of which RBM47 has three RRM structural domains that can play an important role as tumor suppressors in posttranscriptional regulation, mainly by inhibiting EMT and Wnt/β-catenin signaling (85). Low RBM47 expression is significantly associated with a poor prognosis in two subtypes of claudin-low breast cancer and basal breast cancer. In addition, RBM47 binds mainly to the intron and 3'UTR of the target mRNA, with the strongest binding occurring in the 3'UTR (47). RBM47 increases the stability of Dkk1 mRNA in breast cancer cells through direct binding to the noncoding region at the 3' end of Dkk1 mRNA. Dkk1 is a secreted protein that suppresses tumor metastasis and is also an inhibitor of Wnt signaling, which has been shown to promote breast cancer progression. RBM47 can increase Dkk1 secretion, which in turn inhibits Wnt signaling, thereby reducing the tumorigenic fitness of metastatic breast cancer cells (47). As a result, RBM47 inhibits the progression and metastasis of breast cancer.

Heterogeneous ribonucleoprotein D (HnRNP D), also known as Au-rich element RNA binding protein 1 (AUF1), is localized to the 3' untranslated region (3'UTR) of many unstable mRNAs and consists of four different protein isoforms: $p40^{AUF1}$ and $p37^{AUF1}$ are commonly found in the cytoplasm and nucleus, whereas the $P45^{AUF1}$ and $p42^{AUF1}$ isoforms are predominantly found in the nucleus (26). These isoforms have a high affinity for unstable sequences of mRNA and AU-rich (AREs) sequences located in the 3'UTR of mRNAs, and therefore, HnRNP D promotes mRNA decline through ARE-mediated decline (AMD) (26, 80). c-Yes is a member of the c-Src family of tyrosine kinases. In MDA-MB-231 human breast cancer cells, downregulation of c-Yes expression levels leads to overexpression of the small molecule heat shock protein 27 (Hsp27), immediately followed by increased invasive ability *in vitro* and metastatic behavior *in vivo* (27). The expression regulation of c-Yes may be mediated by regulatory sequences in the 3'UTR because the c-Yes 3'-UTR can interact with AUF1 and HuR, which may accelerate mRNA degradation, ultimately leading to the downregulation of c-Yes (27).

The HnRNPs E1 and E2, also commonly referred to as poly (C)-binding proteins PCBP1 and PCBP2, are composed of HnRNP K/J and HnRNP K homology structural domain (KH) alpha-complex proteins (CP1-4 or PCBP1-4α) (28). PCBP1 stabilizes p27 mRNA mainly by binding to the p27 3'UTR through its Kh1 structural domain, which enhances its translation, promotes p27 protein expression, induces cell cycle arrest, inhibits cell proliferation, and ultimately suppresses tumorigenesis *in vitro* and *in vivo*. Conversely, knockdown of PCBP1 in turn accelerates p27 mRNA degradation, causes low p27 (cell cycle inhibitor) protein levels and leads to the development of breast cancer. It has been reported that PCBP1 expression is downregulated in breast cancer (29). In addition, both UFD1 and NT5E knockdown inhibit cell proliferation, colon formation, migration and invasion in breast cancer. Overexpression of PCBP2 promotes the proliferation and metastasis of breast cancer cells by maintaining the mRNA stability of UFD1 and NT5E. PCBP2 binds to the 3'UTR of UFD1 and NT5E to upregulate the expression of these two downstream genes, which ultimately promotes the development of breast cancer (30).

The RNA binding protein DND1 is an evolutionarily conserved RBP that maintains the stability of BIM mRNA by binding to its 3'UTR and competitively inhibits the interaction between miR-221 and BIM, resulting in increased expression of BIM and promoting apoptosis in breast cancer cells (48). When DND1 is knocked down in breast cancer cells, it promotes the decline of BIM mRNA due to the increased binding of miR-221 to the Bim-3'UTR, thereby inhibiting apoptosis or leading to a poor prognosis in breast cancer patients. Conversely, DND1 protects BIM expression from miR-221 inhibition by competitive binding to BIM, thereby promoting apoptosis in breast cancer cells, but the expression level of DND1 is reduced in breast neoplasmss (48).

Zipcode Binding Protein 1 (ZBP1, also known as IMP-1 or IGF2BP1) belongs to a family of conserved RNA-binding proteins containing four HnRNP K (KH) structural domains and two RNA recognition motifs and is an mRNA regulatory factor (86). The expression of ZBP1 and β-catenin (associated with cell migration and proliferation) is synergistically regulated. ZBP1 binds to β-catenin mRNA *in vivo*, increasing the stability of β-catenin mRNA and inhibiting cell proliferation and migration. In metastatic breast cancer cell lines and tumors, the expression of ZBP1 is downregulated, leading to cell proliferation and migration (49, 50). Conversely, in breast cancer cells, IMP1 binds to the ACACCC motif of lncRNA UCA1 through the KH34 structural domain of the protein, destabilizing UCA1, promoting the decay of UCA1, and causing suppression of the UCA1-induced invasive phenotype (51). miR-122-5p is a suppressor of mRNAs associated with cell invasion, and UCA1 is a sponge for endogenous miR-122-5p. IMP1 binding to UCA1 destabilizes UCA1 and blocks the association between UCA1 and miR-122-5p, which in turn reduces the sponging effect of UCA1 on miRNAs, ultimately allowing the oncogenic effect of UCA1 to be diminished (51). IMP2 and IMP3 promote epithelial-mesenchymal transition (EMT) and metastasis, and they are overexpressed in TNBC. miR-200a, a family of tumor suppressor miRNAs, is downregulated in TNBC and maintains a stable epithelial phenotype by directly targeting the E-cadherin repressors ZEB1 and ZEB2, thereby significantly inhibiting EMT and metastasis (53). IMP2 and IMP3 are direct targets of miR-

200a. IMP2 and IMP3 destabilize progesterone receptor (PR) mRNA by recruiting the CCR4-NOT transcriptional complex subunit 1 (CNOT1) complex and repressing miR-200a transcription. Overexpression of IMP2 and IMP3 repress miR-200a by post transcriptionally regulating PR mRNA stability to suppress miR-200a expression. Conversely, PR-induced miR-200a can also inhibit the expression of IMP2 and IMP3 by directly targeting their 3'UTR regions (53).

HuR is a tumor maintenance gene that allows malignant transformation, tumor growth and metastasis of RBPs.HuR binds to the 3'UTR of many proto-oncogenes and unstable AREs to regulate the stability and enhance the translation of target mRNAs, and it is also a key regulator affecting their translocation from the nucleus to the cytoplasm (87). Overall, in breast cancer cell lines, HuR has been shown to bind to mRNAs encoding 38 proteins that are associated with pathways of cell cycle arrest, angiogenesis and proliferation, and apoptosis, such as HuR, through stabilization of cell cycle protein-dependent kinase inhibitor 1 (p21), CDK1, CDK7, hypoxia-inducible factor 1α (HIF-1-α), calmodulin, vascular endothelial growth factor A (VEGF-A), MMP9, ER, HOX-A5, IL-8, FOXO1, CD9, CXCR-4, erbB2, SiRT1, and SOCS3, among other mRNAs, thereby increasing their protein levels, but HUR downregulates the mRNA levels of Wnt5a, tumor protein 63-delta Np63 (TP63), breast cancer type 1 susceptibility protein (BRCA1) and insulin growth factor 1 receptor (IGF1R) (54). Therefore, silencing and overexpression of HuR regulate the development of breast cancer.

LA-associated protein 7 (LARP7), a La family RNA-binding protein that controls RNAPII-suspended 7SK RNA, contains two RNA-binding domains: the RNA recognition motif (RRM) and the HTH La-type RNA-binding domain, which binds to and stabilizes 30 hairpins of 7SK RNA (the most abundant noncoding RNA in mammalian cells), forming the core of 7SK snRNP (7SK small ribonucleoprotein) (59). LARP7 is expressed at low levels in invasive breast cancer tissues and cells; therefore, when a reduction in LARP7 expression is observed, P-TEFb (positive transcriptional elongation factor b) in 7SK snRNP is released, and P-TEFb is reassigned to the transcriptionally active super elongation complex, allowing P-TEFb activation and EMT transcription factors (including FOXC2, Slug Twist1 and ZEB2) to be transcriptionally increased, which ultimately promotes breast cancer invasion, metastasis and EMT (60).

Tristetraprolin (TTP, also known as ZFP36) is an RNA-binding protein containing a tandem CCCH zinc finger structural domain and a proline-rich structural domain and a conserved carboxy-terminal sequence that normally binds to AU-rich elements (AREs) in the 3'-UTR of mRNA, causing the mRNA to depolymerize the poly(A) tail and leading to the degradation of its own mRNA (61, 62). In ERBB2 (oncogenic gene, also known as her2/Neu)-positive breast cancer, the RAS-MAPK kinase pathway is one of the signaling cascades activated by ERBB2 and synergizes with the PI3K/AKT pathway. The MAPK pathway stimulates TTP phosphorylation and becomes less active when it is phosphorylated, preventing deadenylation through the retention of 14-3-3 protein, thus failing to promote mRNA decay, leading to enhanced mRNA stability and translation and promoting the formation of cancer features, including proliferation, invasion, angiogenesis, metastasis and drug resistance (62).

Wig1 (also known as ZMAT3) is a direct target of the oncogene p53 and can encode a double-stranded RNA-binding zinc finger protein that inhibits cell proliferation by binding to p53 mRNA, stabilizing the AU-rich elements (AREs) in the 3'UTR of p53 mRNA and promoting its translation. p53 may also inhibit cell proliferation through Wig-1 by blocking HnRNP A2/B1, thus inhibiting cell proliferation through an unknown mechanism (63–65). Therefore, downregulation of Wig-1 may contribute to the development of breast cancer.

RBPs regulate the poly(A) tail length of mRNAs of breast cancer-related genes through alternative polyadenylation

Alternative polyadenylation (APA) is an event leading to the formation of mRNA 3' UTR isoforms that can produce shorter or longer mRNA isoforms by 3'-terminal cleavage and polyadenylation (CPA). The 3' UTR was observed to be generally longer in breast cancer cells and is an important regulator of gene expression regulation (88). RBPs can also regulate the cleavage and CPA of target mRNAs by competing for or enhancing the binding of polyadenylation machinery proteins to their target sites, and thus, other auxiliary proteins, including RBPs, as well as polyadenylation machinery proteins strictly regulate polyadenylation (88).

Cytoplasmic polyadenylation element binding proteins (CPEBs), consisting of four paralogs (CPEB1-4) containing two zinc fingers and two RNA recognition motifs (RRMS), as well as a regulatory N-terminal region, are a family of RNA-binding proteins that directly mediate intracytoplasmic polyadenylation. They bind to target mRNAs through a mechanism of translational repression or cytoplasmic polyadenylation, allowing cytoplasmic polyadenylation elements (CPEs) to regulate the poly(A) tail length of mRNAs to regulate the translation of mRNAs (89). CPEB1 absence in breast cancer not only leads to the loss of polarity of mammary epithelial cells but also lengthens poly(A) and increases the polyadenylation and translation efficiency of MMP9 mRNA (tumor metastasis-promoting factor), which promotes the metastasis of breast cancer (66). CPEB2 plays a key role in the development of ER-positive breast cancer by regulating the poly(A) tail length of CPE-containing mRNAs, which in turn regulates the translation of mRNAs downstream of steroid hormone signaling, culminating in mammary gland development and ductal breast carcinogenesis (90). Overexpression of CPEB4 is associated with tumor growth, vascularization, migration, invasion and metastasis

in breast cancer patients, causing an upregulation of Vimentin expression and promoting EMT, invasion and migration of breast cancer cells. However, the specific role and mechanisms of CPEB4 in breast cancer have not been fully investigated and reported in this regard (91, 92).

RBPs affect breast cancer by regulating RNA subcellular localization

Nucleolin is a multifunctional RNA-binding protein (RBP) with multiple subcellular localizations, consisting of an amino-terminal charge region, a central region consisting of four RNA-binding regions, and multiple functional structural domains of a carboxy-terminal glycine/arginine (GAR) structural domain that drives subcellular localization mainly through the interaction of the protein with the kinesin light chain (93). In contrast, RBPs in numerous mammals have a GAR structural domain, which is a key determinant of the subcellular localization of the nucleolus, with implications for both their cellular function and disease-related occurrence (93). A few known cancer-associated ncRNAs interact with RBPs, such as AUF1, HuR, TTP, and IGF2BP1, which regulate ncRNA stability and subcellular localization in multiple ways (94).

HnRNPK is an abundant nuclear RNA binding protein in which lncRNA MLXIPL with a long internal exon containing multiple HnRNPK binding sites is strongly enriched in the nucleus of various human cell lines, and knockdown of HNRNPK strongly affects MCF7 cells (37, 38). In addition, a short sequence from the Alu element can bind to HnRNPK and increase its nuclear accumulation (37).

In addition to regulating the level of synthesis of specific proteins, CPEB1 coordinates the translational position of mRNAs through the regulation of their subcellular localization, while its regulated RNA localization is important for cell polarity. For example, CPEB1 mediates the apical localization of ZO-1 mRNA, a key tight junction component encoded by this mRNA in mouse mammary epithelial cells. This process is manifested by impaired colocalization of the tight junction protein ZO-1 and the tip protein syntaxin-3 and increased mislocalization of ZO-1 and the basal protein E-cadherin, ultimately leading to loss of cytosolic polarity in mammary epithelial cells, allowing epithelial-mesenchymal transition (EMT) and increased metastasis (66).

ZBP1 (IGF2BP1 or IMP1) acts as an RNA regulator associated with many cellular processes, including cell proliferation, cell polarity, induction of tumorigenesis and metastasis, binding to β-catenin (mRNA associated with cell proliferation and migration) to enable its activation, and leading to uncontrolled β-catenin by regulating the localization of β-actin mRNA. Disruption of β-catenin signaling allows the maintenance of cell polarity and directional movement, thereby inhibiting breast cancer cell chemotaxis and metastasis (49, 52). The extent to which IMP2 and IMP3 are involved in RNA localization is unclear, but IMP2 can bind to many nuclear-encoded mRNAs associated with mitochondrial function and may help localize transcripts to the mitochondria in a similar manner to that mediated by IMP1 and IMP3, transporting cytoskeletal and adhesion protein transcripts to the frontier of motile cells, while IMP2 binding to mitochondria regulates respiratory complex formation and facilitates oxidative phosphorylation (OXPHOS) (95). Therefore, we need to further investigate the functions of IMP2 and IMP3 in RNA localization in breast cancer and their mechanisms.

Translational regulation of RBPs in breast cancer

Certain known RBPs (such as the splicing factors EFTUD2 and PRPF8) regulate different translational efficiencies by selective binding to 5'UTR structures; in addition, the use of other UTRs may expose the upstream ORFs of translation (UORF) or affect the stable binding sites of mRNA translation and/or miRNA (96). RBPs can facilitate mRNA translational control by recognizing the internal ribosome entry site (IRES) motif (a structural RNA element in the mRNA 5'UTR) and the translational (BAT) element activated by TGF-β in a cap-independent manner (97–99). Thus, RBPs are involved in various stages of translation, such as initiation, elongation and termination, and, concurrently, may bind to the 5'UTR or 3'UTP to regulate translation efficiency.

HnRNP E1 can regulate the translation of specific proteomes directly or indirectly by binding to RNA: (1) binding of HnRNP E1 to specific targets, which directly inhibits translation by preventing translation elongation; (2) relying on selective splicing; and (3) positively regulating translation by binding to the 3'UTR of transcripts (31). In particular, the ribonucleoprotein (MRNP) complex binds to the 33-nucleotide TGFβ-activated translation element (BAT) in the 3'UTR of the mRNA, thereby silencing the translation of the mRNA encoding the mesenchymal protein. HnRNP E1 is a key component of the BAT-binding mRNP complex (31). In addition, HnRNP E1 can prevent the release of eEF1A1 from the ribosomal A site after GTP hydrolysis by binding to the 3'UTR BAT element of eukaryotic elongation factor-1A1 (eEF1A1), bringing translation elongation to a halt and leading to translational silencing of the two EMT transcripts DAB2 and ILEI (97, 98). TGFβ activates a nonclassical kinase cascade reaction that induces protein kinase BB/Akt2-mediated phosphorylation of HnRNP E1 at serine 43, resulting in release of the mRNP complex from the BAT element and restoration of translation (32). Both TGFβ stimulation and silencing of HnRNP E1 in breast cancer increase the translation of ILEI (oncogenic factor associated with EMT and tumorigenesis), which mediates signaling through STAT3,

thereby inducing the formation of BCSCs (breast cancer stem cells) and promoting EMT (33).

HuR may accelerate the initiation of mRNA translation by binding to the 3'-UTR of the target mRNA through interaction with eIF3a (a subunit of the eukaryotic translation initiation factor 3 complex) to regulate protein synthesis (55). HuR not only promotes the translation of p53 mRNA directly but also increases p53 protein synthesis by blocking UV-induced miRNA miR-125b, which has the effect of inhibiting p53 translation (56). In addition, HuR both stimulates XIAP IRES activity and promotes translation of endogenous XIAP mRNA, resulting in elevated levels of XIAP protein and achieving enhanced cytoprotective effects. XIAP is a member of the endogenous inhibitor of apoptosis (IAP) protein family (99). Taken together, HuR may regulate the efficiency of translation through binding to the corresponding breast cancer target mRNA 3'UTR or 5'UTP, which in turn regulates the development of breast cancer.

Eukaryotic translation initiation factor 4E (EIF4E), one of the components of the translation initiation complex EIF4F, recognizes and binds the m^7G cap at the 5' end of mRNA and is a key factor in initiating translation, while its phosphorylation increases mesenchymal markers such as N-calmodulin, wave proteins and fibronectin, which in turn promote tumor invasion, EMT and metastasis (67). When the mRNA unravels, ribosomes are recruited into the mRNA, and translation begins. Overexpression of EIF4E in cancer elevates c-MYC and Cyclin D1 protein levels, which promote proliferation and inhibit apoptosis. Because of the low abundance of eIF4E, it is suggested that it plays a role in translation by regulating the efficiency of mRNA translation (67, 68). The phosphorylation of EIF4E is regulated to some extent by MAP kinase integrated kinase MNK1/2 at serine 209, so the phosphorylation of EIF4E can be blocked by MNK inhibitors. Simultaneously, the synthesis of Cyclin D1 is reduced, and the proliferation and metastasis of breast cancer cells are inhibited by MNK inhibitors (69, 70).

The ACHN gene (also known as La-associated protein 6; LARP6) is a protrusion-rich RNA-binding protein that is also enriched in translation initiation and elongation factors in front of the protrusion and is a key point of translation for local ribosomal protein-encoding mRNAs (RP-mRNAs), promoting migrating cell RP synthesis, protein synthesis and ribosome biogenesis. In human breast cancer, LARP6 overexpression is associated with epithelial-to-mesenchymal transition (EMT) (57, 58).

Function of RBPs in breast cancer

The occurrence of breast cancer may be associated with many factors, including genetic and environmental factors, and RBPs can be involved in the development of breast cancer by regulating the expression levels of proto-oncogenes and oncogenes. Aberrant expression of these RBPs can affect every stage of breast cancer, including proliferation, apoptosis, angiogenesis, senescence, and EMT/invasion/metastasis, and thus, their roles are complex and diverse (Figure 2).

RBPs play a proliferative role in breast cancer cells

Most of these RBPs are associated with cell proliferation, and excessive and abnormal proliferation is key to the development of cancer and may gradually evolve into malignancy.

It has been shown that in breast cancer cells, aberrant activation of LIN28 not only represses let-7 to enable it to function as an oncogene but also promotes and maintains the proliferation of breast cancer cells by directly or indirectly stimulating the expression of tumor growth-related genes (including HER2 and HMGA1) after transcription (16, 100).

The HnRNP family of HnRNPA1 and HnRNPI were reported to be overexpressed in breast cancer and to regulate selective splicing of PKM to promote tumor cell proliferation (25). In contrast, HnRNP D, also known as AUF1, controls tumor proliferation by regulating the translation level of Myc mRNA (101). Overexpression of HnRNP K in breast cancer cells significantly increases target c-myc promoter activity and c-Myc protein and HnRNP K protein levels and promotes breast cancer cell proliferation in a nondependent anchoring manner (38).

IGF2BP1 inhibits cell proliferation by regulating the targets of mRNAs associated with breast cancer, such as binding to β-catenin mRNA and improving its stability (49, 50). IGF2BP3 accelerates the proliferation of breast cancer cells not only by regulating the target of the corresponding mRNA but also by competitively binding with miR-3614-3p to the 3'UTR of the host gene TRIM25 and protecting TRIM25 mRNA from miR-3614-mediated degradation (102).

CPEB1 regulates the translation of CPE-containing mRNAs by regulating their poly(A) tail length, thereby affecting cell proliferation (90). CPEB4 is overexpressed in breast cancer cells and alters the proliferative state of the tumor by affecting the expression level of its target mRNA (91, 92).

HuR promotes breast cancer proliferation through mRNAs that regulate the cell cycle or proliferation-related genes and pathways, such as CDK2 and Cyclin E1 (54, 103). LARP6, an oncogene, is highly expressed in myoepithelial cells and mammary basal cell-like invasive ductal carcinoma of the breast and is also aberrantly expressed in MDA-MB-231 breast cancer cells, promoting cell proliferation (104).

Sam68 can promote cell proliferation by regulating the selective splicing of multiple genes, such as Bcl-xL, Cyclin D1, and CD44 (46). In breast cancer, Sam68 is overexpressed, and acetylation of Sam68 and enhancement of its binding to poly(U) RNA by the acetyltransferase CBP can exert a proliferative effect on tumor cells when acetylation of Sam68 and enhancement of RNA binding activity are present (105).

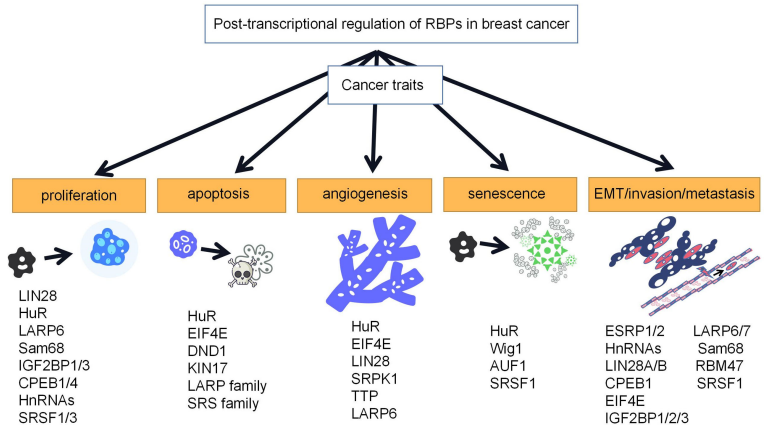


FIGURE 2
Roles of RBPs in breast cancer. RBPs play important roles in proliferation, apoptosis, angiogenesis, senescence, and EMT/invasion/metastasis of breast cancer. Representative RBPs for breast cancer traits are listed in the schematic diagram.

The splicing factor SRSF1 is upregulated in human breast tumors and acts as a target involved in gene expression regulation, cell cycle and proliferation control, as well as cell death and survival, such as through selective splicing (AS). Overexpression of one such heterodimer, exon 9, including CASC4, promotes an increased follicle size and proliferation (106). In addition, the TDP43/SRSF3 complex controls specific splicing events, and TDP43 (TAR DNA-binding protein 43) is an important splicing regulator; when the TDP43 or SRSF3 gene is knocked out, reduced proliferation of mammary epithelial cells is mediated by splicing regulation of Numb exon 12 (107).

Role of RBPs in apoptosis of breast cancer cells

Cancer cells have the ability to not only continuously proliferate but also prevent cell death. Normal cells undergo apoptosis; however, cancer cells perpetually evade apoptosis, thus maintaining the activity of cancer cells and promoting further tumor development. Some of these RBPs are involved in this anti-apoptotic effect by regulating apoptosis-related mRNAs in breast cancer target cells, such as Myc, Mcl-1, p53, Bcl-2 and other mRNAs (108, 109).

HuR affects apoptosis in breast cancer cells by regulating mRNAs that stabilize anti-apoptotic genes, such as mRNAs for p53, bcl-2, Fas, and TNF (54–56). HuR can also influence the anti-apoptotic effects of cells by stimulating XIAP IRES activity and promoting the translation of endogenous XIAP mRNA (99).EIF4E is involved in regulating the expression levels of c-Myc and Bcl-xL to influence apoptosis (110). DND1 expression is downregulated in breast cancer cells and is associated with a poor patient prognosis, and it promotes apoptosis by inducing BIM mRNA expression through competitive interactions with miR-221 (48). In breast cancer, downregulation of KIN17 inhibits cell proliferation and promotes apoptosis, which is associated with increased Caspase3/7 activity (111). The LARP family affects cell growth by controlling the stability of cell survival genes (e.g., Bax and Bcl-2) (104). The SRS family affects apoptosis mainly by regulating the selective splicing of tumor-associated genes (such as BIM and BIN1) (39, 78).

RBPs affect angiogenesis in breast cancer

Both normal cells and cancer cells need oxygen and nutrients, especially cancer cells, which need larger amounts. The process of cancer cell metastasis requires passage through blood vessels, so angiogenesis is necessary for tumor development. Angiogenesis is promoted by angiogenic activators, such as vascular endothelial growth factor, tumor necrosis factor-α, and hypoxia-inducible factor-1α (HIF-1α) (112). RBPs are involved in regulating the expression of angiogenic factors and play an important role in tumor progression.

HuR is involved in regulating the expression of several angiogenesis-related genes, including vascular endothelial growth factor α (VEGFα), HIF1α and platelet response element 1 (TSP1), a known anti-angiogenic gene. Surprisingly, overexpression of HuR in ER-breast cancer leads to an increase in TSP1 and a decrease in VEGF expression, resulting in reduced tumor angiogenesis, so the exact mechanism of the antiangiogenic effect against HuR is not fully understood but may involve an interaction between HuR and microRNAs (113). EIF4E may be an important regulator of angiogenic factor (such

as IL-8 and VEGF) production in breast cancer cells, affecting angiogenesis by regulating the translation of its target mRNAs (VEGF, Cyclin D1 and FGF2), and is associated with a poor prognosis in breast cancer (114, 115). LIN28 affects angiogenesis by regulating the expression level of let-7d (116). In breast cancer, SRPK1 can mediate SRSF1 phosphorylation and promote angiogenesis by regulating VEGF premRNA splicing to generate proangiogenic isoforms (117). Regulation of the mRNA half-life plays an important role in breast cancer. TTP, an RNA-binding protein 1 and KH-type splicing regulatory protein that normally promotes mRNA degradation, reduces the half-life of VEGF mRNA and slows the growth of RAS-transformed cell-derived nude mouse xenograft tumors, in turn reducing the microvessel density in tumors and leading to the inhibition of tumor growth and angiogenesis (118). LARP6 is overexpressed in breast cancer and promotes angiogenesis by upregulating the expression of MMP-9 and VEGF (57, 104).

Role of RBPs in the senescence of breast cancer

Cellular senescence is a biological process influenced by multiple factors that can lead to permanent cell cycle arrest. RBPs can lead to abnormal gene expression during cellular senescence, which in turn regulates the senescence of tumor cells.

In immortalized MCF-10A mammary epithelial cells, HuR can specifically bind to two U-rich elements in the 3'UTR of p63 mRNA, which in turn downregulates the expression level of the tumor suppressor △Np63 and slows cellular senescence (119). Wig1 promotes the degradation of p21 mRNA by binding to the stem–loop structure near the miRNA target site, thereby reducing the expression of p21 and inhibiting cellular senescence (120). AUF1 inhibits the senescence of breast cancer cells by participating in the degradation of the senescence-related genes p16, p53, and p21 (121). SRSF1 stabilizes p53 by recruiting the RPL5-MDM2 complex and increases p53 protein expression and activity, leading to premature cellular senescence (122).

RBPs and breast cancer EMT with invasion and metastasis

During cancer development, RBPs can promote EMT in tumors through various regulatory mechanisms, and when EMT occurs, they inhibit intercellular adhesion and cell polarity, which also promote cancer invasion and metastasis.

ESRP1 and/or ESRP2 further promote EMT by regulating the selective splicing of Rac1 and CD44. In breast cancer, the reduction of ESRP1 changes the variant expression of CD44v from CD44v to CD44, thus inhibiting its metastasis in the lung (42, 43). In addition, HnRNP M can promote the expression of mesenchymal-specific CD44v through competitive interaction with ESRP1, thereby promoting breast cancer metastasis (25, 34).

Members of the HnRNP family can promote EMT and tumor invasion and metastasis. HnRNP E1 regulates the splicing of EMT-related genes and silences their translation in a TGF-β-dependent manner by binding to C-rich elements in the 3'UTR of certain mRNAs, including CD44 and PNUTS. In normal mouse mammary epithelial cells (NMuMG), when HnRNP E1 is silenced, it increases migration and invasiveness *in vitro* and promotes the formation of distant metastases *in vivo* (123). In breast cancer, HnRNP-K is highly expressed and promotes metastasis by inducing the extracellular matrix, cell motility, angiogenesis-related genes and invasive signaling pathways, such as the regulation of cell migration *via* the Ras/MEK/ERK-MMP-3 pathway (124). HnRNP A1 affects the expression of SREBP1, suppresses E-cadherin, and promotes formation of the Snail/HDAC1/2 complex by regulating the processing of miRNA-18a (pri-mir-18a), leading to EMT in breast cancer cells (23).

Overexpression of LIN28A/B is associated with breast cancer tumor migration and invasion, and the mechanism may be related to the let-7 gene (17, 18). In normal mouse mammary epithelial cells (NMuMG), KHSRP can inhibit TGF-β-mediated EMT by activating miR-192-5p, thereby reducing EMT-associated factors (20). CPEB1 is negatively associated with breast cancer metastasis, and mechanistically, knockdown of CPEB1 can contribute to breast cancer metastasis through polyadenylation and translation of MMP9 mRNA (66).In breast cancer, EIF4E increases mesenchymal markers by regulating its phosphorylation, which in turn promotes tumor EMT, invasion and metastasis (67).

IGF2BP1 binds to target mRNAs, such as β-catenin or lncRNA UCA1, by regulating their stabilization and localization, thereby inhibiting metastatic cell invasion and migration, but IGF2BP1 is expressed at low levels in metastatic breast cancer (49–51). In triple-negative breast cancer, IGF2BP2 and 3 contribute to cell migration and invasion by recruiting the CNOT1 complex to destabilize PR mRNA and thereby synergistically promote cell migration and invasion (53).

LARP6, a member of the La-associated protein (LARP) family, is aberrantly expressed in MDA-MB-231 breast cancer cells, resulting in a series of physiological responses with enhanced invasive behavior in *in vitro* and *in vivo* xenograft models, including proliferation, platelet pseudopod formation, EMT, invasion, MMP-9 and VEGF expression, angiogenesis and tumor growth (57). LARP7 is expressed at low levels in breast cancer; therefore, elevated levels of this protein are associated with overall improvement and longer recurrence-free survival. It has been found that short hairpin silencing of LARP7 in MCF10A cells can upregulate the expression levels of P-TEFb-mediated EMT and metastatic genes (such as Slug, Twist1 and ZEB2), thereby promoting tumor invasion and metastasis (104).

It has been shown that Sam68 can induce the BRK/ERK5/Sam68 complex through the activation of Met receptors (and ErbB receptors), which function to reprogram cellular mRNA splicing, thereby promoting protein expression and ultimately favoring breast cancer cell migration (45). RBM47 inhibits tumor progression and metastasis by increasing the secretion of DKK1, which in turn inhibits tumor progression and metastasis (47). SRSF1 promotes EMT, invasion and migration of breast cancer by generating the expression of splice variants lacking the BH3 structural domain (39).

RBPs as biomarkers of breast cancer and their future development prospects for clinical treatment

With the in-depth study of RBPs in breast cancer in recent years, there is a new understanding of their function and mechanism in regulating RNAs, which are closely related to breast cancer proliferation, invasion, metastasis, MET and drug resistance.

RBPs as biomarkers and potential therapeutic targets for breast cancer

Through a large amount of clinical data and literature in recent years, it has been shown that many RBPs can serve as biomarkers and potential therapeutic targets for breast cancer. For example, CPEB4, which is overexpressed in breast cancer, can induce MET and metastasis in breast cancer cells and may become a potential molecular marker for treatment and prognosis prediction in advanced breast cancer (91). It has been shown that DND1 can inhibit the binding of miRNAs to BIM in breast cancer cells and highlighted that DND1 can promote apoptosis in breast cancer cells; thus, DND1 may be a potential therapeutic target for breast cancer (48). It has also been found that NONO is a key regulator of breast cancer proliferation, regulating the expression of the cell proliferation-related genes Skp2 and E2F8 at the posttranscriptional level, and it may become a new diagnostic marker and therapeutic target for advanced breast cancer (125). In addition, the RNA-binding protein PSF promotes the proliferation of ER-positive breast cancer cells by regulating the expression of ERα, TRA2B, aberrant spindle-like microcephaly associated protein (ASPM), and SEC1 family structural domain 2 (SCFD2) mRNAs at the posttranscriptional level, and it may be a potential diagnostic and therapeutic target for hormone-resistant breast cancer and primary breast cancer, as well as a potential poor prognostic factor for ER-positive breast cancer (126). According to an experimental validation, downregulation of the expression of three RBPs (MRPL12, MRPL13 and POP1) resulted in significant inhibition of breast cancer cell survival and migration *in vitro*, suggesting their potential to be designed as biomarkers and/or therapeutic targets for breast cancer (127). There are data supporting that Sam68 is overexpressed in human breast cancer cell lines and tissues and may play an important role in promoting proliferation and cell cycle progression in human breast cancer, so sam68 could be used as a prognostic or diagnostic biomarker for breast cancer treatment. Silencing sam68 plays an antiproliferative role, mainly through activation of the FOXO/p21/p27 pathway and inactivation of the Akt/GSK-3β signaling pathway, so it is also a potential target for the future treatment of breast cancer (46). Finally, EIF4A3, an important RBP, is overexpressed in breast cancer and regulates the cell cycle by binding to SEPT9 premRNA to promote circ-septin 9 (SEPT9) expression, so it may also serve as a diagnostic marker or therapeutic target for breast cancer (128, 129).

Therapeutic approaches for cancer RBPs and future development directions

Previous reports have shown that RBPs play an important role not only in the expression of normal cells but also in the regulation of breast cancer development. In recent decades, there have been no specific drugs directly targeting RBPs for treatment, but recent developments have revealed that we can target RBPs directly or indirectly with a variety of different approaches. These strategies may involve RNA–protein or protein–protein interactions, cellular pathways, and protein aggregation, among others. Direct therapeutic strategies revolve around the inhibition or overexpression of specific RBPs, while indirect approaches include the use of small molecules, oligonucleotide-based strategies, oligonucleotide aptamers, synthetic peptides and other potential strategies for targeting RBPs in cancer, with the use of small molecules being the most common strategy for targeting RBPs (Figure 3) (7, 130).

Small molecule drugs can target RBP function in various human diseases, including breast cancer, and have been clinically tested and reported to have anticancer effects. Small molecules can be used to inhibit RBP function in breast cancer by binding to RBD. Taking EIF4E as an example, the binding of EIF4E to the cap structure is used as a target (7). Ribavirin, a guanosine ribonucleoside analog, was initially found to mimic the cap structure and subsequently compete with endogenous mRNA for binding EIF4E, blocking the transport and translation of EIF4E-regulated oncogenes (such as Cyclin D1) to reduce tumor formation *in vivo* and *in vitro* (131). It has shown good preclinical efficacy and potential efficacy in clinical trials in metastatic breast cancer (132). In addition, use of the N-7 benzylguanosine monophosphate tryptamine phosphoramidate prodrug (4Ei-1) prevents EIF4E cap binding

and triggers proteasomal degradation of EIF4E, thereby sensitizing breast cancer to gemcitabine chemotherapy (133). Several small molecules, 4EGI-1, 4E1RCat and 4E2RCat, have been designed to disrupt the interaction between EIF4E and EIF4G to inhibit cap-dependent translation and promote apoptosis of tumor cells *in vitro* and *in vivo*, with significant antitumor effects, especially in breast cancer xenograft models (7, 134).

Another anticancer strategy involves the use of oligonucleotide-based strategies, including short-stranded antisense oligonucleotides (ASOs), small interfering RNAs (SiRNAs), and aptamers. ASOs can disrupt protein production by blocking ribosome binding to inhibit translation of target mRNA or binding to RNA *via* Watson-Crick base-pairing, which in turn promotes the degradation of target RNA (*via* RNAase H-mediated degradation), altering RNA metabolism, or upregulating the expression levels of certain genes; therefore, therapeutic ASOs are considered a promising approach for targeted treatment of TNBC (7, 135). For example, in breast cancer mouse transplant tumors, the second-generation antisense oligonucleotide 4E-ASO4 inhibits EIF4E by modifying it to provide nuclease resistance, shows its antitumor activity and is well tolerated with no adverse effects on liver function or body weight (136). SiRNA-based therapies involve the introduction of synthetic SiRNAs encapsulated in nanocarriers into target cells to induce RNAi, thereby inhibiting the expression of specific mRNAs. Thus, SiRNA-mediated gene silencing effects are produced by directing the degradation of specific mRNAs (135). The SiRNA of HuR was loaded into folic acid (FA)-coupled nanoparticles, and the formulation was found to be effective in reducing HuR expression and cell proliferation and to synergistically enhance antitumor effects with reduced cytotoxicity. Furthermore, HuR silencing sensitizes triple-negative breast cancer cells to radiation therapy due to its ability to induce oxidative stress and DNA damage (7). In addition to HuR, SiRNA against EIF4E not only inhibits growth and promotes apoptosis in human breast cancer cells, but also enhances the cytotoxic effect of cisplatin (137). Aptamers can fold into sequence-specific three-dimensional structures that can recognize their unique targets and have antibody-like functions (7). The aptamer AS1411 (formerly known as AGRO100), targeting RBP nucleolin, is a 26-nucleotide DNA-based aptamer that forms a stable G-quadruplex structure that is resistant to nucleases and was the first aptamer to be used in cancer clinical trials. Nucleolin regulates several essential cellular processes, namely, RNA polymerase I transcription, proper folding of mature and prethoracic RNA, mRNA translation, and mRNA stability, and it is overexpressed in cancer (138, 139). AS1411 binds to the external structural domain of the nucleolus and inhibits tumor growth in *in vitro* and *in vivo* xenograft models of breast, lung and kidney cancer (139).

Other potential strategies to target RBPs for the treatment of breast cancer include circRNAs and CRISPR-based therapies. circRNAs act as miRNAs or RBP sponges in cancer, altering gene expression levels by regulating transcription and splicing and acting as translation templates. Some circRNAs can also induce the proliferation and progression of TNBC by regulating the transcription of tumor-associated signaling pathways and related genes (140). For example, circ-1073 binds to and

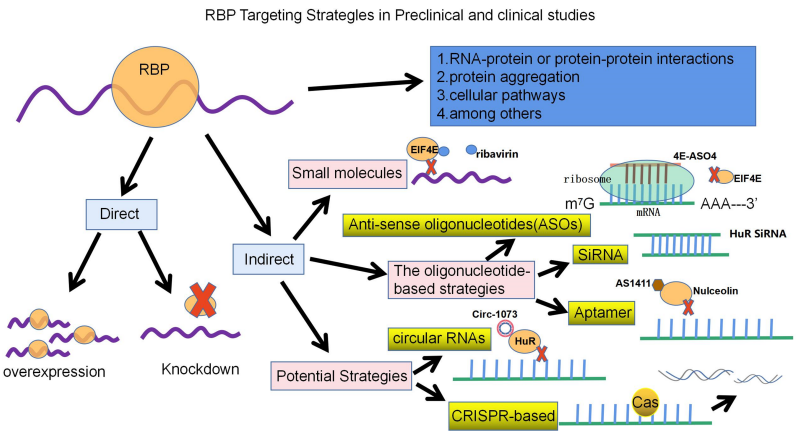


FIGURE 3
With the development of Clip-sequencing, a technique to identify genome-wide RNA binding motifs *in vivo*. These strategies may involve RNA-protein or protein-protein interactions, cellular pathways and protein aggregation, among others. Direct therapeutic strategies revolve around knocking down or overexpressing specific RBPs, while indirect approaches, on the other hand, include the use of small molecules, oligonucleotide-based strategies (ASO, SiRNA, Aptamer) and other potential strategies. The schematic diagram lists some of the RBPs that have made breakthroughs in breast cancer treatment strategy research.

increases the expression of HUR, which in turn increases the levels of cleaved Caspase3/9 and E-cadherin, thereby suppressing the malignant biological behavior of breast cancer (141). Interestingly, a circRNA may contain several loci of one or more RBPs, thus regulating the function of RBPs by acting as an RBP sponge or decoy (142). In the last decade, development of the clustered regularly interspaced short palindromic repeat sequence/CRISPR-associated protein 9 (CRISPR/CAS9) system has also had potential therapeutic applications in cancer therapy. CRISPR can directly target RBPs or their functions in different ways. For example, it can be used to knock down oncogenic RBPs in cancer cells, regulate RBP binding sites in mRNAs, or correct cancer-specific RBP mutations that lead to abnormal splicing of oncogenes (7).

In summary, some therapeutic strategies are still in preclinical and clinical trials for evaluation, and we have a lot of work ahead, so the development of a new therapeutic strategy is long and needs to be supported by expansive clinical data.

Conclusions

With the in-depth study of gene expression abnormalities in cancer and our further understanding of posttranscriptional regulation in cancer, there is a strong interest in RBPs because of their involvement in all aspects of posttranscriptional regulation, including mRNA processing, RNA stability, alternative splicing, alternative polyadenylation, subcellular localization and translation, emphasizing that they play an important role in cancer development. As described in this review, certain RBPs collectively regulate multiple genes in breast cancer through multiple functions, leading to different progression and changes in cancer and, for this reason, to the design of new diagnostic and prognostic biomarkers with potential targets for new therapeutic approaches, allowing us to detect breast cancer earlier and develop rational prognostic treatment strategies.

To summarize, dysfunction of RBPs and consequent abnormalities in posttranscriptional gene expression may contribute to breast cancer development and progression. Although in recent years, a large number of researchers have tried to target RBPs and/or their chaperones in preclinical and clinical studies using small molecules, SiRNAs, ASOs, aptamers and nanoparticle carriers of peptides, only a few RBPs have been used in cancer therapy. Because of the large number of RBPs associated with cancer and the lack of available structure-function studies to predict these targets bioinformatically, there is still a long way to go regarding the development of therapeutic strategies against RBPs.

With the development of in-depth research techniques, such as Clip-sequencing(HITS-Clip), PAR-Clip, RIP-SEq and iCLIP, we have discovered many new RBPs and their partners and conducted functional studies. However, the complexity of interactions between RBPs and other cellular networks, pathways and disease-related processes and the function of RBPs are not incompletely understood and under investigation, thus limiting the associated therapeutic strategies associated. In conclusion, our understanding of RBPs related to breast cancer is still in the initial stage, and a large amount of additional research is needed. It is hoped that RBPs will become an important means of clinical treatment of breast cancer in the future.

Author contributions

XL and JZ searched the literature and wrote the manuscript. LL,WZ,SZ, LC, YW, HZ and JW searched the literature. FG and WC conceived the idea for the review, critically revised the manuscript and provided the final approval. All authors contributed to the article and approved the submitted version.

Funding

The present study was supported by the National Natural Science Foundation of China (grant nos. 82060543 and 82060538), the Yunnan Fundamental Research Projects (grant no. 202101AT070347) and The Yunnan Fundamental Research Projects (grant no. 202201AT070119).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. An J, Peng C, Tang H, Liu X, Peng F. New advances in the research of resistance to neoadjuvant chemotherapy in breast cancer. *Int J Mol Sci* (2021) 22 (17):9644. doi: 10.3390/ijms22179644

2. Ruo SW, Alkayyali T, Win M, Tara A, Joseph C, Kannan A, et al. Role of gut microbiota dysbiosis in breast cancer and novel approaches in prevention, diagnosis, and treatment. *Cureus* (2021) 13:e17472. doi: 10.7759/cureus.17472

3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71:7–33. doi: 10.3322/caac.21654

4. Mondal M, Conole D, Nautiyal J, Tate EW. UCHL1 as a novel target in breast cancer: Emerging insights from cell and chemical biology. *Br J Cancer* (2021) 126 (1):24–33. doi: 10.1038/s41416-021-01516-5

5. Jin H, Du W, Huang W, Yan J, Tang Q, Chen Y, et al. lncRNA and breast cancer: Progress from identifying mechanisms to challenges and opportunities of clinical treatment. *Mol Ther Nucleic Acids* (2021) 25:613–37. doi: 10.1016/j.omtn.2021.08.005

6. Lunde BM, Moore C, Varani G. RNA-Binding proteins: modular design for efficient function. *Nat Rev Mol Cell Biol* (2007) 8:479–90. doi: 10.1038/nrm2178

7. Mohibi S, Chen X, Zhang J. Cancer the'RBP'eutics-RNA-binding proteins as therapeutic targets for cancer. *Pharmacol Ther* (2019) 203:107390. doi: 10.1016/j.pharmthera.2019.07.001

8. Liu-Yesucevitz L, Bassell GJ, Gitler AD, Hart AC, Klann E, Richter JD, et al. Local RNA translation at the synapse and in disease. *J Neurosci* (2011) 31:16086–93. doi: 10.1523/JNEUROSCI.4105-11.2011

9. Van Nostrand EL, Freese P, Pratt GA, Wang X, Wei X, Xiao R, et al. A large-scale binding and functional map of human RNA-binding proteins. *Nature* (2020) 583:711–9. doi: 10.1038/s41586-020-2077-3

10. Wang ZL, Li B, Luo YX, Lin Q, Liu SR, Zhang XQ, et al. Comprehensive genomic characterization of RNA-binding proteins across human cancers. *Cell Rep* (2018) 22:286–98. doi: 10.1016/j.celrep.2017.12.035

11. Wang K, Li L, Fu L, Yuan Y, Dai H, Zhu T, et al. Integrated bioinformatics analysis the function of RNA binding proteins (RBPs) and their prognostic value in breast cancer. *Front Pharmacol* (2019) 10:140. doi: 10.3389/fphar.2019.00140

12. Rehfeld F, Rohde A, Nguyen D, Wulczyn FJC. Lin28 and let-7: ancient milestones on the road from pluripotency to neurogenesis. *Cell Tissue Res* (2015) 359:145–60. doi: 10.1007/s00441-014-1872-2

13. Loughlin F, Gebert L, Towbin H, Brunschweiger A, Hall J, FJNs A, et al. Structural basis of pre-let-7 miRNA recognition by the zinc knuckles of pluripotency factor Lin28. *Nat Struct Mol Biol* (2011) 19:84–9. doi: 10.1038/nsmb.2202

14. Piskounova E, Viswanathan SR, Janas M, LaPierre RJ, Daley GQ, Sliz P, et al. Determinants of microRNA processing inhibition by the developmentally regulated RNA-binding protein Lin28. *J Biol Chem* (2008) 283:21310–4. doi: 10.1074/jbc.C800108200

15. Powers JT, Tsanov KM, Pearson DS, Roels F, Spina CS, Ebright R, et al. Multiple mechanisms disrupt the let-7 microRNA family in neuroblastoma. *Nature* (2016) 535:246–51. doi: 10.1038/nature18632

16. Xiong H, Zhao W, Wang J, Seifer B, Ye C, Chen Y, et al. Oncogenic mechanisms of Lin28 in breast cancer: new functions and therapeutic opportunities. *Oncotarget* (2017) 8:25721–35. doi: 10.18632/oncotarget.14891

17. Balzeau J, Menezes MR, Cao S, Hagan JP. The LIN28/let-7 pathway in cancer. *Front Genet* (2017) 8:31. doi: 10.3389/fgene.2017.00031

18. Viswanathan SR, Powers JT, Einhorn W, Hoshida Y, Ng TL, Toffanin S, et al. Lin28 promotes transformation and is associated with advanced human malignancies. *Nat Genet* (2009) 41:843–8. doi: 10.1038/ng.392

19. Trabucchi M, Briata P, Garcia-Mayoral M, Haase AD, Filipowicz W, Ramos A, et al. The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. *Nature* (2009) 459:1010–4. doi: 10.1038/nature08025

20. Puppo M, Bucci G, Rossi M, Giovarelli M, Bordo D, Moshiri A, et al. miRNA-mediated KHSRP silencing rewires distinct post-transcriptional programs during TGF-beta-Induced epithelial-to-Mesenchymal transition. *Cell Rep* (2016) 16:967–78. doi: 10.1016/j.celrep.2016.06.055

21. Kang D, Lee Y, Lee JS. RNA-Binding proteins in cancer: Functional and therapeutic perspectives. *Cancers (Basel)* (2020) 12(9):2699. doi: 10.3390/cancers12092699

22. Kooshapur H, Choudhury NR, Simon B, Muhlbauer M, Jussupow A, Fernandez N, et al. Structural basis for terminal loop recognition and stimulation of pri-miRNA-18a processing by hnRNP A1. *Nat Commun* (2018) 9:2479. doi: 10.1038/s41467-018-04871-9

23. Shen K, Cao Z, Zhu R, You L, Zhang T. The dual functional role of MicroRNA-18a (miR-18a) in cancer development. *Clin Transl Med* (2019) 8:32. doi: 10.1186/s40169-019-0250-9

24. Michlewski G, Caceres JF. Antagonistic role of hnRNP A1 and KSRP in the regulation of let-7a biogenesis. *Nat Struct Mol Biol* (2010) 17:1011–8. doi: 10.1038/nsmb.1874

25. Yang Q, Zhao J, Zhang W, Chen D, Wang Y. Aberrant alternative splicing in breast cancer. *J Mol Cell Biol* (2019) 11:920–9. doi: 10.1093/jmcb/mjz033

26. Fialcowitz E, Brewer B, Keenan B, Wilson G. A hairpin-like structure within an AU-rich mRNA-destabilizing element regulates trans-factor binding selectivity and mRNA decay kinetics. *J Biol Chem* (2005) 280:22406–17. doi: 10.1074/jbc.M500618200

27. Sommer S, Cui Y, Brewer G, Fuqua SA. The c-yes 3'-UTR contains adenine/uridine-rich elements that bind AUF1 and HuR involved in mRNA decay in breast cancer cells. *J Steroid Biochem Mol Biol* (2005) 97:219–29. doi: 10.1016/j.jsbmb.2005.09.002

28. Makeyev A, Liebhaber SJR. The poly(C)-binding proteins: a multiplicity of functions and a search for mechanisms. *RNA* (2002) 8:265–78. doi: 10.1017/s1355838202024627

29. Shi H, Li H, Yuan R, Guan W, Zhang X, Zhang S, et al. PCBP1 depletion promotes tumorigenesis through attenuation of p27(Kip1) mRNA stability and translation. *J Exp Clin Cancer Res* (2018) 37:187. doi: 10.1186/s13046-018-0840-1

30. Wang X, Guo Q, Wang H, Yuan X, Wang B, Lobie PE, et al. PCBP2 posttranscriptional modifications induce breast cancer progression *via* upregulation of UFD1 and NT5E. *Mol Cancer Res* (2021) 19:86–98. doi: 10.1158/1541-7786.MCR-20-0390

31. Grelet S, Howe PH. hnRNP E1 at the crossroads of translational regulation of epithelial-mesenchymal transition. *J Cancer Metastasis Treat* (2019) 5:16. doi: 10.20517/2394-4722.2018.85

32. Hussey GS, Link LA, Brown AS, Howley BV, Chaudhury A, Howe PH. Establishment of a TGFbeta-induced post-transcriptional EMT gene signature. *PloS One* (2012) 7:e52624. doi: 10.1371/journal.pone.0052624

33. Woosley AN, Dalton AC, Hussey GS, Howley BV, Mohanty BK, Grelet S, et al. TGFbeta promotes breast cancer stem cell self-renewal through an ILEI/LIFR signaling axis. *Oncogene* (2019) 38:3794–811. doi: 10.1038/s41388-019-0703-z

34. Xu Y, Gao XD, Lee JH, Huang H, Tan H, Ahn J, et al. Cell type-restricted activity of hnRNPM promotes breast cancer metastasis *via* regulating alternative splicing. *Genes Dev* (2014) 28:1191–203. doi: 10.1101/gad.241968.114

35. Xue Y, Zhou Y, Wu T, Zhu T, Ji X, Kwon YS, et al. Genome-wide analysis of PTB-RNA interactions reveals a strategy used by the general splicing repressor to modulate exon inclusion or skipping. *Mol Cell* (2009) 36:996–1006. doi: 10.1016/j.molcel.2009.12.003

36. Gautrey H, Jackson C, Dittrich AL, Browell D, Lennard T, Tyson-Capper A. SRSF3 and hnRNP H1 regulate a splicing hotspot of HER2 in breast cancer cells. *RNA Biol* (2015) 12:1139–51. doi: 10.1080/15476286.2015.1076610

37. Lubelsky Y, Ulitsky I. Sequences enriched in alu repeats drive nuclear localization of long RNAs in human cells. *Nature* (2018) 555:107–11. doi: 10.1038/nature25757

38. Mandal M, Vadlamudi R, Nguyen D, Wang RA, Costa L, Bagheri-Yarmand R, et al. Growth factors regulate heterogeneous nuclear ribonucleoprotein K expression and function. *J Biol Chem* (2001) 276:9699–704. doi: 10.1074/jbc.M008514200

39. Anczukow O, Rosenberg AZ, Akerman M, Das S, Zhan L, Karni R, et al. The splicing factor SRSF1 regulates apoptosis and proliferation to promote mammary epithelial cell transformation. *Nat Struct Mol Biol* (2012) 19:220–8. doi: 10.1038/nsmb.2207

40. Jia R, Li C, McCoy JP, Deng CX, Zheng ZM. SRp20 is a proto-oncogene critical for cell proliferation and tumor induction and maintenance. *Int J Biol Sci* (2010) 6:806–26. doi: 10.7150/ijbs.6.806

41. Buoso E, Ronfani M, Galasso M, Ventura D, Corsini E, Racchi M. Cortisol-induced SRSF3 expression promotes GR splicing, RACK1 expression and breast cancer cells migration. *Pharmacol Res* (2019) 143:17–26. doi: 10.1016/j.phrs.2019.03.008

42. Ishii H, Saitoh M, Sakamoto K, Kondo T, Katoh R, Tanaka S, et al. Epithelial splicing regulatory proteins 1 (ESRP1) and 2 (ESRP2) suppress cancer cell motility *via* different mechanisms. *J Biol Chem* (2014) 289:27386–99. doi: 10.1074/jbc.M114.589432

43. Gökmen-Polar Y, Neelamraju Y, Goswami CP, Gu Y, Gu X, Nallamothu G, et al. Splicing factor ESRP1 controls ER-positive breast cancer by altering metabolic pathways. *EMBO Rep* (2019) 20(2):e46078. doi: 10.15252/embr.201846078

44. Rajan P, Gaughan L, Dalgliesh C, El-Sherif A, Robson CN, Leung HY, et al. Regulation of gene expression by the RNA-binding protein Sam68 in cancer. *Biochem Soc Trans* (2008) 36:505–7. doi: 10.1042/BST0360505

45. Locatelli A, Lofgren KA, Daniel AR, Castro NE, Lange CA. Mechanisms of HGF/Met signaling to brk and Sam68 in breast cancer progression. *Horm Cancer* (2012) 3:14–25. doi: 10.1007/s12672-011-0097-z

46. Song L, Wang L, Li Y, Xiong H, Wu J, Li J, et al. Sam68 up-regulation correlates with, and its down-regulation inhibits, proliferation and tumourigenicity of breast cancer cells. *J Pathol* (2010) 222:227–37. doi: 10.1002/path.2751

47. Vanharanta S, Marney CB, Shu W, Valiente M, Zou Y, Mele A, et al. Loss of the multifunctional RNA-binding protein RBM47 as a source of selectable metastatic traits in breast cancer. *Elife* (2014) 3:e02734. doi: 10.7554/eLife.02734

48. Cheng F, Pan Y, Lu YM, Zhu L, Chen S. RNA-Binding protein Dnd1 promotes breast cancer apoptosis by stabilizing the bim mRNA in a miR-221 binding site. *BioMed Res Int* (2017) 2017:9596152. doi: 10.1155/2017/9596152

49. Gu W, Pan F, Singer RH. Blocking beta-catenin binding to the ZBP1 promoter represses ZBP1 expression, leading to increased proliferation and migration of metastatic breast-cancer cells. *J Cell Sci* (2009) 122:1895–905. doi: 10.1242/jcs.045278

50. Gu W, Wells AL, Pan F, Singer RH. Feedback regulation between zipcode binding protein 1 and beta-catenin mRNAs in breast cancer cells. *Mol Cell Biol* (2008) 28:4963–74. doi: 10.1128/MCB.00266-08

51. Zhou Y, Meng X, Chen S, Li W, Li D, Singer R, et al. IMP1 regulates UCA1-mediated cell invasion through facilitating UCA1 decay and decreasing the sponge effect of UCA1 for miR-122-5p. *Breast Cancer Res* (2018) 20:32. doi: 10.1186/s13058-018-0959-1

52. Nwokafor CU, Sellers RS, Singer RH. IMP1, an mRNA binding protein that reduces the metastatic potential of breast cancer in a mouse model. *Oncotarget* (2016) 7:72662–71. doi: 10.18632/oncotarget.12083

53. Kim HY, Ha Thi HT, Hong S. IMP2 and IMP3 cooperate to promote the metastasis of triple-negative breast cancer through destabilization of progesterone receptor. *Cancer Lett* (2018) 415:30–9. doi: 10.1016/j.canlet.2017.11.039

54. Kotta-Loizou I, Vasilopoulos SN, Coutts RH, Theocharis S. Current evidence and future perspectives on HuR and breast cancer development, prognosis, and treatment. *Neoplasia* (2016) 18:674–88. doi: 10.1016/j.neo.2016.09.002

55. Dong Z, Liu J, Zhang JT. Translational regulation of Chk1 expression by eIF3a *via* interaction with the RNA-binding protein HuR. *Biochem J* (2020) 477:1939–50. doi: 10.1042/BCJ20200025

56. Guha A, Ahuja D, Das Mandal S, Parasar B, Deyasi K, Roy D, et al. Integrated regulation of HuR by translation repression and protein degradation determines pulsatile expression of p53 under DNA damage. *iScience* (2019) 15:342–59. doi: 10.1016/j.isci.2019.05.002

57. Shao R, Scully SJJr., Yan W, Bentley B, Mueller J, Brown C, et al. The novel lupus antigen related protein acheron enhances the development of human breast cancer. *Int J Cancer* (2012) 130:544–54. doi: 10.1002/ijc.26015

58. Dermit M, Dodel M, Lee FCY, Azman MS, Schwenzer H, Jones JL, et al. Subcellular mRNA localization regulates ribosome biogenesis in migrating cells. *Dev Cell* (2020) 55:298–313 e10. doi: 10.1016/j.devcel.2020.10.006

59. Zhang F, Yan P, Yu H, Le H, Li Z, Chen J, et al. L ARP7 is a BRCA1 ubiquitinase substrate and regulates genome stability and tumorigenesis. *Cell Rep* (2020) 32:107974. doi: 10.1016/j.celrep.2020.107974

60. Ji X, Lu H, Zhou Q, Luo K. LARP7 suppresses p-TEFb activity to inhibit breast cancer progression and metastasis. *eLife* (2014) 3:e02907. doi: 10.7554/eLife.02907

61. Fu M, Blackshear PJ. RNA-Binding proteins in immune regulation: a focus on CCCH zinc finger proteins. *Nat Rev Immunol* (2017) 17:130–43. doi: 10.1038/nri.2016.129

62. Al-Matouq J, Al-Haj L, Al-Saif M, Khabar KSA. Post-transcriptional screen of cancer amplified genes identifies ERBB2/Her2 signaling as AU-rich mRNA stability-promoting pathway. *Oncogenesis* (2021) 10:61. doi: 10.1038/s41389-021-00351-w

63. Hellborg F, Qian W, Mendez-Vidal C, Asker C, Kost-Alimova M, Wilhelm M, et al. Human wig-1, a p53 target gene that encodes a growth inhibitory zinc finger protein. *Oncogene* (2001) 20:5466–74. doi: 10.1038/sj.onc.1204722

64. Prahl M, Vilborg A, Palmberg C, Jornvall H, Asker C, Wiman KG. The p53 target protein wig-1 binds hnRNP A2/B1 and RNA helicase a *via* RNA. *FEBS Lett* (2008) 582:2173–7. doi: 10.1016/j.febslet.2008.04.065

65. Vilborg A, Glahder J, Wilhelm M, Bersani C, Corcoran M, Mahmoudi S, et al. The p53 target wig-1 regulates p53 mRNA stability through an AU-rich element. *Proc Natl Acad Sci USA* (2009) 106:15756–61. doi: 10.1073/pnas.0900862106

66. Nagaoka K, Fujii K, Zhang H, Usuda K, Watanabe G, Ivshina M, et al. CPEB1 mediates epithelial-to-mesenchyme transition and breast cancer metastasis. *Oncogene* (2016) 35:2893–901. doi: 10.1038/onc.2015.350

67. Yang X, Zhong W, Cao R. Phosphorylation of the mRNA cap-binding protein eIF4E and cancer. *Cell Signal* (2020) 73:109689. doi: 10.1016/j.cellsig.2020.109689

68. Sonenberg N. eIF4E, the mRNA cap-binding protein: from basic discovery to translational research. *Biochem Cell Biol* (2008) 86:178–83. doi: 10.1139/O08-034

69. Robichaud N, Hsu BE, Istomine R, Alvarez F, Blagih J, Ma EH, et al. Translational control in the tumor microenvironment promotes lung metastasis: Phosphorylation of eIF4E in neutrophils. *Proc Natl Acad Sci U S A* (2018) 115:E2202–E9. doi: 10.1073/pnas.1717439115

70. Ramalingam S, Gediya L, Kwegyir-Afful A, Ramamurthy V, Purushottamachar P, Mbatia H, et al. First MNKs degrading agents block phosphorylation of eIF4E, induce apoptosis, inhibit cell growth, migration and invasion in triple negative and Her2-overexpressing breast cancer cell lines. *Oncotarget* (2014) 5:530–43. doi: 10.18632/oncotarget.1528

71. van Kouwenhove M, Kedde M, Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. *Nat Rev Cancer* (2011) 11:644–56. doi: 10.1038/nrc3107

72. Fujita Y, Masuda K, Hamada J, Shoda K, Naruto T, Hamada S, et al. KH-type splicing regulatory protein is involved in esophageal squamous cell carcinoma progression. *Oncotarget* (2017) 8:101130–45. doi: 10.18632/oncotarget.20926

73. Tong L, Luo Y, Wei T, Guo L, Wang H, Zhu W, et al. KH-type splicing regulatory protein (KHSRP) contributes to tumorigenesis by promoting miR-26a maturation in small cell lung cancer. *Mol Cell Biochem* (2016) 422:61–74. doi: 10.1007/s11010-016-2806-y

74. Ladomery M. Aberrant alternative splicing is another hallmark of cancer. *Int J Cell Biol* (2013) 2013:463786. doi: 10.1155/2013/463786

75. Ast G. How did alternative splicing evolve? *Nat Rev Genet* (2004) 5:773–82. doi: 10.1038/nrg1451

76. Bates DO, Morris JC, Oltean S, Donaldson LF. Pharmacology of modulators of alternative splicing. *Pharmacol Rev* (2017) 69:63–79. doi: 10.1124/pr.115.011239

77. De Craene B, Berx G. Regulatory networks defining EMT during cancer initiation and progression. *Nat Rev Cancer* (2013) 13:97–110. doi: 10.1038/nrc3447

78. Zheng X, Peng Q, Wang L, Zhang X, Huang L, Wang J, et al. Serine/arginine-rich splicing factors: the bridge linking alternative splicing and cancer. *Int J Biol Sci* (2020) 16:2442–53. doi: 10.7150/ijbs.46751

79. More DA, Kumar A. SRSF3: Newly discovered functions and roles in human health and diseases. *Eur J Cell Biol* (2020) 99:151099. doi: 10.1016/j.ejcb.2020.151099

80. Geuens T, Bouhy D, Timmerman V. The hnRNP family: insights into their role in health and disease. *Hum Genet* (2016) 135:851–67. doi: 10.1007/s00439-016-1683-5

81. Kedzierska H, Piekielko-Witkowska A. Splicing factors of SR and hnRNP families as regulators of apoptosis in cancer. *Cancer Lett* (2017) 396:53–65. doi: 10.1016/j.canlet.2017.03.013

82. Paronetto MP, Achsel T, Massiello A, Chalfant CE, Sette C. The RNA-binding protein Sam68 modulates the alternative splicing of bcl-x. *J Cell Biol* (2007) 176:929–39. doi: 10.1083/jcb.200701005

83. Frederick MI, Heinemann IU. Regulation of RNA stability at the 3' end. *Biol Chem* (2021) 402:425–31. doi: 10.1515/hsz-2020-0325

84. Garneau NL, Wilusz J, Wilusz CJ. The highways and byways of mRNA decay. *Nat Rev Mol Cell Biol* (2007) 8:113–26. doi: 10.1038/nrm2104

85. Shivalingappa P, Sharma V, Shiras A, Bapat SJM. Biochemistry c. RNA binding motif 47 (RBM47): emerging roles in vertebrate development, RNA editing and cancer. *Mol Cell Biochem* (2021) 476(12):4493–505. doi: 10.1007/s11010-021-04256-5

86. Huang X, Zhang H, Guo X, Zhu Z, Cai H, Kong X. Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) in cancer. *J Hematol Oncol* (2018) 11:88. doi: 10.1186/s13045-018-0628-y

87. Calaluce R, Gubin MM, Davis JW, Magee JD, Chen J, Kuwano Y, et al. The RNA binding protein HuR differentially regulates unique subsets of mRNAs in estrogen receptor negative and estrogen receptor positive breast cancer. *BMC Cancer* (2010) 10:126. doi: 10.1186/1471-2407-10-126

88. Erson-Bensan AE, Can T. Alternative polyadenylation: Another foe in cancer. *Mol Cancer Res* (2016) 14:507–17. doi: 10.1158/1541-7786.MCR-15-0489

89. Fernandez-Miranda G, Mendez R. The CPEB-family of proteins, translational control in senescence and cancer. *Ageing Res Rev* (2012) 11:460–72. doi: 10.1016/j.arr.2012.03.004

90. Pascual R, Martín J, Salvador F, Reina O, Chanes V, Millanes-Romero A, et al. The RNA binding protein CPEB2 regulates hormone sensing in mammary gland development and luminal breast cancer. *Sci Adv* (2020) 6:eaax3868. doi: 10.1126/sciadv.aax3868

91. Lu R, Zhou Z, Yu W, Xia Y, Zhi X. CPEB4 promotes cell migration and invasion *via* upregulating vimentin expression in breast cancer. *Biochem Biophys Res Commun* (2017) 489:135–41. doi: 10.1016/j.bbrc.2017.05.112

92. Sun HT, Wen X, Han T, Liu ZH, Li SB, Wang JG, et al. Expression of CPEB4 in invasive ductal breast carcinoma and its prognostic significance. *Onco Targets Ther* (2015) 8:3499–506. doi: 10.2147/OTT.S87587

93. Doron-Mandel E, Koppel I, Abraham O, Rishal I, Smith TP, Buchanan CN, et al. The glycine arginine-rich domain of the RNA-binding protein nucleolin regulates its subcellular localization. *EMBO J* (2021) 40:e107158. doi: 10.15252/embj.2020107158

94. Jonas K, Calin GA, Pichler M. RNA-Binding proteins as important regulators of long non-coding RNAs in cancer. *Int J Mol Sci* (2020) 21(8):2969. doi: 10.3390/ijms21082969

95. Degrauwe N, Suvà M, Janiszewska M, Riggi N, Stamenkovic IJG. Development. IMPs: an RNA-binding protein family that provides a link between stem cell maintenance in normal development and cancer. *Genes Dev* (2016) 30:2459–74. doi: 10.1101/gad.287540.116

96. Schneider-Lunitz V, Ruiz-Orera J, Hubner N, van Heesch S. Multifunctional RNA-binding proteins influence mRNA abundance and translational efficiency of distinct sets of target genes. *PloS Comput Biol* (2021) 17:e1009658. doi: 10.1371/journal.pcbi.1009658

97. Chaudhury A, Hussey GS, Ray PS, Jin G, Fox PL, Howe PH. TGF-beta-mediated phosphorylation of hnRNP E1 induces EMT *via* transcript-selective translational induction of Dab2 and ILEI. *Nat Cell Biol* (2010) 12:286–93. doi: 10.1038/ncb2029

98. Hussey GS, Chaudhury A, Dawson AE, Lindner DJ, Knudsen CR, Wilce MC, et al. Identification of an mRNP complex regulating tumorigenesis at the translational elongation step. *Mol Cell* (2011) 41:419–31. doi: 10.1016/j.molcel.2011.02.003

99. Durie D, Lewis SM, Liwak U, Kisilewicz M, Gorospe M, Holcik M. RNA-Binding protein HuR mediates cytoprotection through stimulation of XIAP translation. *Oncogene* (2011) 30:1460–9. doi: 10.1038/onc.2010.527

100. Feng C, Neumeister V, Ma W, Xu J, Lu L, Bordeaux J, et al. Lin28 regulates HER2 and promotes malignancy through multiple mechanisms. *Cell Cycle* (2012) 11:2486–94. doi: 10.4161/cc.20893

101. Liao B, Hu Y, Brewer G. Competitive binding of AUF1 and TIAR to MYC mRNA controls its translation. *Nat Struct Mol Biol* (2007) 14:511–8. doi: 10.1038/nsmb1249

102. Wang Z, Tong D, Han C, Zhao Z, Wang X, Jiang T, et al. Blockade of miR-3614 maturation by IGF2BP3 increases TRIM25 expression and promotes breast cancer cell proliferation. *EBioMedicine* (2019) 41:357–69. doi: 10.1016/j.ebiom.2018.12.061

103. Guo X, Connick MC, Vanderhoof J, Ishak MA, Hartley RS. MicroRNA-16 modulates HuR regulation of cyclin E1 in breast cancer cells. *Int J Mol Sci* (2015) 16:7112–32. doi: 10.3390/ijms16047112

104. Stavraka C, Blagden S. The la-related proteins, a family with connections to cancer. *Biomolecules* (2015) 5:2701–22. doi: 10.3390/biom5042701

105. Babic I, Jakymiw A, Fujita DJ. The RNA binding protein Sam68 is acetylated in tumor cell lines, and its acetylation correlates with enhanced RNA binding activity. *Oncogene* (2004) 23:3781–9. doi: 10.1038/sj.onc.1207484

106. Anczukow O, Akerman M, Clery A, Wu J, Shen C, Shirole NH, et al. SRSF1-regulated alternative splicing in breast cancer. *Mol Cell* (2015) 60:105–17. doi: 10.1016/j.molcel.2015.09.005

107. Ke H, Zhao L, Zhang H, Feng X, Xu H, Hao J, et al. Loss of TDP43 inhibits progression of triple-negative breast cancer in coordination with SRSF3. *Proc Natl Acad Sci U S A* (2018) 115:E3426–E35. doi: 10.1073/pnas.1714573115

108. Einstein JM, Perelis M, Chaim IA, Meena JK, Nussbacher JK, Tankka AT, et al. Inhibition of YTHDF2 triggers proteotoxic cell death in MYC-driven breast cancer. *Mol Cell* (2021) 81:3048–64 e9. doi: 10.1016/j.molcel.2021.06.014

109. Gautrey HL, Tyson-Capper AJ. Regulation of mcl-1 by SRSF1 and SRSF5 in cancer cells. *PloS One* (2012) 7:e51497. doi: 10.1371/journal.pone.0051497

110. Pisera A, Campo A, Campo S. Structure and functions of the translation initiation factor eIF4E and its role in cancer development and treatment. *J Genet Genomics* (2018) 45:13–24. doi: 10.1016/j.jgg.2018.01.003

111. Gao X, Liu Z, Zhong M, Wu K, Zhang Y, Wang H, et al. Knockdown of DNA/RNA-binding protein KIN17 promotes apoptosis of triple-negative breast cancer cells. *Oncol Lett* (2019) 17:288–93. doi: 10.3892/ol.2018.9597

112. Zhao D, Zheng S, Wang X, Liu H, Zhao K, Li L, et al. iASPP is essential for HIF-1α stabilization to promote angiogenesis and glycolysis via attenuating VHL-mediated protein degradation. *Oncogene* (2022) 41(13):1944–58. doi: 10.1038/s41388-022-02234-9

113. Gubin MM, Calaluce R, Davis JW, Magee JD, Strouse CS, Shaw DP, et al. Overexpression of the RNA binding protein HuR impairs tumor growth in triple negative breast cancer associated with deficient angiogenesis. *Cell Cycle* (2010) 9:3337–46. doi: 10.4161/cc.9.16.12711

114. Zhou S, Wang GP, Liu C, Zhou M. Eukaryotic initiation factor 4E (eIF4E) and angiogenesis: prognostic markers for breast cancer. *BMC Cancer* (2006) 6:231. doi: 10.1186/1471-2407-6-231

115. Yang S, Hewitt S, Steinberg S, Liewehr D, Swain S. Expression levels of eIF4E, VEGF, and cyclin D1, and correlation of eIF4E with VEGF and cyclin D1 in multi-tumor tissue microarray. *Oncol Rep* (2007) 17:281–7. doi: 10.3892/or.17.2.281

116. De Santis C, Gotte M. The role of microRNA let-7d in female malignancies and diseases of the female reproductive tract. *Int J Mol Sci* (2021) 22(14):7359. doi: 10.3390/ijms22147359

117. Li Q, Zeng C, Liu H, Yung KWY, Chen C, Xie Q, et al. Protein-protein interaction inhibitor of SRPKs alters the splicing isoforms of VEGF and inhibits angiogenesis. *iScience* (2021) 24:102423. doi: 10.1016/j.isci.2021.102423

118. Griseri P, Pages G. Control of pro-angiogenic cytokine mRNA half-life in cancer: the role of AU-rich elements and associated proteins. *J Interferon Cytokine Res* (2014) 34:242–54. doi: 10.1089/jir.2013.0140

119. Yan W, Zhang Y, Zhang J, Cho SJ, Chen X. HuR is necessary for mammary epithelial cell proliferation and polarity at least in part *via* DeltaNp63. *PloS One* (2012) 7:e45336. doi: 10.1371/journal.pone.0045336

120. Kim BC, Lee HC, Lee JJ, Choi CM, Kim DK, Lee JC, et al. Wig1 prevents cellular senescence by regulating p21 mRNA decay through control of RISC recruitment. *EMBO J* (2012) 31:4289–303. doi: 10.1038/emboj.2012.286

121. Al-Khalaf HH, Aboussekhra A. p16(INK4A) induces senescence and inhibits EMT through microRNA-141/microRNA-146b-5p-dependent repression of AUF1. *Mol Carcinog* (2017) 56:985–99. doi: 10.1002/mc.22564

122. Das S, Fregoso OI, Krainer AR. A new path to oncogene-induced senescence: at the crossroads of splicing and translation. *Cell Cycle* (2013) 12:1477–9. doi: 10.4161/cc.24749

123. Howley BV, Mohanty B, Dalton A, Grelet S, Karam J, Dincman T, et al. The ubiquitin E3 ligase ARIH1 regulates hnRNP E1 protein stability, EMT and breast cancer progression. *Oncogene* (2022) 41(12):1679–90. doi: 10.1038/s41388-022-02199-9

124. Gao R, Yu Y, Inoue A, Widodo N, Kaul SC, Wadhwa R. Heterogeneous nuclear ribonucleoprotein K (hnRNP-K) promotes tumor metastasis by induction of genes involved in extracellular matrix, cell movement, and angiogenesis. *J Biol Chem* (2013) 288:15046–56. doi: 10.1074/jbc.M113.466136

125. Iino K, Mitobe Y, Ikeda K, Takayama KI, Suzuki T, Kawabata H, et al. RNA-Binding protein NONO promotes breast cancer proliferation by post-transcriptional regulation of SKP2 and E2F8. *Cancer Sci* (2020) 111:148–59. doi: 10.1111/cas.14240

126. Mitobe Y, Iino K, Takayama KI, Ikeda K, Suzuki T, Aogi K, et al. PSF promotes ER-positive breast cancer progression *via* posttranscriptional regulation of ESR1 and SCFD2. *Cancer Res* (2020) 80:2230–42. doi: 10.1158/0008-5472.CAN-19-3095

127. Liu Y, Sun H, Li X, Liu Q, Zhao Y, Li L, et al. Identification of a three-RNA binding proteins (RBPs) signature predicting prognosis for breast cancer. *Front Oncol* (2021) 11:663556. doi: 10.3389/fonc.2021.663556

128. Lin Y, Zhang J, Cai J, Liang R, Chen G, Qin G, et al. Systematic analysis of gene expression alteration and Co-expression network of eukaryotic initiation factor 4A-3 in cancer. *J Cancer* (2018) 9:4568–77. doi: 10.7150/jca.27655

129. Zhu Y, Ren C, Yang L. Effect of eukaryotic translation initiation factor 4A3 in malignant tumors. *Oncol Lett* (2021) 21:358. doi: 10.3892/ol.2021.12619

130. Kelaini S, Chan C, Cornelius VA, Margariti A. RNA-Binding proteins hold key roles in function, dysfunction, and disease. *Biol (Basel)* (2021) 10(5):366. doi: 10.3390/biology10050366

131. Kentsis A, Topisirovic I, Culjkovic B, Shao L, Borden K. Ribavirin suppresses eIF4E-mediated oncogenic transformation by physical mimicry of the 7-methyl guanosine mRNA cap. *Proc Natl Acad Sci USA* (2004) 101:18105–10. doi: 10.1073/pnas.0406927102

132. Assouline S, Culjkovic B, Cocolakis E, Rousseau C, Beslu N, Amri A, et al. Molecular targeting of the oncogene eIF4E in acute myeloid leukemia (AML): a proof-of-principle clinical trial with ribavirin. *Blood* (2009) 114:257–60. doi: 10.1182/blood-2009-02-205153

133. Li S, Jia Y, Jacobson B, McCauley J, Kratzke R, Bitterman PB, et al. Treatment of breast and lung cancer cells with a n-7 benzyl guanosine monophosphate tryptamine phosphoramidate pronucleotide (4Ei-1) results in chemosensitization to gemcitabine and induced eIF4E proteasomal degradation. *Mol Pharm* (2013) 10:523–31. doi: 10.1021/mp300699d

134. Moerke NJ, Aktas H, Chen H, Cantel S, Reibarkh MY, Fahmy A, et al. Small-molecule inhibition of the interaction between the translation initiation factors eIF4E and eIF4G. *Cell* (2007) 128:257–67. doi: 10.1016/j.cell.2006.11.046

135. Haque S, Cook K, Sahay G, Sun C. RNA-Based therapeutics: Current developments in targeted molecular therapy of triple-negative breast cancer. *Pharmaceutics* (2021) 13(10):1694. doi: 10.3390/pharmaceutics13101694

136. Blagden SP, Willis AE. The biological and therapeutic relevance of mRNA translation in cancer. *Nat Rev Clin Oncol* (2011) 8:280–91. doi: 10.1038/nrclinonc.2011.16

137. Dong K, Wang R, Wang X, Lin F, Shen JJ, Gao P, et al. Tumor-specific RNAi targeting eIF4E suppresses tumor growth, induces apoptosis and enhances

cisplatin cytotoxicity in human breast carcinoma cells. *Breast Cancer Res Treat* (2009) 113:443–56. doi: 10.1007/s10549-008-9956-x

138. Berger CM, Gaume X, Bouvet P. The roles of nucleolin subcellular localization in cancer. *Biochimie* (2015) 113:78–85. doi: 10.1016/j.biochi.2015.03.023

139. Bates PJ, Laber DA, Miller DM, Thomas SD, Trent JO. Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer. *Exp Mol Pathol* (2009) 86:151–64. doi: 10.1016/j.yexmp.2009.01.004

140. Tian T, Zhao Y, Zheng J, Jin S, Liu Z, Wang T. Circular RNA: A potential diagnostic, prognostic, and therapeutic biomarker for human triple-negative breast cancer. *Mol Ther Nucleic Acids* (2021) 26:63–80. doi: 10.1016/j.omtn.2021.06.017

141. Yi Z, Li Y, Wu Y, Zeng B, Li H, Ren G, et al. Circular RNA 0001073 attenuates malignant biological behaviours in breast cancer cell and is delivered by nanoparticles to inhibit mice tumour growth. *Onco Targets Ther* (2020) 13:6157–69. doi: 10.2147/OTT.S248822

142. Kristensen LS, Hansen TB, Veno MT, Kjems J. Circular RNAs in cancer: opportunities and challenges in the field. *Oncogene* (2018) 37:555–65. doi: 10.1038/onc.2017.361

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 18 August 2022
DOI 10.3389/fonc.2022.974523

OPEN ACCESS

EDITED BY
Maria Rosaria De Miglio,
University of Sassari, Italy

REVIEWED BY
Greco Hernández,
National Institute of Cancerology (INCAN), Mexico
Jinan Wang,
University of Kansas, United States

*CORRESPONDENCE
Lufeng Zheng
zhlf@CPU.edu.cn
Hai Qin
18786665889@163.com

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 21 June 2022
ACCEPTED 27 July 2022
PUBLISHED 18 August 2022

CITATION
Chen Y, Qin H and Zheng L (2022) Research progress on RNA–binding proteins in breast cancer.
Front. Oncol. 12:974523.
doi: 10.3389/fonc.2022.974523

Research progress on RNA–binding proteins in breast cancer

Ying Chen[1], Hai Qin[2*] and Lufeng Zheng[1*]

[1]School of Life Science and Technology, Jiangsu Key Laboratory of Carcinogenesis and Intervention, China Pharmaceutical University, Nanjing, China, [2]Department of Clinical Laboratory, Guizhou Provincial Orthopedic Hospital, Guiyang, China

Breast cancer is the most common malignancy in women and has a high incidence rate and mortality. Abnormal regulation of gene expression plays an important role in breast cancer occurrence and development. RNA-binding proteins (RBPs) are one kind of the key regulators for gene expression. By interacting with RNA, RBPs are widely involved in RNA cutting, transport, editing, intracellular localization, and translation regulation. RBPs are important during breast cancer occurrence and progression by engaging in many aspects, like proliferation, migration, invasion, and stemness. Therefore, comprehensively understanding the role of RBPs in breast cancer progression can facilitate early diagnosis, timely treatment, and long-term survival and quality of life of breast cancer patients.

KEYWORDS

breast cancer, RNA-binding proteins, research progress, mRNA, 3'UTR

Introduction

Breast cancer is the most common malignancy affecting women and is a highly heterogeneous disease including several subtypes, which are defined by the differential expression of receptors on the cell surface (1). The progression and occurrence of breast cancer are contributed by the ectopic gene expression, which is regulated transcriptionally, post-transcriptionally, translationally, and post-translationally.

RNA biosynthesis and metabolism is one of the key steps of gene regulation. An increasing number of evidence shows that RNA expression profile in cancer cells is significantly different from that in benign cells, suggesting that the abnormal regulation of RNA metabolism may be related to the occurrence and progression of tumors (2). RNA binding proteins (RBPs) are the major regulators of RNA metabolism and crucial in all steps of gene expression (3). As a kind of unstable and degradable biomacromolecules, mRNAs bind to specific RBPs and form complexes to maintain their stability in cells, within which RBPs control the localization, stability, translation, and degradation of RNA by binding to different regions of mRNAs . In addition, the

binding of RBPs to RNA contributes to RNA metabolism at different stages and regulates its subsequent function. Currently, exceeding 2000 RBPs are known to interact with RNA through different binding surfaces. The roles of abnormally expressed RBPs in human diseases (such as cancer, viral infection) and the potential application of RBPs as a therapeutic target or diagnostic marker represent a rapidly expanding research field (4). RBPs are disordered in various tumors, and affect the expression and function of tumor-related transcripts, so as to play different biological roles in tumor progression, such as proliferation, invasion, migration, stemness, and angiogenesis. This article will review recent advances in RBPs related to breast cancer (Figure 1; Table 1).

RNA-binding motif protein 38 (RBM38)

RBM38, also known as RNPC1, is a member of the RBP family. *RBM38* gene is located on chromosome 20q13 and belongs to the RNA recognition motif (RRM) family of RBP. *RBM38* contains the classical RRM domain and is expressed as RNPC1a with 239 amino acids and RNPC1b with 121 amino acids, respectively. RBM38 exists in various tissues (48), including bone marrow, lymph nodes, human blood, brain, breast, colorectal, lung, and other organs. Additionally, RBM38 has been shown to participate in the progression of breast cancer (49), acute myeloid leukemia (50), colorectal cancer, and renal

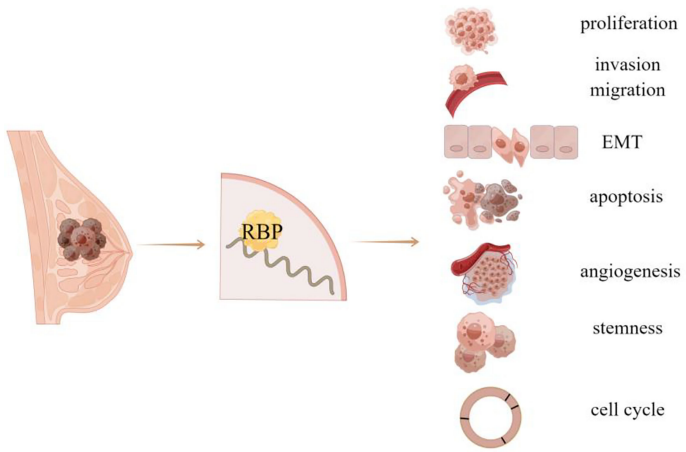


FIGURE 1
RBP is related to the occurrence and progression of breast cancer.

TABLE 1 Summary of the cellular functions of RBPs in breast cancer.

RBP	Expression in breast cancer	Functions	Pathways/targets	References
RBM38	Downregulation	Inhibits proliferation, invasion, migration, EMT; regulates the cell cycle	p53, c-Myc, PTEN, ZO-1, STARD13-correlated ceRNA network	(5–9)
PCBP2	Upregulation	Promotes migration, proliferation, invasion, stemness, EMT and cholesterol synthesis; inhibits apoptosis	UFD1, NT5E, lnc030, SQLE, PI3K/Akt signaling pathways	(10, 11)
QKI	Downregulation	Inhibits self-renewal, EMT, cell contact, proliferation, migration, invasion; regulates the cell cycle and apoptosis	RASA1, MAPK signaling pathways, FOXO1, lncRNA ST8SIA6-AS1	(12–14)
HuR	Upregulation	Promotes invasion, proliferation, migration, angiogenesis; regulates the cell cycle; inhibits apoptosis	Snail, MMP-9, uPAR, FOXQ1, VEGFA, CDK3, lncRNA AGAP2-AS1, MTA1, TNF-α	(15–23)
LIN28	Upregulation	Promotes proliferation, migration, invasion, stemness; regulates aerobic glycolysis, Warburg effect and pH	let-7, CAIX, miR-638, CREB1, VASP, MSI2, YAP1, Hippo signaling pathways	(24–32)
SAM68	Upregulation	Promotes survival, proliferation, migration and invasion; regulates the cell cycle	CBP/β-catenin, Insulin and leptin signaling pathway, MAPK/PI3K signaling pathways, p21 and p27, FOXO, Akt/GSK-3β signal transduction, Rad51, PARP	(33–38)
MSI	Upregulation	Promotes stemness, chemoresistance and proliferation; regulates the cell cycle; inhibits apoptosis and invasion	$p21^{Cip1}$, TAC1, EMT, ERK1/2, TP53INP1, ESR1, Notch	(39–47)

cell carcinoma (51), *via* transcriptionally regulating many downstream targets in different ways (51).

Studies have shown that ectopic expression of RBM38 can inhibit the proliferation of breast cancer cells, while knockdown of RBM38 exhibits an opposite effect *in vivo* and *in vitro* (52). When RBM38 is overexpressed, it inhibits the migration and invasion of breast cancer cells by inducing cell cycle arrest and inhibiting mutant p53-induced epithelial-mesenchymal transition (EMT) (5). EMT is triggered by individual extracellular signals, including extracellular matrix components, such as collagen and hyaluronic acid, and soluble growth factors, like transforming growth factor–β (TGF-β), fibroblast growth factor (FGF), and epidermal growth factor (EGF). TGF-β is one of the most famous EMT inducers (53). Elevated levels of TGF-β in malignant breast cells enhance breast cancer invasion, migration, and immune evasion. It is found that TGF-β induces a significant down-regulation of RBM38 in breast cancer, which is directly regulated by Snail, a transcription factor targeting at the E-box element of the *RBM38* gene promoter region (6). In addition, Zonula occludens-1 (ZO-1) is downregulated in response to TGF-β, which can control endothelial cell-cell tension, cell migration, and barrier formation, while RBM38 positively regulates ZO-1 transcript by directly binding to AU/U rich elements (Ares) on its mRNA 3'-untranslated region (3'UTR), thus inhibiting cell migration and invasion (6).

Furthermore, RBM38 often functions by forming regulatory loops with related genes, for example, Li et al. (7) have shown that RBM38 acted as a tumor suppressor by inhibiting the expression of c-Myc *via* directly targeting the Ares in *c-Myc* mRNA 3'UTR, and thus destabilizing *c-Myc* transcript; In turn, c-Myc inhibits RBM38 expression by directly binding to the E-box motif in the promoter region of *RBM38* gene. RBM38 can also function through the tumor suppressor PTEN, as evident by that RBM38 can enhance the stability of PTEN mRNA and increase the expression of PTEN protein by directly targeting *PTEN* 3'UTR (8). Notably, our previous study also identified novel targets of RBM38 in breast cancer. We found that the expression of RBM38 was positively correlated with the relapse free survival and overall survival of patients with breast cancer, and RBM38 can promote the competing endogenous RNA (ceRNA) network crosstalk among STARD13, CDH5, HOXD10 and HOXD1 (STARD13-correlated ceRNA network) and then inhibit the metastasis of breast cancer cells (9) (Figure 2). Zhu et al. (54) selected 161 cases of breast cancer tissues to explore the relationship between RBM38 expression and distant metastasis and prognosis of breast cancer. The results showed that the high expression of RBM38 was positively correlated with the low rate of distant metastasis and good prognosis in patients with breast cancer (Table 2).

Poly (C) binding protein 2 (PCBP2)

PCBP family plays a central role in transcriptional and translational regulation, including mRNA stability, translation silencing, and translation enhancement (55–57). It has been proved that PCBP plays an important role in tumor progression, including apoptosis, proliferation, invasion, and EMT (58). PCBP2, a member of the PCBP family, is an RBP that can

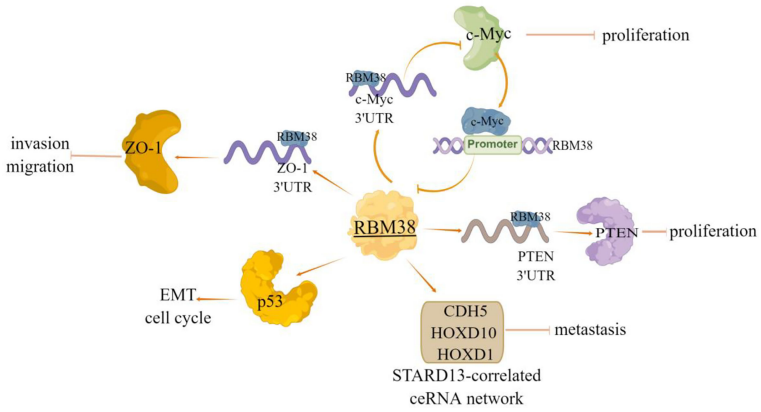


FIGURE 2
The regulatory mechanism of RBM38 in breast cancer. RBM38 can directly bind 3'UTR of *ZO-1* and *PTEN*, positively regulate their transcripts, and inhibit cell migration and invasion. RBM38 inhibits the migration and invasion of breast cancer cells by inducing cell cycle arrest and inhibiting mutant p53-induced EMT. RBM38 can act as a tumor suppressor by forming a regulatory loop with related genes, and inhibit c-Myc expression by directly targeting 3'UTR of *c-Myc* mRNA. In turn, c-Myc inhibits RBM38 expression by directly binding to the E-box motif in the *RBM38* promoter region. RBM38 can promote ceRNA interactions among STARD13, CDH5, HOXD10 and HOXD1 (STARD13-correlated ceRNA network), by promoting the expression of these four genes, inhibit breast cancer cell metastasis. Arrows indicate activation and blunted lines indicate inhibition.

TABLE 2 RBP and clinical relevance.

RBP	Clinical Relevance	References
RBM38	The high expression of RBM38 is positively correlated with the low rate of distant metastasis and good prognosis in patients with breast cancer.	(54)
HuR	Patients with high levels of cytoplasmic HuR have a higher risk of metastasis.	(17)
LIN28	The expression of LIN28 is related to the stage and subtype of advanced disease in patients with breast cancer, and the expression of LIN28 may be an independent prognostic factor.	(30)
MSI	MSI-1 is a negative prognostic marker for disease-free and distant metastasis free survival of breast cancer, which has a negative impact on the overall survival rate. Low expression of MSI-2 is associated with poor prognosis in patients with breast cancer.	(40, 42)

regulate RNA stability *via* directly binding to the single stranded poly (C) motifs of RNAs (10). Several studies have demonstrated the functional role of PCBP2 in the progression of several cancers, including glioma, gastric cancer, pancreatic ductal adenocarcinoma, and hepatocellular carcinoma (58–61). In breast cancer, Thomas et al. (62) used RNA sequencing data to analyze the expression patterns of transcriptional subtypes of eleven estrogen receptor positive (ER+) subtypes and fourteen triple negative (TN) subtypes of breast tumors compared the RNAseq data of 594 cases from the TCGA cohort and identified several RNA processing factors differentially expressing among tumor subtypes and/or regulated by ER, among which *PCBP2* was ranked. A recent study showed that the expression of PCBP2 protein was increased significantly in breast cancer tissues and cell lines, which was due to selective cleavage and polyadenylation (APA) (10). Functionally, PCBP2 promoted the carcinogenesis and metastasis of breast cancer by directly regulating the expression of ubiquitin recognition factor in ER-associated degradation 1 (UFD1) and 5'-nucleotidase ecto (NT5E) *via* binding to their 3'UTRs (10). Additionally, Qin et al. (11) identified that a new long non-coding RNA (lncRNA), named lnc030, is highly expressed in breast cancer stem cells (BCSCs) *in vitro* and *in vivo*. And lnc030 cooperates with PCBP2 to stabilize squalene cyclooxygenase (SQLE) mRNA, resulting in increased cholesterol synthesis, which subsequently facilitates the stemness of BCSCs *via* activating PI3K/Akt signaling (Figure 3).

Quaking (QKI)

QKI belongs to the RBP of signal transduction and activation RNA (STAR) family (63). It was firstly found in and important for the central and peripheral nervous systems. Human *QKI* gene expresses three main alternative splicing transcripts, namely *QKI-5*, *QKI-6* and *QKI-7*, among which QKI-5 is the only subtype located in the nucleus, while QKI-6 and QKI-7 are distributed in the cytoplasm (64). QKI specifically binds to cis elements in 3'UTR (65), and regulates precursor RNA (pre-mRNA) processing, mRNA output, mRNA stability, and protein translation of target genes (66–69). Increasing evidence showed that QKI may be a tumor suppressor in various malignant tumors, including colon cancer, lung cancer, oral cancer, and prostate cancer (70–73), for example, QKI

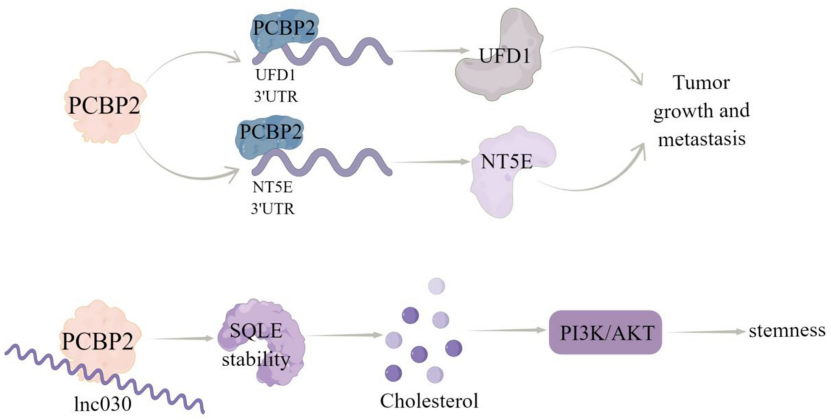


FIGURE 3
The regulatory mechanism of PCBP2 in breast cancer. PCBP2 can directly bind 3'UTR of UFD1 and NT5E, positively regulate their transcripts,and promote tumor growth and metastasis. Lnc030 cooperates with PCBP2 to stabilize SQLE mRNA, increase cholesterol synthesis, activate PI3K/Akt signal transduction, and regulate cholesterol synthesis and stemness properties of BCSCs. Arrows indicate activation and blunted lines indicate inhibition.

impairs the self-renewal and EMT of oral squamous cell carcinoma cells (70–73); QKI can also regulate cell communication and inhibit the progression of clear cell renal cell carcinoma (74). Notably, QKI mRNA and protein are downregulated in breast cancer and decreased QKI expression was significantly associated with ER, PR, and HER2 positive, non-basal-like breast carcinoma and non-triple-negative breast cancer. Meanwhile, QKI expression is positively correlated with the survival of patients, suggesting the prognostic value of QKI in breast cancer patients (12).

Cao et al. (12) have shown that the decreased expression or activity of RASA1 can activate MAPK signaling pathway by reducing the GTPase activity of Ras protein, thereby increasing cell proliferation, inhibiting apoptosis and regulate cell cycle distribution; QKI can directly bind to RASA1 mRNA and enhance RASA1 expression by stabilizing its transcripts, through which QKI overexpression weakens the phosphorylation of MAPK signaling pathway, thereby inhibiting the activation of MAPK pathway and breast cancer progression. In addition, Forkhead box O1 (FoxO1) is a key tumor suppressor for cell proliferation, which can control cell cycle and apoptosis and dysregulation of *FoxO1* expression has been observed in various cancers (13). QKI may cause dysgenesis of FoxO1 through post-transcriptional inhibition, and lead to the occurrence and progression of breast cancer, which is characterized by that QKI can directly bind to the 3'UTR of *FoxO1* and reduce its mRNA stability, this effect is critical for breast cancer occurrence and progression (13).

Furthermore, QKI can bind to ncRNAs to modulate breast cancer progression, like Chen et al. (75) demonstrated that QKI could interact with lncRNA *ST8SIA6-AS1*, to promote proliferation, migration, and invasion of breast cancer cells. And Hu et al. (14) showed that lncRNA *TPT1-AS1* may act as a ceRNA of *miR-330-3p* to upregulate QKI expression, thereby inhibiting the proliferation, migration, and invasion of breast cancer cells. Notably, Gu et al. (76) carried out immunohistochemical evaluation (IHC) of QKI protein expression and prognostic value of 108 patients with breast cancer. The obtained results showed that QKI expression predicted a better prognostic value in BC patients, and the correlation between QKI and EMT was verified in the coexpression analysis of METABRIC datasets (Figure 4).

Hu-antigen R (HuR)

HuR, also known as embryonic lethal abnormal visual protein 1 (ELAVL1), is a

widely expressed post-transcriptional regulatory factor. Although HuR is mainly located in the nucleus, its function of stabilizing and regulating target mRNA translation is tightly related to its translocation to the cytoplasm (77). HuR preferentially binds to mRNA with adenine and uridine rich elements (ARE) or uridine rich sequences, usually locating in 3'UTR (78, 79). ARE is a specific cis element of mRNA, which can target mRNAs for rapid exosomal degradation (80).

HuR has been reported to interact with the mRNA 3'UTR of transcription factor Snail, metallopeptidase MMP-9 (15), and serine protease uPAR (16), among which Snail can induce EMT, while MMP-9 and uPAR are involved in extracellular matrix (ECM) degradation. Therefore, HuR is thought to promote invasion and metastasis by increasing the expression of proteins that induce EMT and degrade ECM. In consistent, inhibition of HuR using the small molecule inhibitor KH-3, can

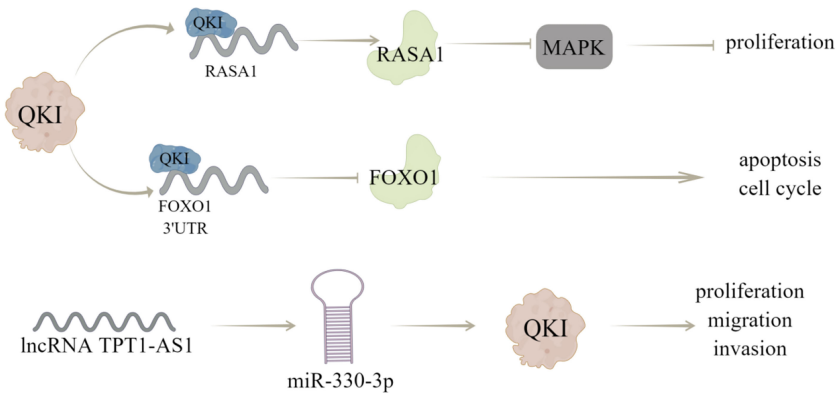


FIGURE 4
The regulatory mechanism of QKI in breast cancer. QKI can directly combine with *RASA1* mRNA to enhance its expression and reduce the phosphorylation of MAPK signaling pathway, thus inhibiting the activation of MAPK pathway and proliferation of breast cancer cells. QKI can directly bind to 3'UTR of *FoxO1*, reduce its mRNA stability, and regulate cell cycle and apoptosis. LncRNA *TPT1-AS1* can act as a ceRNA of *miR-330-3p* to up regulate QKI expression, thus inhibiting the proliferation, migration, and invasion of breast cancer cells. Arrows indicate activation and blunted lines indicate inhibition.

inhibit the invasion of breast cancer cells by destroying the HuR-FOXQ1 mRNA interaction (17). Additionally, Yang et al. (18) proved that HuR protein may be a useful target for the screening of anti-tumor angiogenesis drugs, as they found that a compound ZM-32 could effectively prevent the formation of HuR RRM1/2–VEGFA mRNA complex, thus suppressing the proliferation, migration, growth, and angiogenesis of breast cancer cells. Zhu et al. (19) also revealed the HuR-dependent anti-angiogenic effect of eltrombopag in breast cancer, and further emphasized the effectiveness of HuR inhibitors on tumor inhibition, especially angiogenesis.

Cyclin dependent kinase (CDK) plays a key role in regulating the process of cell cycle. In human cancers, including breast cancer, hepatocellular carcinoma and lymphoma, a series of upstream regulatory factors and downstream substrates of CDK participate in abnormal CDK-related signal transduction (20). HuR can directly bind to and regulate the expression of CDK3 mRNA, thereby promoting the progression of breast cancer (21). In addition, HuR and CDK3 expression levels were positively correlated and significantly up-regulated in breast cancer samples (21). Furthermore, Wang et al. (22) revealed that docetaxel (DTX) induces apoptosis of MCF-7 cells through the SIDT2/NOX4/JNK/HuR axis-mediated TNF-α expression. Notably, HuR can also bind to ncRNA in breast cancer, like Wu et al. (23) determined that HuR could bind to lncRNA *AGAP2-AS1* to stabilize AGAP2-AS1 expression and the AGAP2-AS1-HuR complex upregulates H3K27ac level in *MTA1* promoter region to improve *MTA1* promoter activity and expression, thereby inducing the resistance breast cancer cells to apoptosis (Figure 5). Wu et al. (17) studied 140 samples of patients with breast cancer and found that high cytoplasmic HuR was significantly correlated with high tumor grade, low overall survival and distant disease-free survival. In addition, in the sample, 63.6% of patients with metastasis have high cytoplasmic HuR, indicating that patients with high levels of cytoplasmic HuR have a higher risk of metastasis (Table 2).

Based on the critical roles of HuR in cancer cells, a variety of methods have been developed to inhibit HuR, including inhibiting HuR/mRNA interaction, HuR dimerization/polymerization, HuR nuclear/cytoplasmic shuttle, and HuR expression (81). Meanwhile, some inhibitors of HuR were identified, including MS-444, which can block HuR dimerization and nuclear/cytoplasmic shuttle (82); CMLD2, dihydrotanshinone-I (DHTs) and suramin can regulate the interaction of HuR/mRNA (83–85); Okicenone, trichostatin, 5-aza-2'-deoxycytidine (AZA) can also be used as inhibitors of HuR shuttle (82, 86). Recently, based on the fact that the function of HuR in cancer cells depends on its dimerization and its nuclear/cytoplasmic shuttle, Natalia et al. (81) identified a new kind of HuR polymerization inhibitor SRI-42127, which can inhibit the formation of HuR polymers in glioblastoma exotoxin (PDGx) from primary patients, resulting in proliferation arrest, induction of apoptosis and inhibition of colony formation.

LIN28

LIN28, a highly conserved RBP, has two homologues LIN28A/B in mammals (87) and expressed in various human epithelial tumors, such as lung cancer (88), ovarian cancer (89), hepatocellular carcinoma (90), and colorectal cancer (91). LIN28 is a member of a reprogramming factor that interacts with KLF4, SOX2, and NANOG to induce pluripotency in adult fibroblasts

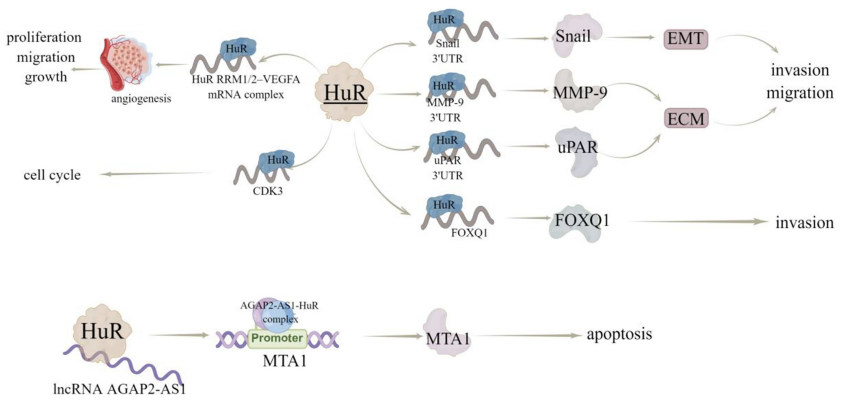


FIGURE 5
The regulatory mechanism of HuR in breast cancer. HuR interacts with 3'UTR of *Snail*, *MMP-9* and *uPAR*, regulates EMT and EMC, and promotes invasion and metastasis. HuR interacts with *FOXQ1* mRNA to inhibit the invasion of breast cancer cells. HuR can form a complex with RRM1/2–VEGFA mRNA to promote tumor growth and angiogenesis. Hur can bind to lncRNA *AGAP2-AS1* to stabilize AGAP2-AS1 expression. AGAP2-AS1-HuR complex up regulates H3K27ac level in *MTA1* promoter region to increase *MTA1* promoter activity and MTA1 expression, thus enhancing resistance to apoptosis. Arrows indicate activation and blunted lines indicate inhibition.

(91). LIN28 can block the production of let-7 miRNA and subsequently disinhibit let-7 miRNA target genes (*RAS*, *myc* and *HMGA2*), which plays an important role in CSC maintenance (92). In consistent, LIN28 is highly enriched in BCSC population and plays an important role in maintaining CSC characteristics (92). Notably, let-7 miRNA family has been identified as a downstream target of LIN28 to inhibit let-7 maturation. The blocking of let-7 biogenesis and subsequent dis-inhibition of let-7 target genes by LIN28 has been proved to be the potential mechanism contributing to LIN28-induced cancer progression and metastasis (93). All let-7 family members are regulated by LIN28 *via* blocking its processing into mature miRNA, conversely LIN28 is also down-regulated by let-7, forming a regulatory-loop (94).

Additionally, abnormal expression of LIN28 and let-7 promotes aerobic glycolysis or Warburg effect in cancer cells (95). Carbonic anhydrase IX (CAIX) is a hypoxia induced transmembrane protein that catalyzes the reversible hydration of carbon dioxide to bicarbonate ions and protons (25). It contributes to the neutralization of intracellular pH, plays a vital role in maintaining favorable intracellular pH (PHI), provides selective advantages for cancer cells and promotes cancer progression (26). In hypoxia breast cancer cell lines, inhibition of CAIX affects let-7/LIN28 axis, thereby affecting the related metabolic pathways and stem cell reprogramming (27). Vasodilator stimulated phosphoprotein (VASP) is an important cytoskeleton related protein belonging to Ena/VASP protein family (28). VASP is a key target protein that regulates the migration of various tumor cells and upregulated in breast cancer tissues and cells (29). VASP silencing inhibits the invasion of breast cancer MDA-MB-231 cells, and *miR-638* can inhibit the expression of VASP (29). LIN28 can also regulate the processing of *miR-638*, thus inhibiting its maturation and promoting VASP expression, while CREB1, as a transcription factor, binds to the promoter of *LIN28* gene and activates the LIN28/miR-638/VASP pathway, which promotes the proliferation and migration of breast cancer cells. Xu et al. (30) collected data from 291 patients with breast cancer, in which the expression level of LIN28 was evaluated by immunohistochemical staining. The found that the positive expression of LIN28 is related to lymph node metastasis, HER-2, estrogen receptor and progesterone receptor, and Kaplan-Meier analysis showed that the overall survival rate of LIN28 positive patients was lower than that of LIN28 negative patients. These data suggest that the expression of LIN28 is related to the stage and subtype of advanced disease in patients with breast cancer, and the expression of LIN28 may be an independent prognostic factor (Table 2).

Notably, LIN28 can also act as a transcription or translation regulator independent of let-7 (31). LIN28 recruit RNA binding protein Musashi-2 (MSI-2) by LIN28 CSD domain and MSI2 RRM domain, which directly induces the mRNA decay of YAP1 upstream kinase and negatively regulates Hippo pathway, resulting in the activation of YAP1, enhancing CSC like characteristics, tumorigenesis and metastasis in TNBC cells (32).

Based on the let-7/LIN28 axis, researchers have found a series of inhibitors of LIN28, such as tetrahydroquinoline (THQ) -containing Povarov scaffolds. By changing the substituents of 2-Benzoic acid, fused rings at positions 3 and 4, and the substituents of phenyl part of tetrahydroquinoline core, the structure of THQ molecule has been optimized, which can be used as inhibitors to destroy the protein-RNA interaction of LIN28-let-7 (96). Trisubstituted pyrrolidone can also act as a small molecule inhibitor to destroy the protein-RNA interaction between Lin28 and let-7 (97). In addition, C1632 is a small molecule inhibitor of LIN28, which can increase let-7 level, reduce PD-L1 and inhibit a variety of cell growth (97). Furthermore, the inhibitor TPEN can make the zinc finger domain of LIN28 unstable (98) (Figure 6).

SAM68

SAM68, also known as KHDRBS1, belongs to the RBP of the STAR family. It acts as a downstream target of Src family kinases in cell cycle, transcriptional regulation, cell survival and apoptosis (99). SAM68 is engaged in the progression of numerous cancers, such as MLL fusion induced leukemia (100), prostate cancer (101), breast cancer (2, 33), colon (102), and renal tumor (34).

Yannick et al. (35) revealed that SAM68 forms a CBP-SAM68 complex in CSC, which compromises histone acetylation of known β-catenin target genes to reduce the self-renewal and induce differentiation in CSC. SAM68 can be recruited into insulin and leptin signaling pathways to mediate its effects on the survival, growth, and proliferation of different cell types. In human breast cancer cell lines MCF7, MDA-MB-231 and BT-474, the number and expression of SAM68 protein was increased under leptin or insulin stimulation, and insulin and leptin can stimulate SAM68 tyrosine phosphorylation (36). Leptin and insulin have been proven to activate MAPK and PI3K signaling pathways in cancer to promote proliferation, cell survival and cell growth (37, 38). Therefore, SAM68 not only mediates cell metabolism stimulated by insulin and leptin, but also participates in leptin- and insulin-dependent activation of MAPK and PI3K signaling pathways in breast cancer cells (36). Additionally, knockdown of endogenous *SAM68* can inhibit cell proliferation and tumorigenicity of breast cancer cells by blocking G1 phase transition to S phase, which is related to upregulation of cyclin dependent kinase inhibitor p21 Cip1 and p27 Kip1, enhanced transactivation of FOXO factor and decreased Akt/GSK-3β signal transduction (2). Notably, Alice et al. (103) showed that all molecular subtypes of breast cancer contain a subset of anti-therapeutic cells, which express high levels of Myc, Sam68 and Rad51, effectively inhibit cell survival. Analysis of a group of

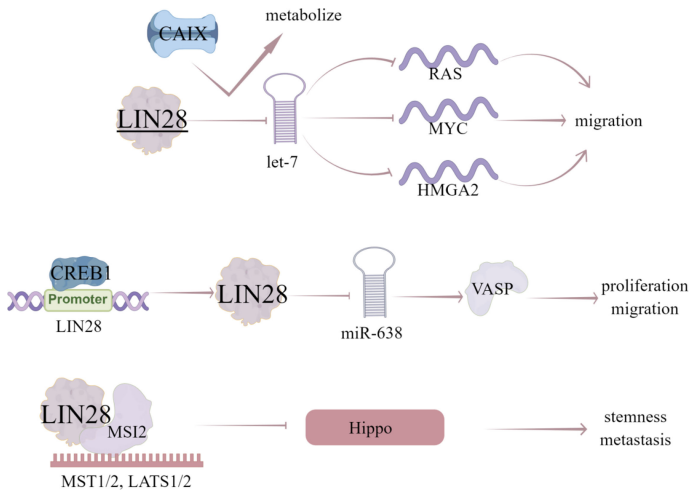


FIGURE 6
The regulatory mechanism of LIN28 in breast cancer. LIN28 can block the production of *let-7* miRNA and disinhibit the target genes of *let-7* miRNA (*RAS, MYC* and *HMGA2*), thus promoting the migration of breast cancer cells. Inhibition of CAIX will affect let-7/LIN28 axis and related metabolic pathways. CREB1 binds to the promoter of *LIN28*, activates LIN28/miR-638/VASP pathway, and promotes the proliferation and migration of breast cancer cells. LIN28 can recruit MSI2, directly induce the mRNA decay of upstream kinase of YAP1, and negatively regulate Hippo pathway, leading to the activation of YAP1, thus enhancing CSC like characteristics, tumorigenesis and metastasis in TNBC cells. Arrows indicate activation and blunted lines indicate inhibition.

breast cancer patients showed that Sam68 was an independent negative factor associated with disease progression. Rad51 targeting significantly reduced the activity of Sam68-silenced breast cancer sphere cells (BCSphCs), and SAM68 is necessary as a coactivator of PARP and a synthetic lethal partner of Rad51 (103). This Sam68-PARP axis can play an important role in controlling the resistance of ER+ cells to endocrine therapy (Figure 7).

Musashi (MSI)

The MSI RNA binding protein family, including two homologues Musashi-1 (MSI-1) and MSI-2, usually regulates mRNA translation and engages in tumorigenesis (104). There are two ribonucleoprotein like RNA-binding domains (RBD) in MSI protein, namely RBD1 and RBD2, which bind single stranded RNA motifs with a central UAG trinucleotide with

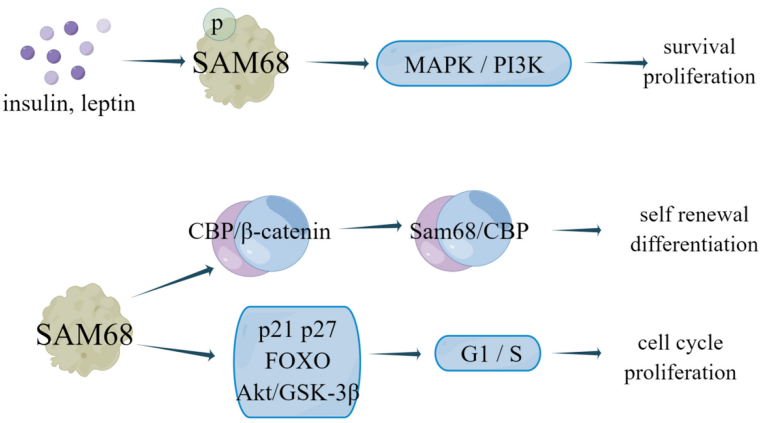


FIGURE 7
The regulatory mechanism of SAM68 in breast cancer. Insulin and leptin stimulation can promote SAM68 tyrosine phosphorylation, activate MAPK and PI3K signaling pathways in cancer, and promote cell proliferation and survival. SAM68 can form CBP-SAM68 complex in CSC, reduces CSC self-renewal and induces differentiation. Knockdown of endogenous SAM68 can inhibit cell proliferation and tumorigenicity of breast cancer cells by blocking G1 phase transition to S phase.

high affinity and specificity (39). Increasing evidences indicated that MSI protein modulates the initiation and progression of various cancer cells, including lung cancer, colorectal cancer, leukemia, glioblastoma, pancreatic cancer, as well as breast cancer (39).

MSI-1, as a prognostic marker in breast cancer, has been identified as a key participant in a variety of malignancies (40). The researchers analyzed the prognostic correlation of MSI-1 with multiple survival results and found that MSI-1 is a negative prognostic marker for disease-free and distant metastasis free survival of breast cancer (40). Expression of specific breast cancer stem cells (BCSCs) is seen in aggressive tumors and MSI-1 has been shown to be one of the BCSC-related genes (41). The authors also found that silencing MSI-1 results in down-regulation of stem cell gene expression and up-regulation of cell cycle and apoptosis regulator p21 (40). Additionally, MSI-1 could also promote Notch signaling, which is a critical signaling pathway to maintain stem cell state, by binding to the mRNA of Numb, the negative regulator of Notch signaling (42). Notably, MSI-1 can downregulate the 26S proteasome by binding to the mRNA of NF-YA, the transcriptional factor regulating 26S proteasome subunit expression, thus providing an additional route by which the degradation of Notch-ICD is prevented and Notch signaling is sustained in BCSCs (43). Furthermore, loss of MSI-1 expression resulted in decreased proliferation and treatment resistance of breast cancer cells and increased apoptosis by competitively binding to tachykinin (TAC1) mRNA with miR-130a and miR-206 to stabilize and increase its translation (44).

MSI2 is related to tumorigenesis and tumor progression of some human cancers. It is also reported that MSI2 can also inhibit the progress of EMT in breast cancer, and the low expression of MSI2 is related to the poor prognosis of breast cancer patients (45). Li et al. (46) investigated the expression and phenotypic function of two major alternative splicing MSI2 isoforms (MSI2a and MSI2b) and showed that MSI2 expression was significantly down regulated in TNBC tissues compared with normal tissues, in which MSI2a is the predominant functional isoform of MSI2 proteins in TNBC, as evident by the fact that overexpression of MSI2a inhibits TNBC cell invasion and extracellular signal regulated kinase 1/2 (ERK1/2) activity *in vitro* and *in vivo*. In addition, MSI2 directly regulates estrogen receptor 1 (ESR1) expression, which is a well-known therapeutic target, by binding to the specific sites in ESR1 RNA and increasing the stability of ESR1 protein, thus affecting the growth of breast cancer cells (47) (Figure 8; Table 2).

Conclusion

A large amount of evidence shows that the imbalance of RBPs occurs in various cancer types and affects every step of cancer development. With the progress of science and technology, new RBPs are constantly being reported, and the functions of RBPs will

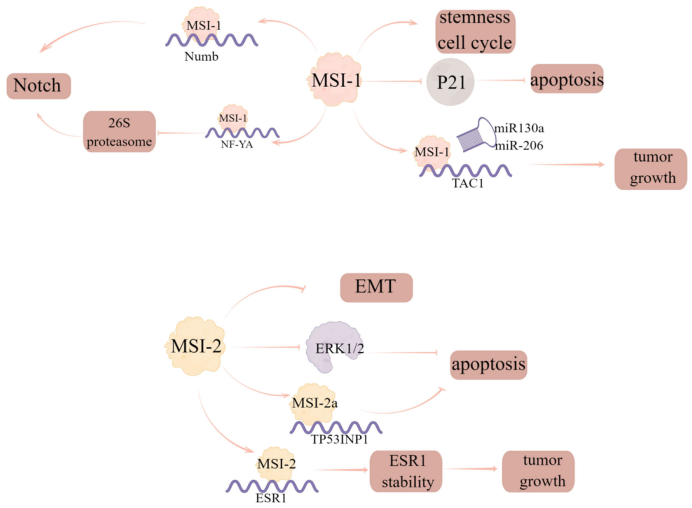


FIGURE 8
The regulatory mechanism of MSI in breast cancer. Silencing MSI-1 results in down-regulation of stem cell gene expression and up-regulation of cell cycle and apoptosis regulator p21. MSI1 competes with miR130a and -206 for interaction with TAC1 mRNA to stabilize and increase its translation and increase tumor growth. MSI-1 can promote Notch signal by binding to the mRNA of the negative regulator of Notch signal, numb, and can also down regulate 26S proteasome by binding to the mRNA of NF-YA to prevent the degradation of Notch ICD. MSI2 can inhibit EMT progression in breast cancer, and MSI2a expression inhibits TNBC invasion by stabilizing TP53INP1 mRNA and inhibiting ERK1/2 activity. MSI2 directly regulates ESR1 and affects the growth of breast cancer cells.

be enriched. Currently, the differential expression of many RBPs has been reported in breast cancer, which means that RBPs may become a new marker for tumor diagnosis and prognosis in the future. Meanwhile, inhibitors and compounds targeting the interaction between RBPs and target proteins are also emerging. As described in this review, small molecule inhibitor KH-3 and compound ZM-32 can inhibit the interaction between HuR and downstream mRNA, thus inhibiting the progression of breast cancer. Taken together, this review lists the roles of several RBPs and their target genes in the proliferation, cycle, apoptosis, migration and invasion of breast cancer cells. There are still few articles related to drug resistance, which can become a new research direction, and the complex regulatory network of RBP has not been fully understood. We need to have a more comprehensive understanding of the role of RBPs in breast cancer, which is expected to become a target for breast cancer therapy in the near future.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

This work was supported by the National Natural Science Foundation of China (82173842) and the Priority Academic Program Development (PAPD) of Jiangsu Higher Education Institutions. The presented figures were drawn by Figdraw.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Bou ZM, Ghorayeb T, Saliba F, Allam S, Bou ZM, Yaghi M, et al. Triple negative breast cancer: Updates on classification and treatment in 2021. *Cancers (Basel)* (2022) 14(5):1253. doi: 10.3390/cancers14051253

2. Song L, Wang L, Li Y, Xiong H, Wu J, Li J, et al. Sam68 up-regulation correlates with, and its down-regulation inhibits, proliferation and tumourigenicity of breast cancer cells. *J Pathol* (2010) 222(3):227–37. doi: 10.1002/path.2751

3. Ding Z, Yang HW, Xia TS, Wang B, Ding Q. Integrative genomic analyses of the RNA-binding protein, RNPC1, and its potential role in cancer prediction. *Int J Mol Med* (2015) 36(2):473–84. doi: 10.3892/ijmm.2015.2237

4. Sternburg EL, Karginov FV. Global approaches in studying RNA-binding protein interaction networks. *Trends Biochem Sci* (2020) 45(7):593–603. doi: 10.1016/j.tibs.2020.03.005

5. She X, Lin Y, Liang R, Liu Z, Gao X, Ye J. RNA-Binding motif protein 38 as a potential biomarker and therapeutic target in cancer. *Onco Targets Ther* (2020) 13:13225–36. doi: 10.2147/OTT.S278755

6. Wu J, Zhou XJ, Sun X, Xia TS, Li XX, Shi L, et al. RBM38 is involved in TGF-beta-induced epithelial-to-mesenchymal transition by stabilising zonula occludens-1 mRNA in breast cancer. *Br J Cancer* (2017) 117(5):675–84. doi: 10.1038/bjc.2017.204

7. Li XX, Shi L, Zhou XJ, Wu J, Xia TS, Zhou WB, et al. The role of c-Myc-RBM38 loop in the growth suppression in breast cancer. *J Exp Clin Cancer Res* (2017) 36(1):49. doi: 10.1186/s13046-017-0521-5

8. Zhou XJ, Wu J, Shi L, Li XX, Zhu L, Sun X, et al. PTEN expression is upregulated by a RNA-binding protein RBM38 *via* enhancing its mRNA stability in breast cancer. *J Exp Clin Cancer Res* (2017) 36(1):149. doi: 10.1186/s13046-017-0620-3

9. Zheng L, Zhang Z, Zhang S, Guo Q, Zhang F, Gao L, et al. RNA Binding protein RNPC1 inhibits breast cancer cell metastasis *via* activating STARD13-correlated ceRNA network. *Mol Pharm* (2018) 15(6):2123–32. doi: 10.1021/acs.molpharmaceut.7b01123

10. Wang X, Guo Q, Wang H, Yuan X, Wang B, Lobie PE, et al. PCBP2 posttranscriptional modifications induce breast cancer progression *via* upregulation of UFD1 and NT5E. *Mol Cancer Res* (2021) 19(1):86–98. doi: 10.1158/1541-7786.MCR-20-0390

11. Qin Y, Hou Y, Liu S, Zhu P, Wan X, Zhao M, et al. A novel long non-coding RNA lnc030 maintains breast cancer stem cell stemness by stabilizing SQLE mRNA and increasing cholesterol synthesis. *Adv Sci (Weinh)* (2021) 8(2):2002232. doi: 10.1002/advs.202002232

12. Cao Y, Chu C, Li X, Gu S, Zou Q, Jin Y. RNA-Binding protein QKI suppresses breast cancer *via* RASA1/MAPK signaling pathway. *Ann Transl Med* (2021) 9(2):104. doi: 10.21037/atm-20-4859

13. Yu F, Jin L, Yang G, Ji L, Wang F, Lu Z. Post-transcriptional repression of FOXO1 by QKI results in low levels of FOXO1 expression in breast cancer cells. *Oncol Rep* (2014) 31(3):1459–65. doi: 10.3892/or.2013.2957

14. Hu C, Fang K, Zhang X, Guo Z, Li L. Dyregulation of the lncRNA TPT1-AS1 positively regulates QKI expression and predicts a poor prognosis for patients with breast cancer. *Pathol Res Pract* (2020) 216(11):153216. doi: 10.1016/j.prp.2020.153216

15. Huwiler A, Akool E, Aschrafi A, Hamada FM, Pfeilschifter J, Eberhardt W. ATP potentiates interleukin-1 beta-induced MMP-9 expression in mesangial cells *via* recruitment of the ELAV protein HuR. *J Biol Chem* (2003) 278(51):51758–69. doi: 10.1074/jbc.M305722200

16. D'Alessio S, Blasi F. The urokinase receptor as an entertainer of signal transduction. *Front Biosci (Landmark Ed)* (2009) 14(12):4575–87. doi: 10.2741/3550

17. Wu X, Gardashova G, Lan L, Han S, Zhong C, Marquez RT, et al. Targeting the interaction between RNA-binding protein HuR and FOXQ1 suppresses breast cancer invasion and metastasis. *Commun Biol* (2020) 3(1):193. doi: 10.1038/s42003-020-0933-1

18. Yang LQ, Yu SP, Yang YT, Zhao YS, Wang FY, Chen Y, et al. Muscone derivative ZM-32 inhibits breast tumor angiogenesis by suppressing HuR-mediated VEGF and MMP9 expression. *BioMed Pharmacother* (2021) 136:111265. doi: 10.1016/j.biopha.2021.111265

19. Zhu Y, Yang L, Xu J, Yang X, Luan P, Cui Q, et al. Discovery of the anti-angiogenesis effect of eltrombopag in breast cancer through targeting of HuR protein. *Acta Pharm Sin B* (2020) 10(8):1414–25. doi: 10.1016/j.apsb.2020.02.007

20. Perez DCI, de Carcer G, Malumbres M. A census of mitotic cancer genes: New insights into tumor cell biology and cancer therapy. *Carcinogenesis* (2007) 28 (5):899–912. doi: 10.1093/carcin/bgm019

21. Zhang Z, Huang A, Zhang A, Zhou C. HuR promotes breast cancer cell proliferation and survival *via* binding to CDK3 mRNA. *BioMed Pharmacother* (2017) 91:788–95. doi: 10.1016/j.biopha.2017.04.063

22. Wang LJ, Chiou JT, Lee YC, Chang LS. Docetaxel-triggered SIDT2/NOX4/JNK/HuR signaling axis is associated with TNF-alpha-mediated apoptosis of cancer cells. *Biochem Pharmacol* (2022) 195:114865. doi: 10.1016/j.bcp.2021.114865

23. Wu M, Wen L, Zhou Y, Wu W. Role of lncRNA AGAP2-AS1 in breast cancer cell resistance to apoptosis by the regulation of MTA1 promoter activity. *Technol Cancer Res Treat* (2022) 21:15330338221085361. doi: 10.1177/15330338221085361

24. Bazley FA, Liu CF, Yuan X, Hao H, All AH, De Los AA, et al. Direct reprogramming of human primordial germ cells into induced pluripotent stem cells: Efficient generation of genetically engineered germ cells. *Stem Cells Dev* (2015) 24(22):2634–48. doi: 10.1089/scd.2015.0100

25. Pastorek J, Pastorekova S. Hypoxia-induced carbonic anhydrase IX as a target for cancer therapy: From biology to clinical use. *Semin Cancer Biol* (2015) 31:52–64. doi: 10.1016/j.semcancer.2014.08.002

26. Debreova M, et al. CAIX regulates invadopodia formation through both a pH-dependent mechanism and interplay with actin regulatory proteins. *Int J Mol Sci* (2019) 20(11):2745. doi: 10.3390/ijms20112745

27. Gibadulinova A, et al. CAIX-mediated control of LIN28/let-7 axis contributes to metabolic adaptation of breast cancer cells to hypoxia. *Int J Mol Sci* (2020) 21(12):4299. doi: 10.3390/ijms21124299

28. Ferron F, et al. Structural basis for the recruitment of profilin-actin complexes during filament elongation by Ena/VASP. *EMBO J* (2007) 26 (21):4597–606. doi: 10.1038/sj.emboj.7601874

29. Doppler H, et al. The phosphorylation status of VASP at serine 322 can be predictive for aggressiveness of invasive ductal carcinoma. *Oncotarget* (2015) 6 (30):29740–52. doi: 10.18632/oncotarget.4965

30. Xu C, Jin S, Huang L. Expression of Lin28 is correlated with prognosis and expression of HER-2 and steroid receptors in breast cancer. *Onco Targets Ther* (2019) 12:1105–10. doi: 10.2147/OTT.S190328

31. Balzer E, Heine C, Jiang Q, Lee VM, Moss EG. LIN28 alters cell fate succession and acts independently of the let-7 microRNA during neurogliogenesis in vitro. *Development* (2010) 137(6):891–900. doi: 10.1242/dev.042895

32. Zou H, Luo J, Guo Y, Liu Y, Wang Y, Deng L, et al. RNA-Binding protein complex LIN28/MSI2 enhances cancer stem cell-like properties by modulating hippo-YAP1 signaling and independently of let-7. *Oncogene* (2022) 41(11):1657–72. doi: 10.1038/s41388-022-02198-w

33. Cheung N, Chan LC, Thompson A, Cleary ML, So CW. Protein arginine-methyltransferase-dependent oncogenesis. *Nat Cell Biol* (2007) 9(10):p1208–15. doi: 10.1038/ncb1642

34. Fu K, Sun X, Wier EM, Hodgson A, Hobbs RP, Wan F. Sam68/KHDRBS1-dependent NF-kappaB activation confers radioprotection to the colon epithelium in gamma-irradiated mice. *Elife* (2016) 5:e21957. doi: 10.7554/eLife.21957

35. Benoit YD, Mitchell RR, Risueno RM, Orlando L, Tanasijevic B, Boyd AL, et al. Sam68 allows selective targeting of human cancer stem cells. *Cell Chem Biol* (2017) 24(7):833–844.e9. doi: 10.1016/j.chembiol.2017.05.026

36. Perez-Perez A, Sanchez-Jimenez F, Vilarino-Garcia T, de la Cruz L, Virizuela JA, Sanchez-Margalet V, et al. Sam68 mediates the activation of insulin and leptin signalling in breast cancer cells. *PloS One* (2016) 11(7):e0158218. doi: 10.1371/journal.pone.0158218

37. Coolican SA, Samuel DS, Ewton DZ, McWade FJ, Florini JR. The mitogenic and myogenic actions of insulin-like growth factors utilize distinct signaling pathways. *J Biol Chem* (1997) 272(10):6653–62. doi: 10.1074/jbc.272.10.6653

38. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer* (2002) 2(7):489–501. doi: 10.1038/nrc839

39. Darai N, Mahalapbutr P, Wolschann P, Lee VS, Wolfinger MT, Rungrotmongkol T, et al. Theoretical studies on RNA recognition by musashi 1 RNA-binding protein. *Sci Rep* (2022) 12(1):12137. doi: 10.1038/s41598-022-16252-w

40. Troschel FM, Palenta H, Borrmann K, Heshe K, Hua SH, Yip GW, et al. Knockdown of the prognostic cancer stem cell marker musashi-1 decreases radio-resistance while enhancing apoptosis in hormone receptor-positive breast cancer cells *via* p21(WAF1/CIP1). *J Cancer Res Clin Oncol* (2021) 147(11):3299–312. doi: 10.1007/s00432-021-03743-y

41. Kagara N, Huynh KT, Kuo C, Okano H, Sim MS, Elashoff D, et al. Epigenetic regulation of cancer stem cell genes in triple-negative breast cancer. *Am J Pathol* (2012) 181(1):257–67. doi: 10.1016/j.ajpath.2012.03.019

42. Jadhav S, Ajay AK, Trivedi P, Seematti J, Pellegrini K, Craciun F, et al. RNA-Binding protein musashi homologue 1 regulates kidney fibrosis by translational inhibition of p21 and numb mRNA. *J Biol Chem* (2016) 291(27):14085–94. doi: 10.1074/jbc.M115.713289

43. Lagadec C, Vlashi E, Frohnen P, Alhiyari Y, Chan M, Pajonk F. The RNA-binding protein musashi-1 regulates proteasome subunit expression in breast cancer- and glioma-initiating cells. *Stem Cells* (2014) 32(1):135–44. doi: 10.1002/stem.1537

44. Nahas GR, Patel SA, Ganta T, Greco SJ, Rameshwar P. The RNA-binding protein musashi 1 stabilizes the oncotachykinin 1 mRNA in breast cancer cells to promote cell growth. *FASEB J* (2016) 30(1):149–59. doi: 10.1096/fj.15-278770

45. Katz Y, Li F, Lambert NJ, Sokol ES, Tam WL, Cheng AW, et al. Musashi proteins are post-transcriptional regulators of the epithelial-luminal cell state. *Elife* (2014) 3:e03915. doi: 10.7554/eLife.03915

46. Li M, Li AQ, Zhou SL, Lv H, Wei P, Yang WT. RNA-Binding protein MSI2 isoforms expression and regulation in progression of triple-negative breast cancer. *J Exp Clin Cancer Res* (2020) 39(1):92. doi: 10.1186/s13046-020-01587-x

47. Kang MH, Jeong KJ, Kim WY, Lee HJ, Gong G, Suh N, et al. Musashi RNA-binding protein 2 regulates estrogen receptor 1 function in breast cancer. *Oncogene* (2017) 36(12):1745–52. doi: 10.1038/onc.2016.327

48. Feldstein O, Ben-Hamo R, Bashari D, Efroni S, Ginsberg D. RBM38 is a direct transcriptional target of E2F1 that limits E2F1-induced proliferation. *Mol Cancer Res* (2012) 10(9):1169–77. doi: 10.1158/1541-7786.MCR-12-0331

49. Xue JQ, Xia TS, Liang XQ, Zhou W, Cheng L, Shi L, et al. RNA-Binding protein RNPC1: acting as a tumor suppressor in breast cancer. *BMC Cancer* (2014) 14:322. doi: 10.1186/1471-2407-14-322

50. Wampfler J, Federzoni EA, Torbett BE, Fey MF, Tschan MP. The RNA binding proteins RBM38 and DND1 are repressed in AML and have a novel function in APL differentiation. *Leuk Res* (2016) 41:96–102. doi: 10.1016/j.leukres.2015.12.006

51. Huang W, Wei XL, Ni W, Cao M, Meng L, Yang H. The expression of RNA-binding protein RBM38 decreased in renal cell carcinoma and represses renal cancer cell proliferation, migration, and invasion. *Tumour Biol* (2017) 39 (5):1010428317701635. doi: 10.1177/1010428317701635

52. Zhang M, Xu E, Zhang J, Chen X. PPM1D phosphatase, a target of p53 and RBM38 RNA-binding protein, inhibits p53 mRNA translation *via* dephosphorylation of RBM38. *Oncogene* (2015) 34(48):5900–11. doi: 10.1038/onc.2015.31

53. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol* (2006) 7(2):131–42. doi: 10.1038/nrm1835

54. Zhu L, Sun X, Xi PW, Hu Y, Shi L, Ding Q. Relationship between expression of RBM38 and distant metastasis and prognosis of breast cancer. *Zhonghua Zhong Liu Za Zhi* (2018) 40(8):600–3. doi: 10.3760/cma.j.issn.0253-3766.2018.08.007

55. Andino R, Boddeker N, Silvera D, Gamarnik AV. Intracellular determinants of picornavirus replication. *Trends Microbiol* (1999) 7(2):76–82. doi: 10.1016/S0966-842X(98)01446-2

56. Blyn LB, Towner JS, Semler BL, Ehrenfeld E. Requirement of poly(rC) binding protein 2 for translation of poliovirus RNA. *J Virol* (1997) 71(8):6243–6. doi: 10.1128/jvi.71.8.6243-6246.1997

57. Collier B, Goobar-Larsson L, Sokolowski M, Schwartz S. Translational inhibition *in vitro* of human papillomavirus type 16 L2 mRNA mediated through interaction with heterogenous ribonucleoprotein K and poly(rC)-binding proteins 1 and 2. *J Biol Chem* (1998) 273(35):22648–56. doi: 10.1074/jbc.273.35.22648

58. Zhang X, Hua L, Yan D, Zhao F, Liu J, Zhou H, et al. Overexpression of PCBP2 contributes to poor prognosis and enhanced cell growth in human hepatocellular carcinoma. *Oncol Rep* (2016) 36(6):3456–64. doi: 10.3892/or.2016.5167

59. Wan C, Gong C, Zhang H, Hua L, Li X, Chen X, et al. beta2-adrenergic receptor signaling promotes pancreatic ductal adenocarcinoma (PDAC) progression through facilitating PCBP2-dependent c-myc expression. *Cancer Lett* (2016) 373(1):67–76. doi: 10.1016/j.canlet.2016.01.026

60. Evans JR, Mitchell SA, Spriggs KA, Ostrowski J, Bomsztyk K, Ostarek D, et al. Members of the poly (rC) binding protein family stimulate the activity of the c-myc internal ribosome entry segment in vitro and *in vivo*. *Oncogene* (2003) 22 (39):8012–20. doi: 10.1038/sj.onc.1206645

61. Hu CE, Liu YC, Zhang HD, Huang GJ. The RNA-binding protein PCBP2 facilitates gastric carcinoma growth by targeting miR-34a. *Biochem Biophys Res Commun* (2014) 448(4):437–42. doi: 10.1016/j.bbrc.2014.04.124

62. Stricker TP, Brown CD, Bandlamudi C, McNerney M, Kittler R, Montoya V, et al. Robust stratification of breast cancer subtypes using differential patterns of transcript isoform expression. *PloS Genet* (2017) 13(3):e1006589. doi: 10.1371/journal.pgen.1006589

63. Vernet C, Artzt K. STAR, a gene family involved in signal transduction and activation of RNA. *Trends Genet* (1997) 13(12):479–84. doi: 10.1016/S0168-9525(97)01269-9

64. Ebersole TA, Chen Q, Justice MJ, Artzt K. The quaking gene product necessary in embryogenesis and myelination combines features of RNA binding and signal transduction proteins. *Nat Genet* (1996) 12(3):260–5. doi: 10.1038/ng0396-260

65. Larocque D, Pilotte J, Chen T, Cloutier F, Massie B, Pedraza L, et al. Nuclear retention of MBP mRNAs in the quaking viable mice. *Neuron* (2002) 36(5):815–29. doi: 10.1016/S0896-6273(02)01055-3

66. Ryder SP, Williamson JR. Specificity of the STAR/GSG domain protein Qk1: Implications for the regulation of myelination. *RNA* (2004) 10(9):1449–58. doi: 10.1261/rna.7780504

67. Galarneau A, Richard S. Target RNA motif and target mRNAs of the quaking STAR protein. *Nat Struct Mol Biol* (2005) 12(8):691–8. doi: 10.1038/nsmb963

68. Ryder SP, Frater LA, Abramovitz DL, Goodwin EB, Williamson JR. RNA Target specificity of the STAR/GSG domain post-transcriptional regulatory protein GLD-1. *Nat Struct Mol Biol* (2004) 11(1):20–8. doi: 10.1038/nsmb706

69. Liu Z, Luyten I, Bottomley MJ, Messias AC, Houngninou-Molango S, Sprangers R, et al. Structural basis for recognition of the intron branch site RNA by splicing factor 1. *Science* (2001) 294(5544):1098–102. doi: 10.1126/science.1064719

70. Yang G, Fu H, Zhang J, Lu X, Yu F, Jin L, et al. RNA-Binding protein quaking, a critical regulator of colon epithelial differentiation and a suppressor of colon cancer. *Gastroenterology* (2010) 138(1):231–40.e1-5. doi: 10.1053/j.gastro.2009.08.001

71. de Miguel FJ, Pajares MJ, Martinez-Terroba E, Ajona D, Morales X, Sharma RD, et al. A large-scale analysis of alternative splicing reveals a key role of QKI in lung cancer. *Mol Oncol* (2016) 10(9):1437–49. doi: 10.1016/j.molonc.2016.08.001

72. Lu W, Feng F, Xu J, Lu X, Wang S, Wang L, et al. QKI impairs self-renewal and tumorigenicity of oral cancer cells *via* repression of SOX2. *Cancer Biol Ther* (2014) 15(9):1174–84. doi: 10.4161/cbt.29502

73. Zhao Y, Zhang G, Wei M, Lu X, Fu H, Feng F, et al. The tumor suppressing effects of QKI-5 in prostate cancer: A novel diagnostic and prognostic protein. *Cancer Biol Ther* (2014) 15(1):108–18. doi: 10.4161/cbt.26722

74. Zhu Z, Wei D, Li X, Wang F, Yan F, Xing Z, et al. RNA-Binding protein QKI regulates contact inhibition *via* yes-associate protein in ccRCC. *Acta Biochim Biophys Sin (Shanghai)* (2019) 51(1):9–19. doi: 10.1093/abbs/gmy142

75. Chen Z, Huang J, Feng Y, Li Z, Jiang Y. Profiling of specific long non-coding RNA signatures identifies ST8SIA6-AS1 AS a novel target for breast cancer. *J Gene Med* (2021) 23(2):e3286. doi: 10.1002/jgm.3286

76. Gu S, Chu C, Chen W, Ren H, Cao Y, Li X, et al. Prognostic value of epithelial-mesenchymal transition related genes: SLUG and QKI in breast cancer patients. *Int J Clin Exp Pathol* (2019) 12(6):2009–21.

77. Hinman MN, Lou H. Diverse molecular functions of hu proteins. *Cell Mol Life Sci* (2008) 65(20):3168–81. doi: 10.1007/s00018-008-8252-6

78. Brennan CM, Steitz JA. HuR and mRNA stability. *Cell Mol Life Sci* (2001) 58(2):266–77. doi: 10.1007/PL00000854

79. Lopez DSI, Zhan M, Lal A, Yang X, Gorospe M. Identification of a target RNA motif for RNA-binding protein HuR. *Proc Natl Acad Sci U.S.A.* (2004) 101(9):2987–92. doi: 10.1073/pnas.0306453101

80. Doller A, Pfeilschifter J, Eberhardt W. Signalling pathways regulating nucleo-cytoplasmic shuttling of the mRNA-binding protein HuR. *Cell Signal* (2008) 20(12):2165–73. doi: 10.1016/j.cellsig.2008.05.007

81. Filippova N, Yang X, Ananthan S, Calano J, Pathak V, Bratton L, et al. Targeting the HuR oncogenic role with a new class of cytoplasmic dimerization inhibitors. *Cancer Res* (2021) 81(8):2220–33. doi: 10.1158/0008-5472.CAN-20-2858

82. Meisner NC, Hintersteiner M, Mueller K, Bauer R, Seifert JM, Naegeli HU, et al. Identification and mechanistic characterization of low-molecular-weight inhibitors for HuR. *Nat Chem Biol* (2007) 3(8):508–15. doi: 10.1038/nchembio.2007.14

83. Muralidharan R, Mehta M, Ahmed R, Roy S, Xu L, Aube J, et al. HuR-targeted small molecule inhibitor exhibits cytotoxicity towards human lung cancer cells. *Sci Rep* (2017) 7(1):9694. doi: 10.1038/s41598-017-07787-4

84. D'Agostino VG, Lal P, Mantelli B, Tiedje C, Zucal C, Thongon N, et al. Dihydrotanshinone-I interferes with the RNA-binding activity of HuR affecting its post-transcriptional function. *Sci Rep* (2015) 5:16478. doi: 10.1038/srep16478

85. Kakuguchi W, Nomura T, Kitamura T, Otsuguro S, Matsushita K, Sakaitani M, et al. Suramin, screened from an approved drug library, inhibits HuR functions and attenuates malignant phenotype of oral cancer cells. *Cancer Med* (2018) 7(12):6269–80. doi: 10.1002/cam4.1877

86. Hostetter C, Licata LA, Witkiewicz A, Costantino CL, Yeo CJ, Brody JR, et al. Cytoplasmic accumulation of the RNA binding protein HuR is central to tamoxifen resistance in estrogen receptor positive breast cancer cells. *Cancer Biol Ther* (2008) 7(9):1496–506. doi: 10.4161/cbt.7.9.6490

87. Moss EG, Tang L. Conservation of the heterochronic regulator Lin-28, its developmental expression and microRNA complementary sites. *Dev Biol* (2003) 258(2):432–42. doi: 10.1016/S0012-1606(03)00126-X

88. Yin J, Zhao J, Hu W, Yang G, Yu H, Wang R, et al. Disturbance of the let-7/LIN28 double-negative feedback loop is associated with radio- and chemo-resistance in non-small cell lung cancer. *PloS One* (2017) 12(2):e0172787. doi: 10.1371/journal.pone.0172787

89. Hsu KF, Shen MR, Huang YF, Cheng YM, Lin SH, Chow NH, et al. Overexpression of the RNA-binding proteins Lin28B and IGF2BP3 (IMP3) is associated with chemoresistance and poor disease outcome in ovarian cancer. *Br J Cancer* (2015) 113(3):414–24. doi: 10.1038/bjc.2015.254

90. Qiu JL, Huang PZ, You JH, Zou RH, Wang L, Hong J, et al. LIN28 expression and prognostic value in hepatocellular carcinoma patients who meet the Milan criteria and undergo hepatectomy. *Chin J Cancer* (2012) 31(5):223–32. doi: 10.5732/cjc.011.10426

91. Tu HC, Schwitalla S, Qian Z, LaPier GS, Yermalovich A, Ku YC, et al. LIN28 cooperates with WNT signaling to drive invasive intestinal and colorectal adenocarcinoma in mice and humans. *Genes Dev* (2015) 29(10):1074–86. doi: 10.1101/gad.256693.114

92. Peng F, Li TT, Wang KL, Xiao GQ, Wang JH, Zhao HD, et al. H19/let-7/LIN28 reciprocal negative regulatory circuit promotes breast cancer stem cell maintenance. *Cell Death Dis* (2017) 8(1):e2569. doi: 10.1038/cddis.2016.438

93. Farzaneh M, Attari F, Khoshnam SE. Concise review: LIN28/let-7 signaling, a critical double-negative feedback loop during pluripotency, reprogramming, and tumorigenicity. *Cell Reprogram* (2017) 19(5):289–93. doi: 10.1089/cell.2017.0015

94. Heo I, Joo C, Cho J, Ha M, Han J, Kim VN. Lin28 mediates the terminal uridylation of let-7 precursor MicroRNA. *Mol Cell* (2008) 32(2):276–84. doi: 10.1016/j.molcel.2008.09.014

95. Ma X, Li C, Sun L, Huang D, Li T, He X, et al. Lin28/let-7 axis regulates aerobic glycolysis and cancer progression *via* PDK1. *Nat Commun* (2014) 5:5212. doi: 10.1038/ncomms6212

96. Goebel GL, Hohnen L, Borgelt L, Hommen P, Qiu X, Lightfoot H, et al. Small molecules with tetrahydroquinoline-containing povarov scaffolds as inhibitors disrupting the protein-RNA interaction of LIN28-let-7. *Eur J Med Chem* (2022) 228:114014. doi: 10.1016/j.ejmech.2021.114014

97. Borgelt L, Li F, Hommen P, Lampe P, Hwang J, Goebel G, et al. Trisubstituted pyrrolinones as small-molecule inhibitors disrupting the protein-RNA interaction of LIN28 and let-7. *ACS Med Chem Lett* (2021) 12(6):893–8. doi: 10.1021/acsmedchemlett.0c00546

98. Wang L, Rowe RG, Jaimes A, Yu C, Nam Y, Pearson DS, et al. Small-molecule inhibitors disrupt let-7 oligouridylation and release the selective blockade of let-7 processing by LIN28. *Cell Rep* (2018) 23(10):3091–101. doi: 10.1016/j.celrep.2018.04.116

99. Chen X, Zhang L, Yuan M, Kuang Z, Zou Y, Tang T, et al. Sam68 promotes the progression of human breast cancer through inducing activation of EphA3. *Curr Cancer Drug Targets* (2020) 20(1):76–83. doi: 10.2174/1568009619666190718124541

100. Bielli P, Busa R, Paronetto MP, Sette C. The RNA-binding protein Sam68 is a multifunctional player in human cancer. *Endocr Relat Cancer* (2011) 18(4):R91–R102. doi: 10.1530/ERC-11-0041

101. Busa R, Paronetto MP, Farini D, Pierantozzi E, Botti F, Angelini DF, et al. The RNA-binding protein Sam68 contributes to proliferation and survival of human prostate cancer cells. *Oncogene* (2007) 26(30):4372–82. doi: 10.1038/sj.onc.1210224

102. Fu K, Sun X, Wier EM, Hodgson A, Liu Y, Sears CL, et al. Sam68/KHDRBS1 is critical for colon tumorigenesis by regulating genotoxic stress-induced NF-kappaB activation. *Elife* (2016) 5:e15018. doi: 10.7554/eLife.15018

103. Turdo A, Gaggianesi M, Di Franco S, Veschi V, D'Accardo C, Porcelli G, et al. Effective targeting of breast cancer stem cells by combined inhibition of Sam68 and Rad51. *Oncogene* (2022) 41(15):2196–209. doi: 10.1038/s41388-022-02239-4

104. Kudinov AE, Karanicolas J, Golemis EA, Boumber Y. Musashi RNA-binding proteins as cancer drivers and novel therapeutic targets. *Clin Cancer Res* (2017) 23(9):p2143–53. doi: 10.1158/1078-0432.CCR-16-2728

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 19 August 2022
DOI 10.3389/fonc.2022.946029

OPEN ACCESS

EDITED BY
Claudia Mello-Thoms,
The University of Iowa, United States

REVIEWED BY
Dong-Sook Kim,
Health Insurance Review and Assessment Service, South Korea
Rainer Leonhart,
University of Freiburg, Germany

*CORRESPONDENCE
Shwn-Huey Shieh
shshieh@mail.cmu.edu.tw

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 17 May 2022
ACCEPTED 13 July 2022
PUBLISHED 19 August 2022

CITATION
Yang C-M, Sung F-C, Mou C-H, Liao C-H, Wang P-H and Shieh S-H (2022) Anxiety and depression risk in Taiwan women with breast cancer and cervical cancer.
Front. Oncol. 12:946029.
doi: 10.3389/fonc.2022.946029

Anxiety and depression risk in Taiwan women with breast cancer and cervical cancer

Chiu-Ming Yang[1,2], Fung-Chang Sung[1,3,4], Chih-Hsin Mou[3], Chun-Hui Liao[5], Po-Hui Wang[6] and Shwn-Huey Shieh[1,7,8]*

[1]Department of Health Services Administration, China Medical University College of Public Health, Taichung, Taiwan, [2]Department of Public Health, China Medical University College of Public Health, Taichung, Taiwan, [3]Management Office for Health Data, China Medical University Hospital, Taichung, Taiwan, [4]Department of Food Nutrition and Health Biotechnology, Asia University, Taichung, Taiwan, [5]Department of Psychiatry, China Medical University College of Medicine, Taichung, Taiwan, [6]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan, [7]Department of Nursing, China Medical University Hospital, Taichung, Taiwan, [8]Department of Nursing, Asia University, Taichung, Taiwan

Background: Studies comparing mental disorder risks between women with breast cancer and cervical cancer are lacking. This study compared risks of developing anxiety and depression between women with breast cancer (BC cohort) and women with cervical cancer (CC cohort) using insurance claims data of Taiwan.

Methods: From the 2000 to 2016 data, we identified a BC cohort and BC controls (N = 96,862) and a CC cohort and CC controls (N = 26,703), matched by propensity scores. Incident mental disorders and the Cox method estimated the related cancer cohort to control cohort hazard ratios (HRs), and 95% confidence intervals (CIs) were estimated by the end of 2016.

Results: Compared to the CC cohort, the BC cohort had slightly higher incident anxiety (15.9 versus 15.5 per 1,000 person-years) and depression (6.92 vs. 6.28 per 1,000 person-years). These mental disorders were higher in respective cancer cohorts than controls. The BC cohort to BC control adjusted HRs of anxiety and depression were 1.29 (95% CI = 1.25–1.33) and 1.78 (95% CI = 1.69–1.87), respectively. The corresponding adjusted HRs for the CC cohort were 1.12 (95% CI = 1.06–1.18) and 1.29 (95% CI = 1.18–1.41). The combined incidence rates of both disorders were 1.4-fold greater in the BC cohort than in BC controls (22.8 vs. 15.8 per 1,000 person-years), and 1.2-fold greater in the CC cohort than in the CC controls (21.7 vs. 18.3 per 1,000 person-years).

Conclusion: Women with breast cancer or cervical cancer are at an elevated likelihood of developing anxiety and depression disorders. These incident disorders are slightly higher in those with breast cancer.

KEYWORDS

anxiety, breast cancer, cervical cancer, depression, retrospective cohort study

Introduction

Patients with anxiety and/or depression are in depressed mood and at aversion to social activity (1, 2). With more than 264 million people of all ages being affected, depression has become an important burden in medical care and public health worldwide (3, 4). These disorders can also lead to subsequent health disorders and shortened life expectancy (4). Depression and anxiety may result from biological or psychological factors with a complex interaction of socioeconomic factors. People who have health ailments or gone through adverse life events are at an elevated risk to develop anxiety and depression. In fact, depressed mood is frequently developed as a reaction to a catastrophic disorder perceived to threaten the life and wellbeing, such as in patients with cancer (5–10). Patients may suffer from cancer treatment side effects, chronic pain, self-esteem and body image, changes in quality of life and family life, and fears of recurrence, subsequent disorders, and death (11–15). Anxiety, depression, and other mood disorders are thus prevalent in cancer patients (6–8, 16–18).

Breast cancer and cervical cancer rank as the first and fourth common female cancers, respectively, with disparities in incidence and mortality worldwide (19, 20). Breast cancer is more prevalent in women in Western countries than in women in developing counties (19), whereas nearly 90% of women with cervical cancer were identified in low-income and middle-income populations (20). The breast and cervix represent the image of appearance and femininity of women. Treatments for cancer can have a negative impact on their self-image. Women with breast cancer and cervical cancer are at a high risk to be dissatisfied by changes in body image during and after the treatment procedure. Patients may also suffer from changes of sexual function and fertility, which can lead to not only sexual disfunction but also psychological adaptation (21, 22). Concurrent treatments for both cancers have been highly effective. However, survival rates have large differences internationally (23). The breast cancer survival may range from 40% in South Africa to 90% or higher in high-income countries (24). The gap of survival rates was even greater for cervical cancer, ranging from 50% to 70% (25). The 5-year survival rates also vary by the stage of the disease at diagnosis and sociodemographic status of patients. The racial disparity of survival rates was greater for cervical cancer than for breast cancer (59.8%–73.7% versus 82.2%–91.5%) in 2011–2017 in the US (26). The psychological adaptation may differ between women with breast cancer and women with cervical cancer and is associated with the disparities. Anxiety, depression, and other mood disorders developed in women with breast cancer and cervical cancer may vary by prevalence and survival among populations.

A systemic review based on seven studies found that the depression could last for years after treatment in women with CC (27). A German study interviewed a nationwide random sample of 2,141 patients with cancer and found that patients with breast cancer had the highest prevalence of mental disorder. A systematic review based on 17 studies found that the prevalence rates of anxiety ranged from 17.9% to 33.3% and of depression from 9.4% to 66.1% in breast cancer survivors (9). Studies have also associated depression with elevated cancer mortality (10).

Studies comparing the psychological adaptation between women with breast cancer and women with cervical cancer are in demand for Asian women. Cervical cancer and breast cancer ranked earlier as the first and second most common female cancers in Taiwan, with age-adjusted incidence rates of 26.27 and 20.95 per 100,000 in 1988–1993, respectively (28). Breast cancer overtook cervical cancer in 1993–1997 with incidence rates of 28.99 versus 26.82 per 100,000, respectively. The incidence gap between the two cancers increased consistently, shifting to 71.91 and 8.72 per 100,000, respectively, in 2013–2016, with cervical cancer ranking the eighth common female cancer. We suspected that the risk of developing mental disorders might be greater in women with breast cancer than women with cervical cancer, although the 5-year survival rate for breast cancer was greater than that for cervical cancer (86.8% versus 72.5%) (29). Thus, the purpose of this study was to compare the risk of developing anxiety and depression between women with breast cancer and women with cervical cancer using the insurance claims data of Taiwan.

Methods and materials

Data source

We used insurance claims databases and cancer registry databases from 1996 to 2016 available at the Health and Welfare Data Science Center, Ministry of Health and Welfare of Taiwan. The insurance claims data consisted of information on demographic status of insured population, longitudinal medical records of outpatient and inpatient cares, including treatments and medications provided, and costs of cares. More than 99% of residents in Taiwan have been covered in this compulsory single-payer healthcare program (30). Diseases were coded with International Classification of Diseases, Clinical Modification Ninth Revision, (ICD-9-CM), before 2016 and Tenth Revision (ICD-10-CM) since 2016. All identifications of all three data sets had been changed into the same surrogate numbers before the databases were released to users. This study was approved by the Ethical Research Committee at China Medical University and Hospital (H107257). Because personal identifications in the data files had been scrambled to protect privacy, patient consents were waived.

Study design

From claims data with healthcare records in the period of 2000–2016, all women aged 18 and above were identified to establish 2 pairs of cancer cohorts and control cohorts. After excluding women with cancer history and mental disorders diagnosed before 2000, we identified 96,862 women with breast cancer and 26,703 women with cervical cancer as the breast cancer cohort (BC cohort) and the cervical cancer cohort (CC cohort), respectively (Figure 1). The date with the cancer diagnosed was defined as the index date. Among 7,250,914 women without the history of cancer and mental disorders, we randomly selected 96,862 women as the BC cohort's controls (BC controls) and 26,703 women as the CC cohort's controls (CC controls), matched by the propensity score. Multivariable logistic regression estimated the propensity score for each woman with variables of age, income, urbanization level of residential areas, diagnosis year, and Charlson comorbidity index (CCI). We estimated the CCI with the sum of weighted values of comorbidities: one point was scored for myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcer disease, mild liver disease, and diabetes; two points for hemiplegia, moderate or severe renal disease, diabetes with end organ damage, leukemia, and lymphoma; three points for moderate or severe liver disease; and six points for AIDS (31).

Outcome

From the databases, we identified mental health disorders of anxiety (ICD-9: 300.0; ICD-10-CM: 110 F40 and F41) and depression: ICD-9-CM: 296.0-296.8, 300.4, and 311.X; ICD-10-CM: F32.9, F30-F33, F34.8-F34.9, and F39), which appeared in the outpatient records for at least twice or in the inpatient records for at least once. Follow-up time in person-years was calculated for each woman from the index date until the mental health disorder diagnosis, withdrawal from the insurance, death, or the end of 2016.

Data analysis

This study used SAS Software 9.4 in Windows (SAS Institute, Cary, NC, USA) to analyze data, and 118 used $P < 0.05$ to indicate the significance level in comparisons. Data analysis first compared the baseline distributions, between the

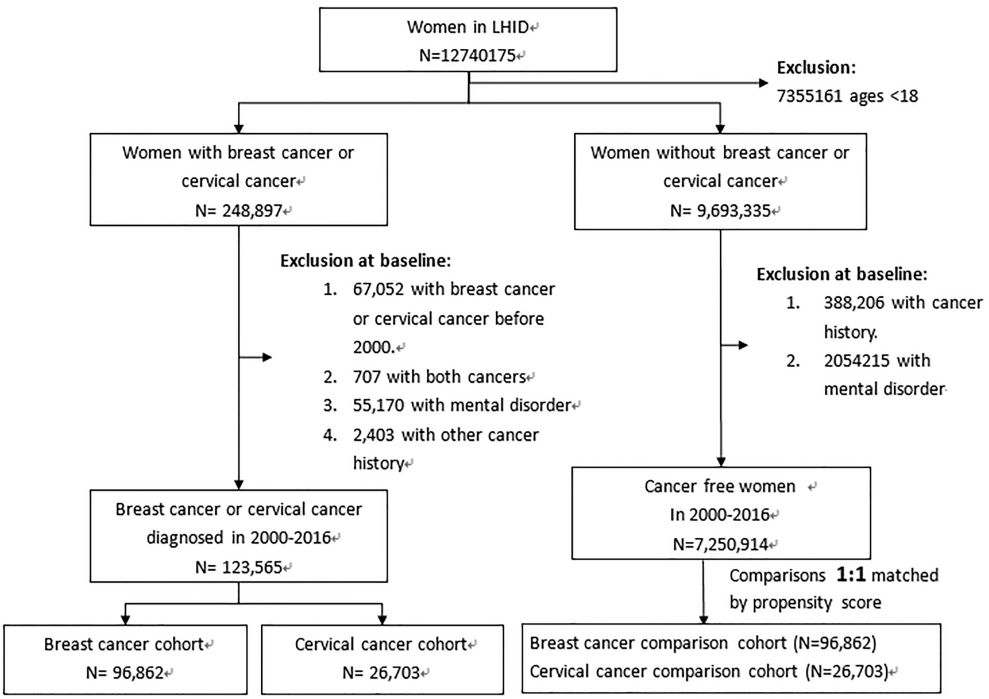


FIGURE 1
Flowchart for establishing study cohorts.

BC cohort and BC controls, and between the CC cohort and CC controls, including age, occupation, income, urbanization level of residential area, and CCI. The standardized mean difference of each variable between each pair of cancer cohort and control cohort was calculated to indicate the significance level. The Kaplan–Meier method was used to estimate the combined cumulative incident anxiety and depression between each pair of the cancer cohort and the control cohort. Differences were examined by the log-rank test. R software (R Foundation for Statistical Computing, Vienna, Austria) was used to plot the cumulative incidence. We calculated the incidence number and rate of each type of mental disorder for each cohort (Table 2). Cox proportional hazard regression analysis was used to calculate the cancer cohort to the control cohort aHR of each type of disorder. Adjustment was performed by the matched pair. The BC cohort to the CC cohort aHRs was also calculated for the two types of disorder, controlling for age, occupation, income, urbanization, and CCI score. Incidence rates of anxiety and depression (per 1,000 person-years) were then pooled as the overall rate calculated for each cohort by the baseline variables. Cox proportional hazard regression analysis was also used to calculate the cancer cohort to the control cohort adjusted hazard ratios (aHRs) and 95% confidence intervals (CI) by these variables. Adjustment was performed by the matched pair.

Results

Table 1 shows that distributions of all baseline variables were not different between the BC cohort and BC controls and between the CC cohort and CC controls. The cancer cohort was slightly older than their controls in both pairs (mean ages 52.3 versus 51.7 years for the BC pair and 56.6 versus 56.1 for the CC pair). Nearly 30% of women in the CC pairs and 15% of women in the BC pairs were the elderly. Compared to the BC cohort, women in the CC cohort had less white-collar jobs (18% versus 32%) with more lower incomes (52% versus 36%), living in less urbanized areas (52% versus 40%) and having a higher portion of women with a CCI score of 1 and above (14.0% versus 9.20%).

Figure 2 shows that after the 17-year follow-up, the cumulative incidence of the two mental disorders combined in the BC cohort was approximately 3.9% higher than the BC control cohort (23.9% versus 20.0%) (log-rank test $p < 0.0001$),

TABLE 1 Distributions of baseline characteristics compared between breast cancer cohort and BC control cohort and between cervical cancer cohort and CC control cohort.

Variable	Breast cancer N = 96,862		BC control N=96,862		Standardization difference	Cervical cancer N = 26,703		CC control N = 26,703		Standardization difference
Age, years	n	%	n	%		n	%	n	%	
18-39	11,988	12.4	11,964	12.4	0.001	2,915	10.9	2,908	10.9	0.001
40-49	31,919	33.0	32,199	33.2	0.006	6,555	24.6	6,592	24.7	0.003
50-64	37,843	39.1	37,906	39.1	0.001	9,270	34.7	9,151	34.3	0.009
65-74	11,485	11.9	11,243	11.6	0.009	5,335	20.0	5,234	19.6	0.010
75+	3,627	3.74	3,550	3.67	0.010	2,628	9.84	2,818	10.5	0.012
Mean (SD)	52.3	(11.9)	51.7	(13.8)	0.046	56.6	(14.4)	56.1	(16.0)	0.034
Occupation										
Homemaker	26,383	27.2	26,122	27.0	0.006	8,923	33.4	9,026	33.8	0.008
White collar	20,911	31.9	30,913	31.9	0.000	4,881	18.3	4,782	17.9	0.010
Blue collar	28,101	29.0	28,346	29.3	0.006	9,452	35.4	9,485	35.5	0.003
Other	11,467	11.8	11,481	11.9	0.000	3,447	12.9	3410	12.8	0.004
Income, NTD										
≤20,000	35,152	36.3	35,141	36.3	0.000	13,777	51.6	13,845	51.9	0.005
20,001-39,999	40,279	41.6	40,387	41.7	0.002	9,669	36.2	9,669	36.2	0.000
40,000+	21,431	22.1	21,334	22.0	0.002	3,257	12.2	3,189	11.9	0.008
Urbanization										
Urban	57,723	59.6	58,045	59.9	0.007	12,811	48.0	12,857	48.2	0.003
Suburban	30,659	31.7	3,791	31.8	0.003	10,118	37.9	10,215	38.3	0.007
Rural	8,480	8.75	8,026	8.29	0.017	3,774	14.1	3,631	13.6	0.015
CCI score										
0	87,948	90.8	89,857	92.8	0.072	22,961	86.0	23,408	87.7	0.050
1	6,053	6.25	5,106	5.27	0.042	2,281	8.54	2,086	7.81	0.027
2+	2,861	2.95	1,899	1.96	0.064	1,461	5.47	1,209	4.53	0.043

SD, standard deviation; Other: unemployed or retired; CCI, Charlson comorbidity index.

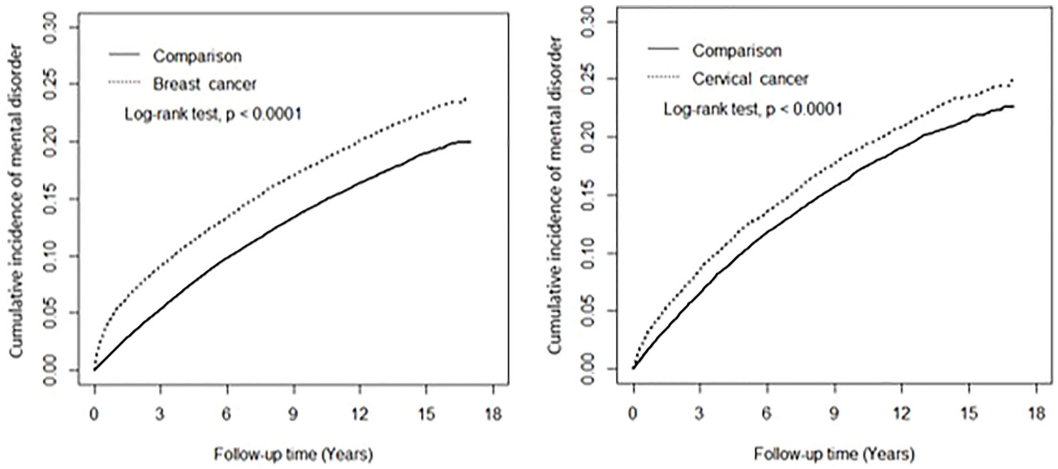


FIGURE 2
Cumulative incident anxiety and depression combined among study cohorts.

and that in the CC cohort was about 2.2% higher than in the CC control cohort (24.7% versus 22.5%) (log-rank test $p < 0.0001$).

Table 2 shows that the incidence of anxiety was higher than that of depression in each cohort. The incidence of anxiety was 1.3-fold higher in the BC cohort than in the BC controls (15.9 versus 12.0 per 1,000 person-years) with an aHR of 1.29 (95% CI = 1.25–1.33). The incidence of depression was 1.8-fold higher in the BC cohort than in the BC controls (6.92 versus 3.77 per 1,000 person-years) with an aHR of 1.78 (95% CI = 1.69–1.87). The incidence rates of both anxiety and depression were slightly lower in the CC cohort than in the BC cohort, whereas these incidence rates were slightly higher in the CC controls than in the BC controls. The CC cohort to CC control aHRs for these two disorders were 1.12 (95% CI = 1.06–1.18) and 1.29 (95% CI = 1.18–1.41), respectively. Compared to the CC cohort, the incidence rates of both anxiety and depression were slightly higher in the BC cohort, but significant for depression (aHR = 1.09, 95% CI = 1.01–1.17) not for anxiety (aHR = 1.04, 95% CI 158 = 0.99–1.09) (Table 3).

During the study period, the overall incidence of anxiety and depression was nearly 1.44-fold higher in breast cancer women than in the comparisons (22.8 versus 15.8 per 1,000 person-years, or 12,534 versus 10,005 cases), with an aHR of 1.40 (95% CI = 1.37–1.44) (Table 4). The incidence increased with age in both cohorts and peaked in the 65–74-year age, whereas the BC cohort to BC control cohort aHRs decreased with age, from 1.91 at 18–39 years of age to 1.15 in 65–74 years of age. The incidence decreased with income in both cohorts, whereas the HR for the BC cohort increased with income to 1.51 for those with higher incomes. Comorbidity had no contribution to the risk for the BC cohort but contributed an increased incidence for BC controls from 15.6 per 1,000 person-years in those without comorbidity to 20.7 per 1,000 person-years in those with comorbidity. The increased hazard remained significant for the BC cohort.

The overall combined incidence of anxiety and depression was near 1.2-fold higher in the CC cohort than in the comparisons (21.7 versus 18.3 per 1,000 person-years), with an aHR of 1.17 (95% CI =1.11-1.22) (Table 5). The incidence increased with age in both cohorts, whereas the aHR decreased with age from 1.63 (95% CI = 1.39-1.89) at 18-39 years-old to 1.07 (95% CI = 0.96-1.19) in those aged 65-74. The comorbidity associated incidence was higher in the CC cohort than in controls (24.3 versus 21.7 per 1,000 person-years), with an

TABLE 2 Incidence rates of anxiety and depression and cancer cohort to comparisons hazard ratio.

	Breast cancer			BC control			Hazard ratio (95% confidence interval)	
Outcome	**n**	**PY**	**Rate**	**n**	**PY**	**Rate**	**Crude**	**Adjusted**
Anxiety	8,724	549,703	15.9	7,617	632,488	12.0	1.33 (1.30-1.39)	1.29 (1.25-1.33)
Depression	3,806	549,703	6.92	2,385	632,488	3.77	1.83 (1.79-1.89)	1.78 (1.69-1.87)
	Cervical cancer			**CC control**				
Anxiety	2,584	167,182	15.5	2,754	203,189	13.6	1.14 (1.09-1.21)	1.12 (1.06-1.18)
Depression	1,050	167,182	6.28	960	203,189	4.72	1.33 (1.25-1. 41)	1.29 (1.18-1.41)

PY, person-years; Rate, per 1,000 person-years; Adjusted hazard ratio, adjusted for matched pair.
$p < 0.001$ for each hazard ratio.

TABLE 3 Breast cancer cohort to cervical cancer cohort adjusted hazard ratio of mental disorder by type.

Outcome	Crude HR (95% CI)	Adjusted hazard ratio (95% CI)
Anxiety	1.01 (0.94-1.03)	1.04 (0.99-1.09)
Depression	1.08 (1.04-1.11)	1.09 (1.02-1.17)

Adjusted for age, occupation, income, urbanization, and CCI score.

aHR of 1.12 (95% CI = 0.96-1.29) for CC patients, which was not significant.

Discussion

Mental disorder as the consequence of reaction to a catastrophic disorder may vary by type and severity of the disorder, sociodemographic variation, and stage of disorder diagnosed (8, 9, 32, 33). A population study using Japanese medical claims data evaluating mental disorders in women found that 16.9% breast cancer patients and 2.7% cervical cancer patients developed major depressive disorder (MDD) after being diagnosed with the cancers. This contrast indicates that the risk of developing the mood disorder was greater for Japanese women with breast cancer than for those with cervical cancer (33). Disorders of anxiety and depression may go hand in hand associated with the risk and severity of diseases. An anxiety disorder may trigger the occurrence of depression with a strong association with the risk level of disease. In our propensity score-matched cohort study, proportions of patients with depression developed were similar in both BC cohort and CC cohort (3.9%), and anxiety developed was slightly lower in the BC cohort than in the CC cohort (9.0 versus 9.7%). The data showed similar absolute risks of developing these mental disorders in women with BC and in women with CC, although the sample size of the BC cohort was 3.6-fold greater than that of the CC cohort, demonstrating that women in Taiwan are at a higher risk of developing BC than developing CC. The HR of developing anxiety was slightly greater for women with breast cancer than for those with cervical cancer, but not significant, featuring that both types of cancer could trigger anxiety at a similar level. The

TABLE 4 Combined incidence of anxiety and depression and breast cancer cohort to comparison cohort adjusted hazard ratio by age, occupation, income, urbanization, and Charlson comorbidity index score.

Variable	BC control			Breast cancer			Adjusted HR (95% CI)
	Event, n	PY	Rate	Event, n	PY	Rate	
Overall	10,002	632,488	15.8	12,530	549,703	22.8	1.40 (1.37-1.44)***
Age, years							
18-39	854	95,003	8.99	1,400	78,297	17.9	1.91 (1.75-2.07)***
40-49	3,438	234,562	14.7	4,348	19,962	21.8	1.44 (1.38-1.51)***
50-64	4,182	231,962	18.0	5,039	201,127	25.0	1.36 (1.30-1.41)***
65-74	983	44,085	22.3	1,332	51,600	25.8	1.15 (1.06-1.25)***
75+	545	26,876	20.3	411	19,069	21.6	1.07 (0.94-1.22)
Occupation							
Homemaker	2,862	164,289	17.4	3,480	145,502	23.9	1.34 (1.28-1.41)***
White collar	2,633	209,800	12.6	3,563	183,478	19.4	1.51 (1.43-1.58)***
Blue collar	3,370	190,807	17.7	4,147	164,392	25.4	1.40 (1.34-1.46)***
Other	1,137	67,592	16.8	1,340	57,232	23.4	1.35 (1.25-1.46)***
Income							
≤20,000	4,844	270,388	17.9	5,472	230,279	23.8	1.29 (1.24-1.34)***
20,001-39,999	3,409	232,941	14.6	4,639	203,872	22.7	1.51 (1.45-1.58)***
40,000+	1,749	129,159	13.5	2,419	11,553	20.9	1.51 (1.42-1.60)***
Urbanization							
Urban	6,008	382,364	15.7	7,462	334,402	22.3	1.39 (1.34-1.43)***
Suburban	3,119	197,532	15.8	3,859	167,820	23.0	1.41 (1.35-1.48)***
Rural	875	52,592	16.6	1,209	47,481	25.5	1.48 (1.36-1.62)***
CCI score							
0	9,380	602,452	15.6	11,644	510,899	22.8	1.42 (1.39-1.46)***
1+	622	30,036	20.7	886	38,804	22.8	1.12 (1.01-1.24)*

PY, person-years; Rate, per 1,000 person-years; CCI, Charlson comorbidity index.
Adjusted for matched pair. *p < 0.05, ***p < 0.001.

TABLE 5 Combined incidence of anxiety and depression and cervical cancer cohort to comparison cohort adjusted cohort hazard ratio by age, occupation, income, urbanization, and Charlson comorbidity index score.

Variable	CC control			Cervical cancer			HR (95% CI)
	Event, n	PY	Rate	Event, n	PY	Rate	
Overall	3,714	203,189	18.3	3,634	167,182	21.7	1.17 (1.11-1.22)***
Age, years							
18-39	300	27,120	11.1	401	21,653	18.5	1.63 (1.40-1.89)***
40-49	973	59,991	16.2	952	48,342	19.7	1.18 (1.08-1.29)**
50-64	1,385	70620	19.6	1,285	58,275	22.0	1.10 (1.02-1.19)*
65-74	691	28,134	24.6	672	25,667	25.6	1.07 (0.96-1.19)
75+	365	17,324	21.1	324	13,245	24.5	1.15 (0.99-1.34)
Occupation							
Homemaker	1,206	65,362	18.5	1,152	52,531	21.9	1.16 (1.08-1.26)***
White collar	576	40,548	14.2	627	34,288	18.3	1.26 (1.12-1.40)***
Blue collar	1,496	73,822	20.3	1,393	62,784	22.2	1.08 (1.00-1.16)*
Other	436	23,458	18.6	462	17,579	26.3	1.37 (1.20-1.56)***
Income, NTD							
≤20,000	2,318	117,010	19.8	2,233	95,869	23.3	1.15 (1.09-1.22)***
20,001-39,999	1,043	63868	16.3	1,011	52,493	19.3	1.16 (1.06-1.26)**
40,000+	353	22,311	15.8	390	18,820	20.7	1.27 (1.10-1.47)***
Urbanization							
Urban	1,765	98,506	17.9	1,740	80,132	21.7	1.18 (1.11-1.26)***
Suburban	1,439	77,461	18.6	1,308	63,790	20.5	1.09 (1.01-1.17)*
Rural	510	27,223	18.7	586	23,260	25.2	1.32 (1.17-1.49)***
CCI score							
0	3,379	187,765	18.0	3,282	152,701	21.5	1.17 (1.12-1.23)***
1+	335	15,424	21.7	352	14,481	24.3	1.12 (0.96-1.29)

PY, person-years; Rate, per 1,000 person-years; CCI, Charlson comorbidity index.
Adjusted for matched pair. *p < 0.05, **p < 0.01, ***p < 0.001.

HR of developing depression was also slightly greater for women with breast cancer than with cervical cancer, but significant, featuring that BC has a slightly stronger impact than CC in triggering depression. This contrast is much slighter than that in Japanese women.

An earlier study using a smaller randomly selected database of insurance claims data of Taiwan found that women with breast cancer had significant incidence rate ratios of 1.94 for MDD and 1.22 for anxiety, relative to controls (34). Another study using a similar database found that women with cervical cancer were prominent for developing depression with an incidence rate ratio of 1.35 (35). These risk estimates are consistent with our findings. The smallish risk variations among these three studies might be associated with databases used.

Our study showed that the occurrence of both depression and anxiety in BC patients and CC patients shared similar risk characteristics associated with age: both incidence rates increased with age, but the HRs were the greatest for patients of the youngest group. The development of psychiatric disorder has been associated with comorbidities. A meta-analysis based on 40 articles showed that patients with multimorbidity could be up to three times more likely depressed than persons without chronic disorders (36). Our data failed to show this relationship in breast cancer patients, but a higher CCI was associated with slightly increased HR of combined incidence of disorders in cervical cancer patients. Comorbidities generally increase with age and are more prevalent in the elderly, explaining that the combined incidence of these two psychiatric disorders increased with age in all our cohorts. However, the subgroup analysis for HRs among age groups showed that, relative to the same age group of the respective control cohort, younger cancer patients had higher HRs than those of older age, indicating that BC or CC is the major risk factor associated with developing psychiatric disorders in younger patients, probably because comorbidities are less prevalent in younger patients than in the elderly. Therefore, the metal disorder reaction to the catastrophic diseases is stronger for younger patients.

Research has shown that social inequalities can contribute to the severity of diseases at which disadvantaged patients might be more likely to experience mood disorders (37–40). An earlier meta-analysis reported that individuals with the lowest socioeconomic position had an odds ratio of 1.81 being depressed relative to those with the highest socioeconomic

position (38). The European Health Interview Survey in Spain showed that MDD in women is strongly associated with socioeconomic disadvantage, including those retired and homemakers (39). A recent meta-analysis based on 40 studies in China reported that the lifetime prevalence of MDD was the highest in participants with the lowest education or those living in rural areas (40). Our data also showed that women from disadvantaged backgrounds experienced greater incident rates of anxiety and depression, the highest for women with blue collar jobs or with a lower income in both the BC cohort and the BC controls. In the CC cohort and CC controls, CC patients of unemployed or retired or of low income were at a higher risk, and these patients are more likely older.

This study had the advantage of using a large population-based health insurance data, from which we could perform a robust cohort study design to randomly select controls matched by propensity score. The matching capacity helped minimize potential bias. The large sample sizes allowed multivariate analyses to assess the mental disorder risks associated with the sociodemographic category. There were also some limitations in this study. The claims data provided no information on the cancer stage. We therefore were unable to examine the incidence of mental disorder by the severity of cancers. Information on lifestyle was also unavailable to be included in data analysis. However, the propensity matching design and CCI use in this study could reduce potential bias, in addition to being controlled by occupation, income, and residential area. Furthermore, based on clinical diagnoses in the claims data, cancer patients with mild mental disorder symptoms might not be diagnosed. The risks of anxiety and depression may be underestimated for both cancer cohorts and comparison cohorts.

Conclusion

Our study found that women in Taiwan are at a much higher risk of developing breast cancer than developing cervical cancer. The risks of further developing anxiety and depression are slightly higher in women with breast cancer than in women with cervical cancer, but the risk difference is significant for depression, but not for anxiety. Risks of both disorders increase with age, but relatively the hazards of developing the mental disorders were greater for youngers. Women with a less well-off economic status are also at a relatively elevated risk.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

This study was approved by the Ethical Research Committee at China Medical University and Hospital (H107257). Because Personal identifications in the data files had been scrambled to protect privacy, Patient consents were waived.

Author contributions

Conception: C-MY, F-CS, S-SH. Design: C-MY, F-CS, C-HM, C-HL. Data analysis: C-HM. Results interpretation: C-MY, C-HM, F-CS. Data evaluation: C-HL, P-HW, S-SH. Drafting the article: C-MY, F-CS, S-SH. Manuscript revision: all authors. C-HM, F-CS, and S-SH had access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

Funding

This study is supported in part by Taiwan Ministry of Health and Welfare Clinical Trial Center (MOHW110-TDU-B-212-124004), China Medical University Hospital (DMR-111-228) and China Medical University (CMU110-S-18), Taiwan. The funders had no role in the study design, data collection and analysis, the decision to publish, or preparation of the manuscript.

Acknowledgments

We are grateful to the Health Data Science Center and China Medical University Hospital for providing administrative, technical and funding support for using the data.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Yang X, Fang Y, Chen H, Zhang T, Yin X, Man J, et al. Global, regional and national burden of anxiety disorders from 1990 to 2019: results from the global burden of disease study 2019. *Epidemiol Psychiatr Sci* (2021) 30:e36. doi: 10.1017/S2045796021000275

2. Kupferberg A, Bicks L, Hasler G. Social functioning in major depressive disorder. *Neurosci Biobehav Rev* (2016) 69:313–32. doi: 10.1016/j.neubiorev.2016.07.002

3. WHO Depression. *World health organization, Geneva* (2021). Available at: https://www.who.int/news-room/fact-sheets/detail/depression (Accessed September 13, 2020).

4. Gilman SE, Sucha E, Kingsbury M, Horton NJ, Murphy JM, Colman I. Depression and mortality in a longitudinal study: 1952-2011. *CMAJ* (2017) 189:E1304–10. doi: 10.1503/cmaj.170125

5. Koch-Gallenkamp L, Bertram H, Eberle A, Holleczek B, Schmid-Hopfner S, Waldmann A, et al. Fear of recurrence in long-term cancer survivors-do cancer type, sex, time since diagnosis, and social support matter? *Health Psychol* (2016) 35:1329–33. doi: 10.1037/hea0000374

6. Massie MJ. Prevalence of depression in patients with cancer. *J Natl Cancer Inst Monogr* (2004) 32:57–71. doi: 10.1093/jncimonographs/lgh014

7. Mehnert A, Brahler E, Faller H, Harter M, Keller M, Schulz H, et al. Four-week prevalence of mental disorders in patients with cancer across major tumor entities. *J Clin Oncol* (2014) 32:3540–6. doi: 10.1200/JCO.2014.56.0086

8. Carreira H, Williams R, Muller M, Harewood R, Bhaskaran K. Adverse mental health outcomes in breast cancer survivors compared to women who did not have cancer: a systematic review. *JNCI* (2018) 110:djy177. doi: 10.1186/s13643-017-0551-2

9. Maass SW, Roorda C, Berendsen AJ, Verhaak PF, de Bock GH. The prevalence of long-term symptoms of depression and anxiety after breast cancer treatment: A systematic review. *Maturitas* (2015) 82:100–8. doi: 10.1016/j.maturitas.2015.04.010

10. Pinquart M, Duberstein PR. Depression and cancer mortality: a meta-analysis. *Psychol Med* (2010) 40:1797–810. doi: 10.1017/S0033291709992285

11. Hartl K, Janni W, Kastner R, Sommer H, Strobl B, Rack B, et al. Impact of medical and demographic factors on long-term quality of life and body image of breast cancer patients. *Ann Oncol* (2003) 14:1064–71. doi: 10.1093/annonc/mdg289

12. Gartner R, Jensen MB, Nielsen J, Ewertz M, Kroman N, Kehlet H. Prevalence of and factors associated with persistent pain following breast cancer surgery. *JAMA* (2009) 302:1985–92. doi: 10.1001/jama.2009.1568

13. DiSipio T, Rye S, Newman B, Hayes S. Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and meta-analysis. *Lancet Oncol* (2013) 14:500–15. doi: 10.1016/S1470-2045(13)70076-7

14. Kim Y, Kashy DA, Wellisch DK, Spillers RL, Kaw CK, Smith TG. Quality of life of couples dealing with cancer: dyadic and individual adjustment among breast and prostate cancer survivors and their spousal caregivers. *Ann Behav Med* (2008) 35(2):230–8. doi: 10.1007/s12160-008-9026-y

15. Inhestern L, Bergelt C. When a mother has cancer: strains and resources of affected families from the mother's and father's perspective - a qualitative study. *BMC Womens Health* (2018) 18:72. doi: 10.1186/s12905-018-0562-8

16. Mitchell AJ, Chan M, Bhatti H, Halton M, Grassi L, Johansen C, et al. Prevalence of depression, anxiety, and adjustment disorder in oncological, haematological, and palliative-care settings: a meta-analysis of 94 interview-based studies. *Lancet Oncol* (2011) 12:160–74. doi: 10.1016/S1470-2045(11)70002-X

17. Krebber AM, Buffart LM, Kleijn G, Riepma IC, de Bree R, Leemans CR, et al. Prevalence of depression in cancer patients: a meta-analysis of diagnostic interviews and self-report instruments. *Psychooncology* (2014) 23:121–30. doi: 10.1002/pon.3409

18. Zahid JA, Grummedal O, Madsen MT, Gögenur I. Prevention of depression in patients with cancer: A systematic review and meta-analysis of randomized controlled trials. *J Psychiatr Res* (2020) 120:113–23. doi: 10.1016/j.jpsychires.2019.10.009

19. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2018) 68:394–424. doi: 10.3322/caac.21492

20. Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical cancer. *Lancet* (2019) 393:169–82. doi: 10.1016/S0140-6736(18)32470-X

21. Ye S, Yang J, Cao D, Lang J, Shen KA. Systematic review of quality of life and sexual function of patients with cervical cancer after treatment. *Int J Gynecol Cancer* (2014) 24:1146–57. doi: 10.1097/IGC.0000000000000207

22. Boquiren VM, Esplen MJ, Wong J, Toner B, Warner E, Malik N. Sexual functioning in breast cancer survivors experiencing body image disturbance. *Psychooncology* (2016) 25:66–76. doi: 10.1002/pon.3819

23. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, et al. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. *Lancet* (2018) 391:1023–75. doi: 10.1016/S0140-6736(17)33326-3

24. WHO. *Breast cancer* (2021). Geneva: World Health Organization. Available at: https://www.who.int/news-room/fact-sheets/detail/breast-cancer (Accessed July 25, 2021).

25. WHO. *Cervical cancer* (2018). Geneva: World Health Organization. Available at: http://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/ (Accessed July 25, 2021).

26. *SEER cancer statistics review* (2020). Available at: https://seer.cancer.gov/csr/1975_2018/.

27. Tsatsou I, Parpa E, Tsilika E, Katsaragakis S, Batistaki C, Dimitriadou E, et al. A systematic review of sexuality and depression of cervical cancer patients. *J Sex Marital Ther* (2019) 45:739–54. doi: 10.1080/0092623X.2019.1610125

28. Huang YC, Chen YH. Cancer incidence characteristic evolution based on the national cancer registry in Taiwan. *J Oncol* (2020) 2020:1408793. doi: 10.1155/2020/1408793

29. Health promotion administration, ministry of health and welfare, in: *Cancer registry report-annual report* . Available at: https://cris.hpa.gov.tw/pagepub/Home.aspx?itemNo=cr.a.10 (Accessed July 4, 2022).

30. Hsing AW, Ioannidis JP. Nationwide population science: Lessons from the taiwan national health insurance research database. *JAMA Internal Med* (2015) 175:1527–9. doi: 10.1001/jamainternmed.2015.3540

31. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classification of prognostic comorbidity for longitudinal studies: development and validation. *J Chron Dis* (1987) 40:373–83. doi: 10.1016/0021-9681(87)90171-8

32. Lu D, Andersson TM, Fall K, Hultman CM, Czene K, Valdimarsdóttir U, et al. Clinical diagnosis of mental disorders immediately before and after cancer diagnosis: a nationwide matched cohort study in Sweden. *JAMA Oncol* (2016) 2:1188–96. doi: 10.1001/jamaoncol.2016.0483

33. Akechi T, Mishiro I, Fujimoto S, Murase K. Risk of major depressive disorder in Japanese cancer patients: A matched cohort study using employer-based health insurance claims data. *Psychooncology* (2020) 29:1686–94. doi: 10.1002/pon.5509

34. Hung YP, Liu CJ, Tsai CF, Hung MH, Tzeng CH, Liu CY, et al. Incidence and risk of mood disorders in patients with breast cancers in Taiwan: a nationwide population-based study. *Psychooncology* (2013) 22:2227–34. doi: 10.1002/pon.3277

35. Shyu IL, Hu LY, Chen YJ, Wang PH, Huang BS. Risk factors for developing depression in women with cervical cancer: a nationwide population-based study in Taiwan. *Int J Womens Health* (2019) 11:135–41. doi: 10.2147/IJWH.S193003

36. Read JR, Sharpe L, Modini M, Dear BF. Multimorbidity and depression: a systematic review and meta-analysis. *J Affect Disord* (2017) 15:36–46. doi: 10.1016/j.jad.2017.06.009

37. Lorant V, Deliège D, Eaton W, Robert A, Philippot P, Ansseau M, et al. Socioeconomic inequalities in depression: a meta-analysis. *Am J Epidemiol* (2003) 157:98–112. doi: 10.1093/aje/kwf182

38. Muntaner C, Eaton WW, Miech R, O'Campo P. Socioeconomic position and major mental disorders. *Epidemiol Rev* (2004) 26:53–62. doi: 10.1093/epirev/mxh001

39. Arias-de la Torre J, Vilagut G, Martín V, Molina AJ, Alonso J. Prevalence of major depressive disorder and association with personal and socio-economic factors. results for Spain of the European health interview survey 2014-2015. *J Affect Disord* (2018) 15:239. doi: 10.1016/j.jad.2018.06.051

40. Zhao YJ, Jin Y, Rao WW, Zhang QE, Zhang L, Jackson T, et al. Prevalence of major depressive disorder among adults in china: a systematic review and meta-analysis. *Front Psychiatry* (2021) 12:659470. doi: 10.3389/fpsyt.2021.659470

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 31 August 2022
DOI 10.3389/fonc.2022.980793

OPEN ACCESS

EDITED BY
Claudia Mello-Thoms,
The University of Iowa, United States

REVIEWED BY
Robert Nishikawa,
University of Pittsburgh, United States
Ziba Gandomkar,
The University of Sydney, Australia

*CORRESPONDENCE
Meredith A. Jones
Meredith.jones@ou.edu

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 28 June 2022
ACCEPTED 04 August 2022
PUBLISHED 31 August 2022

CITATION
Jones MA, Islam W, Faiz R, Chen X and Zheng B (2022) Applying artificial intelligence technology to assist with breast cancer diagnosis and prognosis prediction.
Front. Oncol. 12:980793.
doi: 10.3389/fonc.2022.980793

Applying artificial intelligence technology to assist with breast cancer diagnosis and prognosis prediction

Meredith A. Jones[1]*, Warid Islam[2], Rozwat Faiz[2], Xuxin Chen[2] and Bin Zheng[2]

[1]School of Biomedical Engineering, University of Oklahoma, Norman, OK, United States, [2]School of Electrical and Computer Engineering, University of Oklahoma, Norman, OK, United States

Breast cancer remains the most diagnosed cancer in women. Advances in medical imaging modalities and technologies have greatly aided in the early detection of breast cancer and the decline of patient mortality rates. However, reading and interpreting breast images remains difficult due to the high heterogeneity of breast tumors and fibro-glandular tissue, which results in lower cancer detection sensitivity and specificity and large inter-reader variability. In order to help overcome these clinical challenges, researchers have made great efforts to develop computer-aided detection and/or diagnosis (CAD) schemes of breast images to provide radiologists with decision-making support tools. Recent rapid advances in high throughput data analysis methods and artificial intelligence (AI) technologies, particularly radiomics and deep learning techniques, have led to an exponential increase in the development of new AI-based models of breast images that cover a broad range of application topics. In this review paper, we focus on reviewing recent advances in better understanding the association between radiomics features and tumor microenvironment and the progress in developing new AI-based quantitative image feature analysis models in three realms of breast cancer: predicting breast cancer risk, the likelihood of tumor malignancy, and tumor response to treatment. The outlook and three major challenges of applying new AI-based models of breast images to clinical practice are also discussed. Through this review we conclude that although developing new AI-based models of breast images has achieved significant progress and promising results, several obstacles to applying these new AI-based models to clinical practice remain. Therefore, more research effort is needed in future studies.

KEYWORDS

breast cancer, machine learning, deep learning, computer aided detection, computer aided diagnosis, mammography

Introduction

The latest cancer statistics data for the USA estimates that in 2022, 31% of cancer cases detected in women are breast cancer with 43,250 cases resulting in death. This accounts for 15% of total cancer-related deaths (1). Thus, breast cancer remains the most diagnosed cancer among women with the second highest mortality rate. Over the past three decades, population-based breast cancer screening has played an important role in helping detect breast cancer in the early stage and reduce the mortality rate. From 1989 to 2017, the mortality rate of breast cancer dropped 40% which translates to 375,900 breast cancer deaths averted (2). Even though the mortality rate continues to decline, the rate of decline has slowed from 1.9% per year from 1998-2011 to 1.3% per year from 2011-2017 (2). However, the efficacy of population-based breast cancer screening is a controversial topic due to the low cancer prevalence (≤0.3%) in annual breast cancer screening resulting in a low cancer detection yield and high false-positive rate (3). This high false positive rate is indicative of a high rate of unnecessary biopsies which is not only an economic burden but also leads to unnecessary patient anxieties which often result in women being less likely to continue with routine breast cancer screening (4). Conversations pertaining to the benefits and harms of screening mammography as well as its efficacy in decreasing breast cancer mortality as screening exams do not reduce the incidence of advanced/aggressive cancers are now common (5). For example, detection of ductal carcinoma *in situ* (DCIS) or early invasive cancers that will never progress or be of risk to the patient are occurring at a disproportionately higher rate than aggressive cancers. This is referred to as overdiagnosis and often results in unnecessary treatment that may cause more harm than the cancer itself (6). Thus, improving the efficacy of breast cancer detection and/or diagnosis remains an extremely pressing global health issue (7).

While advances in medical imaging technology and progress towards better understanding the complex biological and chemical nature of breast cancer have greatly contributed to the large decline in breast cancer mortality, breast cancer is a complex and dynamic process, making cancer management a difficult journey with many hurdles along the way. The cancer detection and management pipeline has many steps, including detecting suspicious tumors, diagnosing said tumors as malignant or benign, staging the subtype and histological grade of a cancer, developing an optimal treatment plan, identifying tumor margins for surgical resections, evaluating and predicting response to chemo or radiation therapies, or predicting risk of future occurrence or reoccurrence. In this clinical pipeline, medical imaging plays a crucial role in the decision-making process for each of these tasks. Traditionally, radiologists will rely on qualitative or semi-quantitative information visually extracted from medical images to detect suspicious tumors, predict the likelihood of malignancy, and evaluate cancer prognosis. The clinically relevant information may include enhancement patterns, presence or absence of necrosis or blood, density and size of suspicious tumors, tumor boundary margin spiculation, or location of the suspicious tumor. However, interpreting and integrating information visually detected from medical images to make a final diagnostic decision is not an easy task.

Although mammography is the most frequently employed imaging modality in breast cancer screening, its performance is often unsatisfactory with lower sensitivity (i.e., missing 1 in 8 cancers during interpretation) and very high false positive rates (i.e., <30% of biopsies are malignant) (8). Thus, the downfalls of mammography have led to an increase in the use of other adjunct imaging modalities in clinical practice including ultrasound (US) and dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) (9, 10). Digital breast tomosynthesis (DBT) is a newer modality that is commonly used in which X-ray images are taken over multiple angles in a limited range (i.e., ± 15° and the acquired scanning data is reconstructed into quasi-3D breast images to reduce the impact of dense breast tissue overlap in 2D mammograms (11). Additionally, several other new imaging modalities including contrast enhanced spectral mammography (CESM) (9, 10), phase contrast breast imaging (12), breast computed tomography (13), thermography and electrical impedance tomography of breast imaging (14), and molecular breast imaging (15), have also been investigated and tested in many prospective studies or clinical trials. However, using more imaging modalities for breast cancer detection and diagnosis increases the workload of radiologists in busy clinical practice. Over the last three decades, computer-aided detection and diagnosis (CAD) schemes are being rapidly developed to optimize the busy clinical workflow by assisting radiologists in more accurately and efficiently reading and interpreting multiple images from multiple sources (16, 17).

In the literature, CAD is often differentiated as computer-aided detection (CADe) or computer-aided diagnosis (CADx). The goal of CADe schemes is to reduce observational oversight by drawing the attention of radiologists to suspicious regions in an image. Commercialized CADe schemes of mammograms have been in clinical use since 1998 (18). One study reported that in 2016 CADe was used in about 92% of screening mammograms read in the United States (18, 19). Despite the wide scale clinical adoption, the utility of CADe schemes for breast cancer screening is often questioned (20–22). On the other hand, the goal of computer-aided diagnosis (CADx) schemes is to characterize a suspicious area and assign it to a specific class. US FDA approved the first CADx scheme of breast MR images, QuantX by Qlarity Imaging (Chicago, IL) in 2017 (23). The goal of QuantX is to assist radiologists in deciding if a lesion is malignant or benign by providing a probability estimation of malignancy. This software has yet to be extensively adopted and requires much more clinical testing.

Despite great research efforts and the availability of commercialized CAD tools, the added clinical value of CAD schemes and ML-based prediction models for breast images is limited. Thus, more novel research efforts are needed to explore new approaches (24). While using radiological features from medical images to infer phenotypic information has been done for many years, recent rapid advances in bioinformatics coupled with the advent of high performing computers has led to the field of radiomics. Radiomics involves the computation of quantitative image-based features that can be mined and used to predict clinical outcomes (25). In medical imaging, radiomic techniques are used to extract a large number of features from a set of medical images to quantify and characterize the size, shape, density, heterogeneity, and texture of the targeted tumors (26). Then, a statistics-based feature analysis tool such as Lasso regression or a machine learning (ML) based pipeline is applied to identify small sets of features that are more clinically relevant to the specific application. One method to ensure the extracted features contain some clinical relevance is to segment the tumor region and extract features from there. Despite the relative simplicity of extracting relevant radiomics features, automated tumor segmentation remains a major challenge. Thus, many radiomics-based schemes use manual or semi-automated tumor segmentation. Additionally, recent enthusiasm for deep learning based artificial intelligence (AI) technology has led to new approaches for developing CAD schemes which are being rapidly explored and reported in the literature (27). Several studies have compared CAD schemes using conventional radiomics and deep learning methods to investigate their advantages and limitations (28, 29). Deep learning (DL) based CAD schemes are appealing as majority of such CAD schemes eliminate the need for tedious error prone segmentation steps and no longer need to compute and select optimal radiomic features since deep learning models can extract features directly from the medical images (30). However, despite the challenge of how to achieve high scientific rigor when developing AI-based deep learning models (31), applying AI technology to develop CAD schemes has become the mainstream technique of the CAD research community. Additionally, new AI-based models are being expanded to include broad clinical applications in realms beyond cancer detection and diagnosis, such as prediction of short-term cancer risk and prognosis or clinical outcome.

In order to help researchers better understand state-of-the-art research progress and existing technical challenges, several review articles have recently been published with a variety of goals, such as a review of deep learning (DL) models developed for breast lesion detection, segmentation, and classification (27), radiomics models developed to classify breast lesions and monitor treatment efficacy (32), and how to optimally apply DL models to three commonly used breast imaging modalities (mammograms, ultrasound, and MRI) (33). The focus of this review paper is different from the previously published review articles for the following reasons. First, our paper details the recent advances in both radiomics and DL-based AI technologies to develop new prediction models. Second, this review paper does not review and discuss CADe (lesion detection or segmentation) schemes. It focuses on three more challenged application realms namely, prediction of breast cancer risk, tumor classification (diagnosis) and cancer prognosis (treatment response). Third, to help readers better understand the scientific rationales of applying new AI-based models of medical image to predict breast cancer risk, classify breast lesions, and predict cancer prognosis, this paper reviews recent studies that demonstrate the important relationship between medical image features and the tumor environment (genomic biomarkers), which supports the physiological relevance of radiomics based studies. Last, based on this review process, we are able to summarize several important conclusions that may benefit future research efforts in medical imaging of breast cancer. For this purpose, the rest of this paper is organized as follows. Section two briefly discusses the correlation of extracted medical image features and the tumor environment, followed by section three that surveys recent studies, which detail novel image-based applications of both radiomics and DL-based new AI-supported CAD schemes in three application fields. Lastly, section four discusses and summarizes key points that can be learned or observed from this review paper and future perspectives in developing CAD schemes of breast images.

Relationship between medical image features and tumor environment

A major focus of breast cancer research in the medical imaging field is uncovering the relationships between medical image features and the tumor microenvironment to better predict clinical outcomes (Table 1). Since traditional CAD schemes involve handcrafting a set of features, it is important to understand what kind of descriptors correlate with cancer specific genomic biomarkers, based on radiomic concepts (25), so that optimal and descriptive handcrafted feature sets can be chosen. Additionally, if an image-based marker is widely established as a biomarker for a specific hallmark of cancer such as sustaining proliferative signaling, evading growth suppressors, invasion and metastasis, angiogenesis, or resisting cell death, then monitoring changes in that image-based marker overtime will have high degree of predictive power in many aspects of the cancer management pipeline (32).

For example, many studies investigated the correlation between image-based biomarkers and tumor mechanisms of angiogenesis. As tumors grow and metastasize, there is a decrease in the amount of available oxygen due an increase in demand, resulting in a hypoxic environment (33, 48–51). To adapt to the newly hypoxic environment, the tumor will enter an

TABLE 1 Studies of correlating image-based features with tumor physiology.

Year	Author	Imaging Modality	Image Based Features Extracted	Physiological Features	Relevant Results
2015	Li et al. (34)	DCE-MRI	Quantitative Kinetic Features: K^{trans}, K_{ep}, V_e, ADC	MVD and Proliferation	K^{trans}, K_{ep}, and ADC closely correlate with MVD and Proliferation
2021	Xiao et al. (35)	DCE-MRI	Shape, intensity, and texture features Semi-Quantitative Kinetic Features: PE, SER, FTV, WF	MVD	MVD associates with SER, WF, and radiomic features
2019	Mori et al. (36)	DCE-MRI	Semi-Quantitative Kinetic Features: IER, SER, TIE Quantitative Kinetic Features: EMM derived metrics: A, α, Aα, AUC30	MVD	A, α, Aα, AUC30, and TIE significantly correlate with MVD
2016	Kim et al. (37)	DCE-MRI	Quantitative Kinetic Features: K^{trans}, K_{ep}, V_e,	MVD and VEGF	MVD correlates with V_e and there is significant association between K^{trans}, tumor size, and MVD
2014	Li et al. (38)	DCE-MRI	Semi-Quantitative Kinetic Features: longest dimension, tumor volume, SER, initialAUC Quantitative Kinetic Features: K^{trans}, K_{ep}, V_e, v_p, and τ^i	pathological response to chemotherapy	SER and K_{ep} are significantly different between responders and non-responders ($p<0.05$) and can be used to predict breast cancer response to NACT
2007	Yu et al. (39)	DCE-MRI	Quantitative Kinetic Features: K^{trans}, K_{ep} Tumor size	response to chemotherapy based on RECIST	Tumor size significantly correlates with K^{trans} and K_{ep} and change in tumor size is a better response predictor than both K^{trans} or K_{ep}
2020	Kang et al. (40)	DCE-MRI	Quantitative Kinetic Features: K^{trans}, k_{ep}, v_e, and v_p	ER, PR, HER2, Ki67, p53, EGFR, CK5/6 and lymphovascular space invasion	High K^{trans} and k_{ep} associate with poor prognostic histopathologic factors
2019	Braman et al. (41)	DCE-MRI	Texture and statistical features	HER2+	DCE-MRI texture and statistical features can identify molecular subtype of HER2+ breast cancer from HER2- breast cancers
2016	da Rocha et al. (42)	Mammography	Texture features from the local binary pattern of images	Malignant or benign lesion	GLCM features derived from the Local Binary Pattern have the best results for lesion classification ACC: 88.31% SEN: 85% SPE: 91.89%
2015	Zhu et al. (43)	DCE-MRI	Size, shape, morphological, enhancement texture, kinetic curves, enhancement-variance	miRNA expression, protein expression, gene mutations, transcriptional activities, and gene copy number variation	Transcriptional activities of various genetic pathways positively associate with tumor size, blurred tumor margin and irregular tumor shape, The miRNA expressions associates with the tumor size and enhancement texture
2018	Drukker et al. (44)	DCE-MRI	Semi-Quantitative Kinetic Features: Most enhancing tumor volume (METV)	recurrence free survival based on clinical examination after surgery	METV from pre-NACT and early treatment scans associate with recurrence-free survival
2006	Varela et al. (45)	Mammography	Texture features to characterize contrast and spiculations from the interior, border, and outer area of the mass	Malignant or benign lesions	Features from the mass border and outer regions contain the most information for distinguishing lesions.
2020	La Forgia et al. (46)	CESM	Statistical features	ER, PR, HER2, Ki67, Grade, Triple-negative	Statistical radiomic features extracted from CESM can predict histological outcomes
2017	Wu et al. (47)	DCE-MRI	Semi-Quantitative Kinetic features: FTV features, BPE features Morphological and texture features	molecular subtypes based on IHC	DCE-MRI based features may be able to non-invasively determine the subtype of a breast cancer

SEN, sensitivity; SPE, Specificity; ACC, Overall accuracy.

angiogenic state which changes the microvasculature. In this state the tumor will switch on angiogenic growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGF) to stimulate the formation of new capillaries so that oxygen and nutrients can adequately feed the tumor (48). This process is known as angiogenesis, which is a hallmark of most cancers that can be characterized by non-hierarchical, immature, and highly permeable vasculature that looks obviously different from normal vasculature (52). Traditionally, angiogenesis is indirectly quantified as micro-vessel density (MVD) after immunohistochemical staining of tumor tissue. While high MVD has been established as a

biomarker of poor prognosis and correlated with increased levels of angiogenesis, quantification of MVD is subject to inter- and intra-reader variability, making MVD a non-reproducible and non-standardized marker (53). Thus, development of a quick and non-invasive biomarker that can differentiate between highly immature angiogenic vasculature and normal vasculature has been a hot research topic over the past decade (48, 54).

DCE-MRI is a non-invasive method to detect and characterize the tumor microenvironment. Specifically, dynamic/kinetic image features computed from DCE-MRI characterize the permeability and perfusion kinetics of the tumor microvasculature which can reflect tumor angiogenesis. Many studies have been conducted to correlate quantitative and semi-quantitative DCE-MRI based kinetic features with MVD to demonstrate the relationship between DCE-MRI and tumor angiogenesis (34–37). Peak signal enhancement ratio (peak SER) and washout fraction (WF) are two semi-quantitative metrics extracted from the contrast enhancement curve that reflect the clearance of a contrast agent from the tumor. These metrics directly relate to a highly angiogenic state as rapid washout will occur with a large number of immature and leaky vessels (35). Extracting quantitative features from DCE-MRI requires a pharmacokinetic analysis which requires at high temporal resolution, often resulting in a poor spatial resolution. Clinical DCE-MRI scans prioritize spatial resolution as opposed to temporal resolution, which makes it difficult to do a fully quantitative analysis of clinical DCE-MRI scans. Most studies that have a goal of quantitative analysis of DCE imaging may not be appropriate for clinical use. However, studies have shown that quantitative DCE-MRI parameters such as, K^{trans} and K_{ep}, correlated well with angiogenesis markers and can be used to predict response to treatment or risk of recurrence (34). Physiologically, K_{ep} is a marker of the efflux of contrast agent. High K_{ep} values indicate two observations of tumor microenvironment. The first indicates a strong blood flow with highly permeable vessels which represents existence of an irregular and highly vascularized space associated with tumor angiogenesis. The second indicates the smaller extravascular extracellular space, meaning large quantities of the contrast agent cannot accumulate here; this is expected as there will be an increase in cell density in the tumor environment (38). Technical details pertaining to the extraction of semi-quantitative and fully quantitative kinetic features is beyond the scope of this review, interested readers should explore the following manuscripts for more information (55, 56). While there are many studies exploring the correlations between K^{trans} and K_{ep} and cancer prognosis, there are inconsistent conclusions of the biological relevance of these markers which make studies using kinetic DCE-MRI features non-reproducible (39, 40).

Recent studies demonstrated that radiomics features are thought to be more robust and reproducible than kinetic features computed from breast MRI for different prediction tasks (i.e., classification between malignant and benign tumors, prediction of axillary lymph node metastasis, molecular subtypes of breast cancer, tumor response to chemotherapies and overall survival of patients) (57). For example, malignant tumors as see on mammograms are typically irregular in shape with spiculated margins and architectural distortions while benign tumors are typically rounded with well-defined margins (Figure 1) (58–60). Quantification of these features can help train robust ML classifiers to better differentiate between benign and malignant masses. Features that describe the shape of the tumor may include eccentricity, diameter, convex area, orientation, and more. Shape based features may help differentiate between traditionally round benign tumors and spiculated malignant tumors. While shape features are important, breast compression during mammography makes extraction of these features difficult (60). Features can also be extracted to quantify the spiculations of the tumors which will be particularly helpful for detecting malignant breast tumors (45). First order statistical features are basic metrics that describe the distribution of intensities within an image, this includes mean, standard deviation, variance, entropy, uniformity, and others. For example, entropy quantifies the image histogram randomness which can quantify heterogeneity of the image patterns (61). Texture features belong to the biggest group of radiomics features, which are extremely useful for image recognition and image classification tasks (62, 63). Gray-level cooccurrence matrix (GLCM) based features and gray-level run length matrix (GLRLM) based features are two example of common texture features that characterizes the heterogeneity of intensities within a neighborhood of pixels. Quantification of the heterogeneity of

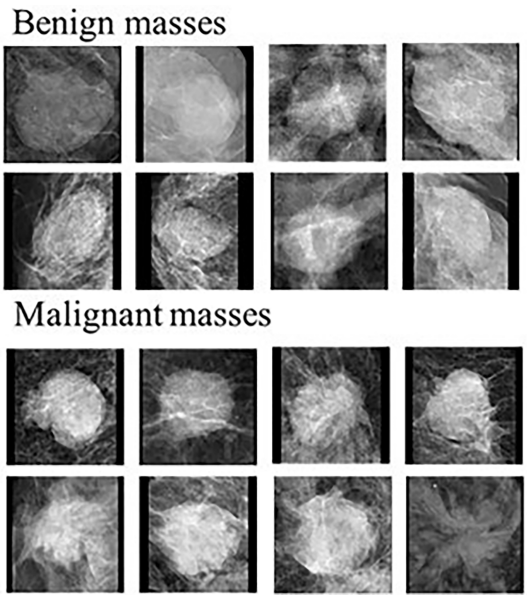
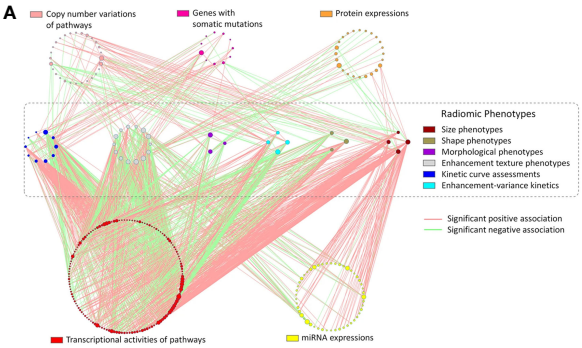


FIGURE 1
Examples of benign and malignant masses seen on mammograms. Modified from (58).

tumors is one of the advantages of radiomics-generated imaging markers as heterogeneity is often very difficult for radiologists to visually capture and quantify in clinical practice.

While identification of physical or biological reasoning for the correlations between image-based markers and cancer specific traits is lacking, there are some studies that do correlate radiomics based features with cancer specific markers that have been obtained from IHC analysis or genomic assays (35, 41). For example, Xiao et al. assessed the correlation between radiomic based DCE-MRI features with MVD in order to identify angiogenesis in breast cancer using DCE-MRI (35). GLCM and GLRLM derived textural features extracted from 3D segmented tumor regions were found to significantly correlate with MVD, therefore, correlate with angiogenesis levels. GLCM derived features from ROIs represented by the local binary patterns were also shown to be extremely useful for distinguishing malignant and benign masses detected on mammograms (42). Radiogenomics is the field that incorporates radiomics based features with patient specific genomic information. Correlation of the image-based features that characterize cancer through genetic information pertaining to tumor hormone receptors and genetic mutations can be very helpful for predicting risk of cancer recurrence and thus help develop optimal personalized treatment plans. Quantitative MRI-based features of tumor size, shape, and blood flow kinetics have been mapped to cancer specific genomic markers (Figure 2) (43, 44, 64). This is a great step forward in development of non-invasive techniques for understanding cancer on a molecular level.

Although DCE-MRI is an important imaging modality used to study the tumor microenvironment and predict tumor staging and/or response to therapies, other modalities have also been investigated for this purpose. For example, contrast enhanced spectral mammography (CESM) has been attracting broad clinical research interest as an alternative to DCE-MRI due to its advantages of low cost, high image resolution, and fast imaging acquisition times. Like DCE-MRI, injection of an intravenous contrast agent in CESM imaging allows for the visualization of contrast enhancement patterns which give insight into the vascular arrangement in the breast tissue. One recent paper reviewed 23 studies that investigated CESM and demonstrated that textural features and/or enhancement patterns obtained from CESM can differentiate between malignant and benign breast lesions as benign lesions often display weak and uniform contrast uptake with enhancing wash-out patterns, while malignant lesions tend to display quick decreasing wash-out patterns (65). As a result, many research studies have recently been conducted and published that compare CESM and DCE-MRI. These studies have demonstrated that CESM could achieve quite comparable performance as DCE-MRI in breast tumor diagnosis (i.e., classifying between malignant and benign tumors) (66), staging or characterizing suspicious breast lesions (46, 67), and



B

	Size phenotypes	Shape phenotypes	Morphological phenotypes	Enhancement texture phenotypes	Kinetic curve assessments	Enhancement-variance kinetics
Transcriptional activities of pathways	173	109	49	374	268	130
Copy number variations of pathways	24	7	7	14	15	21
Mutated genes	3	1	1	15	22	3
miRNA expressions	73	0	0	58	1	0
Protein expressions	10	0	9	17	0	0

FIGURE 2
Results of mapping radiomic features extracted from DCE-MRI images of breast cancer to genomic markers. **(A)** Each line represents a statistically significant association between nodes. Each node represents either a genomic feature or radiomic phenotype. The size of the node reflects the number of connections relative to other nodes in its circle. **(B)** Displays the number of significant associated between the 6 different radiomic categories and the genomic features (43).

predicting or evaluating breast tumor response to neoadjuvant therapy (68). Thus, in the last several years, exploring and extracting image features from CESM also attracts research interest in developing new quantitative image markers or CAD schemes in breast cancer research field (69).

In previous studies, radiomics features are often only extracted from the segmented tumor regions, meaning potentially valuable information of the environment surrounding the tumor and background regions is ignored. To overcome this issue and improve the accuracy of prediction models, several studies report the importance of extracting features from the targeted or global breast parenchyma as these regions may also contain important information relating to cancer state (45, 47). While there has been a wide variety of radiomics features extracted from many different locations for different cancer applications, there is no consensus on what features make up an optimal feature set. Deciding what features should be extracted remains dependent on the goal of the individual study.

Applications of AI-based quantitative image analysis and prediction models

Rapid advances in AI technologies have promoted the development of new quantitative image feature analysis-based

prediction models in breast cancer research. In addition to the conventional CADe and CADx applications, novel AI-based models have also been expanded to new applications. In this section, we review the development and applications of AI-based prediction models in three applications namely, cancer risk prediction, tumor diagnosis or classification, and cancer prognosis prediction or response to treatment (Tables 2–4). There exists an extremely large number of studies pertaining to AI in breast cancer in the three realms mentioned. We apply the following criteria and steps to select the most relevant studies. The titles and abstracts of potentially relevant papers in the literature database (i.e., PubMed and Google Scholar) were first analyzed for terms related to either breast cancer risk (Table 2), breast cancer diagnosis/classification or computer aided diagnosis of breast cancer (Table 3), and breast cancer treatment response or prognosis prediction (Table 4). Papers were then selected if a ML or a DL method was used for predictive modeling and breast image derived features or breast images were used as model inputs. Thus, all studies also use predominantly imaging data as an input to the model. Studies were omitted if there was no explicit methodology of how the model was trained and tested or if the study lacked novelty. Studies that use solely statistical methods or do not report AUC values to make predictions were also omitted from this review. All papers listed in Tables 2–4 are published in the last 8 years. It should be noted that some studies investigate and report performance values for multiple combinations of features or multiple classifiers, we report only the performance results of the best model.

Prediction of breast cancer risk

Women at a high risk for developing breast cancer should undergo supplemental screening exams as early detection is necessary to ensure the best prognosis (97). However, the existing risk models are mainly built based on epidemiological studies that integrate risk factors based on groups of sampled women such as: family history, hormonal and reproductive factors, breast density, obesity, smoking history, and alcohol intake, and output a breast cancer risk estimate (98, 99). By reporting odds ratios or relative risks, these risk models typically do not have discriminatory power applying to individual women. Thus, cancer detection yield in currently defined high risk groups of women remains quite low (< 3%) using mammography plus MRI screening (100). Meanwhile, up to 60% of women diagnosed with breast cancer are not considered high risk patients (101). This coupled with the increased attention to establish a new paradigm of personalized breast cancer screening highlights the need for identifying a non-invasive biomarker or developing AI-based prediction models

TABLE 2 Studies of developing AI-based image feature analysis models to predict breast cancer risk.

Year	Author	Imaging Modality	# of Images	Feature Information	ML Model	Evaluation Metrics
2018	Heidari et al. (70)	Mammography	570	43 features from the discrete cosine transform of the ROI and the spatial domain	SVM	AUC: 0.70 ± 0.04
2015	Sun et al. (71)	Mammography	340	765 texture features from multiscale subregions	SVM RBF Kernel	AUC: 0.729 ± 0.021 PPV: 0.657 (94/140) NPV: 0.755 (151/200)
2018	Mirniaharikandehei et al. (72)	Mammography	1044	8 existing CADe based features	Logistic Regression	MLO based AUC: 0.65 ± 0.017 CC based AUC: 0.586 ± 0.018
2015	Tan et al. (73)	Mammography	870	79 texture and density features	two stage ANN	AUC: 0.725 ± 0.026
2014	Gierach et al. (74)	Mammography	237	38 texture features	Bayesian ANN (BANN)	AUC: 0.72 ± 0.08
2017	Li et al. (75)	Mammography	456	4096 features from last fully connected layer of AlexNet pretrained on ImageNet	SVM	AUC: 0.83
2018	Saha et al. (76)	MRI	133	8 BPE features	multivariate logistic regression	AUC: 0.700
2019	Portnoi et al. (77)	MRI	1656	–	ResNet18 pretrained imageNet and fine tuned	AUC: 0.638 ± 0.094
2019	Yala et al. (78)	Mammography	88994	–	ResNet18	AUC: 0.70 (95% CI: 0.64, 0.73)
2021	Yala et al. (79)	Mammography	275,674	–	MIRAI	AUC: 0.76-0.79 SEN: 26.0%-41.5% SPE: 85.2%-93.1%

AUC, area under ROC curve; SEN, sensitivity; SPE, Specificity; PPV, Positive predictive value; NPV, Negative predictive value.

TABLE 3 Studies of developing new CADx models to classify between malignant and benign breast tumors.

Year	Author	Imaging Modality	# of images	Feature Information	Model	Evaluation Metrics
2020	El-Sokkary et al. (80)	Mammography	322	20 Shape and Texture Features	SVM RBF Kernel	PSO Segmentation ACC: 89.5% GMM Segmentation ACC: 87.5%
2016	Dalmis et al. (81)	MRI	395	23 Shape and Kinetic Features	Random Forest	AUC: 0.8543
2017	Qiu et al. (82)	Mammography	560	–	8 Layer CNN	AUC: 0.790 ± 0.019
2020	Yurttakal et al. (83)	MRI	200	–	multilayer CNN	ACC: 98.33% SEN: 1.0 SPE: 0.9688
2020	Hassan et al. (84)	Mammography	600	–	AlexNet pretrained on ImageNet and fine tuned	ACC: 98.29% SEN: 0.9782 SPE: 0.9876
				–	GoogleNet pretrained on ImageNet and fine tuned	Acc: 95.63% SEN: 0.9047 SPE: 0.9822
2019	Mendel et al. (85)	Mammography and DBT	78	VGG19 pretrained on ImageNet as a Feature Extractor	SVM	Mammography AUC: 0.810 ± 0.05 2D DBT AUC: 0.86 ± 0.04 Key DBT AUC: 0.89 ± 0.04
2021	Caballo et al. (86)	breast CT	284	1354 radiomic features	fusion of radiomic features and CNN based features through MLP	AUC: 0.947
2017	Antropova et al. (87)	Mammography Ultrasound MRI	739 2393 690	VGG19 pretrained on ImageNet as a Feature Extractor and radiomic features	fusion of radiomic features and CNN based features to a SVM RBF Kernel	AUC:0.86 AUC:0.90 AUC:0.89
2015	Tan et al. (88)	Mammography	1896	96 radiomic features	Multistage ANN	AUC: 0.779 ± 0.025
2019	Li et al. (89)	Mammography	182	32 lesion-based features 45 parenchymal features from contralateral breast	Bayesian ANN	AUC: 0.84 ± 0.03
2020	Heidari et al. (90)	Mammography	1000	12 Structural Similarity Index Features	SVM	AUC: 0.84 ± 0.016 ACC: 79.00%
2020	Moon et al. (91)	Ultrasound	1687	–	Ensemble of VGGNet, ResNet, and DenseNet	ACC: 91.10% SEN: 85.14% SPE: 95.77% Precision: 94.03% F1: 89.36% AUC: 0.9697
			697			ACC: 94.62% SEN: 92.31% SPE: 95.60% Precision: 90% F1: 91.14% AUC: 0.9711

AUC, area under ROC curve; SEN, sensitivity; SPE, Specificity; ACC, Overall accuracy; F1, F1 index.

that can better stratify women with high or low risk of developing breast cancer in the short term based on individual testing.

Since previous studies have found that women with dense breast have a higher risk of developing breast cancer (102–106), it then leads that many studies aim to quantify breast density

TABLE 4 Studies of developing new AI-based models to predict tumor response to chemotherapy.

Year	Author	Imaging Modality	# Of Images	Feature Information	ML Model	Evaluation Metrics
2017	Giannini et al. (92)	DCE-MRI	44	27 textural features	Bayesian Classifier	ACC: 70% SPE: 0.72
2015	Michoux et al. (93)	DCE-MRI	69	3 kinetic features, 2 BI-RADS based features, 21 texture- based features	Logistic Regression	ACC: 74% SEN: 0.74 SPE: 0.74
					K-means clustering	ACC: 68% SEN: 0.84 SPE: 0.62
2015	Aghaei et al. (94)	DCE-MRI	68	39 contrast enhanced features from both segmented malignant tumor and background parenchymal enhancement regions	ANN	AUC: 0.96 ± 0.03 ACC: 94% SEN: 0.88 SPE: 0.98
2016	Aghaei et al. (95)	DCE-MRI	151	10 global kinetic features	ANN	AUC: 0.83 ± 0.03
2018	Ravichandran et al. (96)	DCE-MRI	166	–	CNN	AUC: 0.85 ACC: 82%

AUC, area under ROC curve; SEN, sensitivity; SPE, Specificity; ACC, Overall accuracy.

from screening mammograms so that patients can be informed if they have dense breast therefore are at a higher risk. It is the hope that informing women of their breast density and the risks associated with dense breast will encourage supplemental and more frequent screening exams. The American College of Radiology developed the Breast Imaging Reporting and Data System (BI-RADS) to group mammographic density into one of four categories. While BI-RADS has been used extensively, it is often unreliable as the categorization varies between observers. Machine learning and deep learning techniques have been developed that quantify breast density using computerized schemes to make this a more robust metric (107–110). While many studies have shown a correlation between breast density and breast cancer risk (111–113), this metric alone is often not enough to create robust risk assessment models (102, 114). Recent studies indicate that texture-based features may have a higher discriminatory power in stratifying women based on breast cancer risk (107, 115, 116). MRI images from The Cancer Genome Atlas (TCGA) project of the National Cancer Institute (NCI) were used to demonstrate that quantitative radiomic features extracted from breast MRI images can replicate observer-rated breast density based on BI-RADS guideline (117).

In addition to the measured breast density from mammograms, other types of medical images have been explored to develop new imaging markers or AI-based prediction models to predict breast cancer risk in individual women, particularly the short-term risk, which can help better stratify women into different breast cancer screening groups (Table 2). Heidari et al. developed a AI-based prediction scheme to predict the risk of developing breast cancer in the short term (less than 2 years) based on features extracted from negative screening mammograms that had enhanced breast density tissue (70). The dataset used in this study included craniocaudal (CC) views of 570 negative screening mammograms that had a follow up screening exam within 2 years where 285 of these cases were then cancer positive as confirmed by tissue biopsy and 285 cases remained screening negative. The breast area was segmented from each initial negative screening mammogram and enhanced to better visualize the dense tissue as opposed to the fatty tissues. Forty-three global features were computed from the spatial domain and discrete cosine transform domain of both the left and right CC view images. This study takes advantage of the bilateral asymmetry between two breasts when creating the final feature vector that is then used to train a support vector machine (SVM) model which produces a likelihood score that the next sequential screening exam is positive. The results of this scheme were significantly better than the same scheme that does not include the segmentation and dense tissue enhancement step, emphasizing that there is important textural information in the dense tissue of negative screening mammograms that can be used to predict if there is a short-term risk of developing breast cancer.

Like conventional CADe schemes, integrating all four views of screening mammograms enables development of new cancer risk prediction models with increased performance. Mirniaharikandehei et al. investigated the hypothesis that CADe-generated false-positive lesions contain valuable information that can help predict short-term breast cancer risk (72). The motivation for this study is driven by the fact that some

early abnormalities picked up on CADe schemes may have a higher risk of developing into detectable cancers in the short-term (118, 119). All cases used in this study were negative screening exams where some of these cases contained early suspicious tumors that were only considered detectable in a retrospective review of the images. A CADe scheme was applied to right and left CC and mediolateral oblique (MLO) view images and then a feature vector was created that describes the number of initial detection seeds, the number of final false positives, the average, and the sum of all detection scores. To quantify the bilateral asymmetry, the features from the left and right CC or MLO views were summed to create one CC and one MLO view feature vector with four features in each vector. Two independent multinominal logistic regression classifiers were trained, one using the CC view feature vector and another using the MLO view feature vector. The results indicated that using the MLO view model achieved higher prediction accuracy, which indicates image features computed from CC and MLO views are different since mammograms are 2D projection images and fibroglandular tissue may appear quite different along the two projection directions. Since CADe schemes are routinely used in the clinic, this study provides a unique and cost-effective approach for developing CADe generated biomarkers from negative screening exams to help predict short term breast cancer risk. Tan et al. also took advantage of all four views of the breast and the bilateral asymmetry between breasts to predict short term breast cancer risk (73). In this study, eight groups of features were extracted from either the whole breast region or the dense tissue region of the breast to train a two-stage artificial neural network (ANN). Each feature set was used independently and in combination to train the model. The best performing model was developed when the model was trained using GLRLM based texture features computed from the dense breast regions. Both studies demonstrate that using bilateral asymmetry features computed from CC and MLO views is advantageous in that overlapping dense fibroglandular tissue can be visualized in two different configurations, providing more information about the dense tissue which is a known risk factor for breast cancer development. Clinical adoption of computerized models that can predict short-term breast cancer risk will be extremely valuable to stratify women and decide optimal intervals and methods of breast cancer screening (i.e., whether need to add breast MRI to mammography).

Genetic risk factors are also measured and used by epidemiological studies to indicate the lifetime risk of developing breast cancer. One of these genetic risk factors is an autosomal dominant mutation in the BRCA1 or BRCA2 gene. Up to 72% of women who inherit the BRCA1 mutation and 69% of women who inherit the BRCA2 mutation will develop breast cancer in their lifetime (120). Many women are unaware of their BRCA1/2 status when going in for a screening mammogram. Identification of BRCA1/2 status from routine mammographic images will be clinically useful for determining high-risk individuals. Gierach et al. conducted a texture analysis study of breast cancer negative mammograms to differentiate individuals with BRCA1/2 mutations from those without a BRCA1/2 mutation based on 38 texture features extracted from the breast parenchyma on CC view mammograms (74). After performing feature selection, five features were used to train a Bayesian artificial neural network (BANN) model that outputs a likelihood of having a BRCA1/2 mutation which would classify the individual as high risk. Individuals with BRCA1/2 mutations used in this study were on average 10 years younger than the group without BRCA1/2 mutations. When an age-matched testing dataset was used to evaluate the performance of the BANN model and an AUC of 0.72 ± 0.08 was observed. Results of this study demonstrate that radiomic based texture features extracted from negative screening mammograms can help identify women who have BRCA1/2 mutations. The significance of this study highlights that image analysis of screening mammograms can be expanded to include risk stratification in addition to detection of suspicious tumors.

Breast parenchymal patterns are another biomarker that has been established as a tool for cancer risk prediction (104, 105, 116, 121). Extracting texture features from the breast parenchyma provides local descriptors that can characterize the physiological conditions of the breast tissue which may give more insight into breast cancer risk than breast density or BRCA mutation status. Li et al. used deep transfer learning with pre-trained CNNs to extract features directly from the breast parenchyma depicted on the CC view of FFDM images to differentiate between high-risk patients with a BRCA mutation and the low-risk patients and to differentiate between high-risk patients with unilateral cancer and the low-risk patients (75). In this study, regions of interest (ROIs) were selected from the central region directly behind the nipple as this region has been shown to give best results for describing breast parenchyma (116). ROIs were then input to a pretrained CNN and features were extracted from the last fully connected layer. In addition, texture-based features were also extracted from the ROIs so that the results of deep transfer learning-based classifier and traditional radiomic based classifier can be analyzed. A fusion classifier was created that used features extracted from the pretrained deep CNN and traditional texture features. The fusion classifier was able to differentiate BRCA mutation carriers from low-risk women and unilateral cancer patients from low-risk women with an AUC of 0.86 and 0.84, respectively. Additionally, the pre-trained CNN extracted features were able to differentiate between unilateral breast cancer patients and low risk patients significantly better than using traditional texture features, where AUC = 0.82 and AUC = 0.73, respectively. This study demonstrates the advantages of exploring deep learning techniques independently and in combination with conventional machine learning techniques to better stratify patients on breast cancer risk. In addition to extracting one ROI from one mammogram, other studies

investigate the effect of using either multiple ROIs or global features to develop breast cancer risk assessment models. For example, Sun et al. extracted texture features from multiple subregions within the mammogram that had relatively homogeneous densities and fused the features to train an SVM with a radial basis function (RBF) kernel to predict short-term breast cancer risk (71). The classifier trained using multiscale fusion of features extracted from different density subregions showed superior performance to the classifier trained using features extracted from the whole breast. Zheng et al. developed a fully automated scheme that captures the texture of the entire breast parenchyma using a lattice-based approach (122). Using smaller local windows to extract features provided the best performance when compared to single ROI and may lead to improved model performance in predicting breast cancer risk.

Besides analyzing negative mammograms, the level of background parenchymal enhancement (BPE) on breast MRI has also demonstrated power in predicting breast cancer risk (123–125). BPE refers to the volume and intensity enhancement of normal fibroglandular tissue after intravenous contrast is injected. The hypothesis is that high levels of BPE is associated with a high risk of developing breast cancer, hence why radiologists may group women into risk groups based on BPE (126). However, there is high inter-reader variability in radiologist interpretation of BPE suggesting that developing computerized schemes to quantify BPE has the potential to produce a more robust marker to predict breast cancer risk. Saha et al. automatically quantified the BPE from screening MR exams to predict future breast cancer risk within two years using a logistic regression classifier (76). In the study, eight BPE features were extracted from the fibroglandular tissue mask from both the first post-contrast fat-saturated sequence and the T1 nonfat-saturated sequence. Five breast radiologists also reviewed MR images and categorized each case as either minimal, mild, moderate, or marked BPE according to the BI-RADS guideline. The predictive performance of the multivariate logistic regression model trained using quantitative BPE features yielded higher performance than that of the qualitative BPE assessment of the five radiologists, suggesting that computerized quantification of BPE is a more accurate predictor of breast cancer risk.

Several studies have compared new image feature analysis models with pre-existing epidemiology-based statistical models in predicting cancer risk. For example, Portnoi et al. developed a deep learning breast cancer risk prediction model using DCE-MRI taken from a high-risk population (77). The 3D MR images were converted to 2D projection images using the axial view of the maximum intensity projection (MIP) and then used to fine tune a ResNet-19 CNN that had been pretrained on the ImageNet dataset. Results from the MRI-based deep learning model were compared with the Tyrer-Cuzick model and a logistic regression model that used all risk factors from the Tyrer-Cuzick model in addition to the qualitative BPE assessment made by an expert radiologist based on the BI-RADS guidelines. The AUC of the MRI-based deep learning model, Tyrer-Cuzick model, and logistic regression model were reported as, 0.638 ± 0.094, 0.493 ± 0.092, and 0.558 ± 0.108, respectively. Study results demonstrate that new MRI-based deep learning model has higher discriminatory power to predict breast cancer risk than the existing epidemiology-based risk prediction models.

Finally, based on the hypothesis that new imaging markers and the existing epidemiology-based risk factors may contain complementary information, Yala et al. sought to combine traditional risk factors and image-based risk factors extracted from mammograms using deep learning to investigate whether fusion of the two would yield a superior 5-year risk prediction model (78). In this study, ResNet18 was trained, validated, and tested using 71,689, 8,554 and 8,869 images acquired from 31,806, 3,804 and 3,978 patients, respectively. Four different risk prediction models were compared, namely: the Tyrer-Cuzick Model, a logistic regression model using standard clinical risk factors, the deep learning model, and a hybrid model using traditional clinical risk factors and the deep learning model (AUC = 0.62,0.67,0.68, 0.70, respectively). This work laid the foundation for the development of the MIRAI model in 2021 (79), which predicts the risk of developing breast cancer for each year within the next 5 years. All four mammograms acquired in routine screening (LCC, LML, RCC, RML view) are passed as an input to this model which first go through an image encoder, next to an image aggregator, then to a risk factor predictor, followed by an additive-hazard layer. MIRAI model was first trained and validated using 210,819 and 25,644 screening mammography exams from 56,786 and 7,020 patients from Massachusetts General Hospital (MGH), respectively. MIRAI model was then tested on three different testing sets, one acquired from MGH that contained 25,855 exams from 7,005 patients, the second acquired from Karolinska University Hospital in Sweden that contained 19,328 exams from 19,328 patients, and the third acquired from Chang Gung Memorial Hospital in Taiwan that contained 13,356 exams from 13,356 patients, respectively. AUCs obtained from MIRAI model was significantly higher than those yielded by Tyrer-Cuzick model and both the hybrid deep learning model and image based deep learning model developed in 2019 foundational study (81). Thus, MIRAI model is unique for a few reasons, the first being that traditional clinical risk factors are incorporated into the imaging feature analysis model as the previous Yala et al. study (78) demonstrated that addition of this information will improve performance. If traditional risk information is not provided, MIRAI model is still able to predict cancer risk from mammographic image features. This increases its potential clinical utility in clinics that may not record many risk factors used in Tyrer-Cuzick models. Second, MIRAI model focuses

directly on clinical implementation by training the model on a large dataset and validating this model on different datasets.

In summary, the above studies demonstrate that imaging markers computed from breast density distribution, textural features of parenchymal patterns, and parenchymal enhancement patterns are promising to build AI-based models to predict breast cancer risk. Study results have demonstrated that using image-based risk prediction models can perform superiorly to existing cancer risk prediction models that use epidemiological study data only. However, a majority of these state-of-the-art image-based risk models have not been tested or used in clinical practice due to lack of diversity in the training set leading to a model with poor generalizability on data from different locations and different scanners. Thus, these new image-based prediction models need to undergo vigorous and widespread prospective testing in future studies.

Tumor Classification or Diagnosis

Due to the high rates of false-positive recalls and high number of benign biopsy results in current clinical practice using the existing imaging modalities, it is important to investigate new methods to help decrease the false positive recall and benign biopsy rates so that women are more willing to continue participating routine breast cancer screening. Over the past few decades, a variety of AI-based CADx schemes of different types of medical images have been developed aiming to differentiate between malignant and benign tumors more accurately to help radiologists decrease the false-positive recall rates in future clinical practice (Table 3).

In order to classify a detected tumor, many CADx schemes first segment the tumor or a ROI surrounding the suspicious area before computing image features. Some studies rely on semi-automated segmentation using prior knowledge of the tumor location marked by a radiologist as an initial seed, and other studies focus on fully automated segmentation. Dalmis et al. developed an AI-based CADx scheme for DCE-MRI that uses a semi-automated tumor segmentation technique prior to feature extraction. This is done by a multi-seed smart opening algorithm that first has the user identify a seed point and then a region growing algorithm is conducted followed by a morphological opening to segment out the tumor (81). El-Sokkary et al. recently investigate two new methods for the fully automated segmentation of the ROI from the whole breast mammogram prior to feature computation and classification. The first method segments the ROI using a Gaussian Mixture Model (GMM) and the second method uses a particle swarm optimization (PSO) algorithm. Twenty texture and shape features were then extracted from each ROI independently and used to train a non-linear SVM implemented with an RBF kernel. The accuracy of classifying malignant vs benign tumors using PSO-based segmentation and GMM-based segmentation prior to feature extraction was 89.5% and 87.5%, respectively (80).

To mirror the cognitive process of a radiologist in reading and interpreting bilateral and ipsilateral CC and MLO view mammograms of the left and right breasts simultaneously, researchers have developed and tested CAD schemes that integrate tumor image features with the corresponding features computed from the matched ROIs in other mammograms. For example, Li et al. conducted and reported a study in which image features were extracted from the segmented tumor region and the contralateral breast parenchyma; when these two feature sets were combined and used to train a Bayesian artificial neural network (BANN), there significantly improved tumor classification over the BANN trained using just features from the segmented tumor region (AUC = 0.84 vs 0.79, p=0.047) (89).

Identifying matched ROIs from different breasts is a difficult process. To avoid errors in tumor segmentation and image registration when identifying the matched ROIs in different images, researchers have investigated the feasibility of developing CAD schemes based on global image feature analysis of multiple images. For example, Tan et al. developed a CADx scheme using bilateral mammograms to classify screening mammography cases as malignant or benign. Ninety-two handcrafted features were extracted from each of the four view images and then concatenated into separate CC and MLO feature vectors, each containing the features from the left and right breast of the respective views. A multistage ANN was then trained where the first stage had two ANNs that were trained on either the CC feature vector or the MLO feature vector, and the second stage had a singular ANN that combine the classification scores output from both the prior ANNs and outputs a final score that estimates the likelihood of the case being malignant (88). To overcome the potential limitation of losing classification sensitivity from using the whole breast image, Heidari et al. developed a novel case-based CADx scheme that quantified the bilateral asymmetry between breasts using a tree structure-based analysis of the structural similarity index (SSIM). The left and right images are equally divided into four sub-blocks, the SSIM of each pair of two matched regions is calculated and a pair of the matched sub-blocks with the lowest SSIM among the original four pairs of sub-blocks is selected. The selected sub-blocks (one from left image and one from right image) are then divided into four small sub-blocks again to search for a new pair of matched sub-blocks with the smallest SSIM. This process is repeated six times. As a result, the six smallest SSIM features are extracted for each bilateral CC and MLO view images for each case. Then, three SVMs are trained and tested using a 5-fold cross validation method using the six SSIM features computed from the bilateral CC and MLO view images separately and the combined 12 SSIM features. Each SVM produces an outcome score indicating the likelihood of the case being malignant (90). The study demonstrates that using two bilateral images of MLO view yield significantly higher performance than using two bilateral CC view images (AUC = 0.75 ± 0.021 vs. 0.53 ± 0.026). However, fusion of SSIM features computed from both CC

and MLO view images, SVM yields further increased classification accuracy with AUC = 0.84 ± 0.016.

Another popular method to eliminate the tumor segmentation step in CADx schemes is by using convolutional neural networks (CNN). CNNs can automatically learn hierarchical representations of the images directly from the image, eliminating the need for semi-automated or fully automated tumor segmentation and handcrafted feature selection. Due to the limitation of image dataset sizes in the medical imaging field, researchers have developed and trained shallow CNN models (127), which do not require as much training data as a deep CNN models. However, developing an architecture and training a CNN from scratch is still an extremely time-consuming process. Additionally, the robustness of studies using shallow CNNs is often questionable as they are trained on smaller dataset. Qiu et al. trained an eight-layer CNN to predict the likelihood of a mass being malignant, demonstrating that shallow CNNs can be trained fully on medical images (82). Yurttakal et al. trained a CNN with six convolutional blocks followed by five max pooling layers, a dropout layer, one fully connected layer, and a softmax layer to output a probability of malignancy of tumors detected on MR images. The accuracy of this system is 98.33% which outperformed many other studies of similar goals (83). The deeper a model is, the more complex representations can be learned, so the question of how deep a CNN must be to sufficiently capture features for a large classification task remains (128). However, training a deep CNN from scratch is not possible without a large diverse dataset which are not readily available in the medical imaging field.

By recognizing the limitation of shallow CNN models, transfer learning has emerged as a solution to lack of big data in medical imaging. In transfer learning, a CNN is trained in one domain and applied in a new target domain (129). This involves taking advantage of existing CNNs that have been pretrained on a large data set like ImageNet and repurposing them for a new task (130). There are two approaches to transfer learning (Figure 3), one is fine tuning where some layers of a pre-trained model are frozen while other layers will be trained using the target task dataset (131). The other is using a pre-trained network exactly as is to extract feature maps that will be used to train a separate ML model or classifier. The former is beneficial in that it will train the network to have some target specific features, but the latter is advantageous in that it is computationally inexpensive as it does not require any deep CNN training. In one study, Hassan et al. fine-tuned two existing deep CNNs, AlexNet and GoogleNet, that had been pretrained on the ImageNet database to classify tumors as malignant or benign using mammograms (84). The lower layers of each deep CNN were kept frozen, and the last layers of both networks were replaced to accommodate the two-class classification task and trained using the mammograms. Many different experiments were conducted to determine the most optimal hyperparameters for each deep CNN. The mammograms used in this study were a combination of images from four databases including the Curated Breast Imaging Subset of DDSM (CBIS-DDSM), the Mammographic Image Analysis Society (MIAS), INbreast, and mammogram images from the Egyptian National Cancer Institute (NCI), demonstrating the robustness of this fully automated CADx system. In another study, Mendel et al.

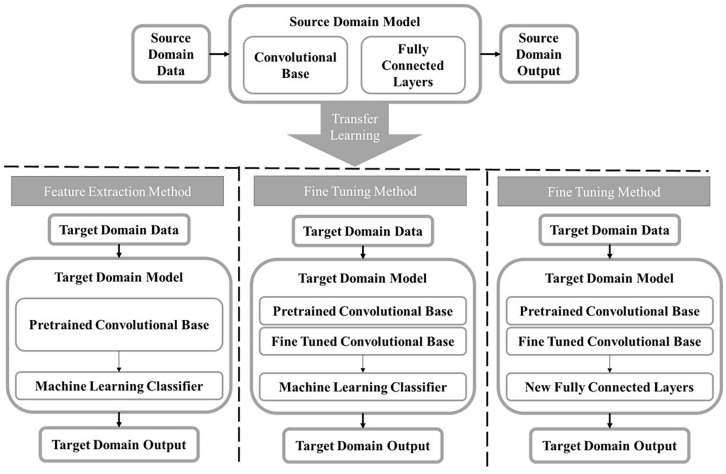


FIGURE 3
A block diagram displaying the transfer learning process. A model is trained in the source domain using a large diverse dataset. The information learned by the model is transferred to the target domain and used on a new task. The two main methods for transfer learning are feature extraction and fine tuning. For the feature extraction method, a feature map is extracted from the convolutional base taken from the source model and used to train a separate machine learning classifier. There are two ways to use transfer learning by fine tuning. The first is freezing the initial layers in the convolutional base from the source model and fine tuning the final layers using the target domain dataset then training a separate classifier. The second method does the same, except instead of training a new machine learning classifier, new fully connected layers will be added and trained using the target domain data.

used transfer learning as a feature extractor to compare the performance of a CADx model trained using DBT images and mammography images, independently. A radiologist placed a ROI around the tumor in corresponding the mammogram, DBT synthesized 2D image, and DBT key image which were then used as an input to the pre-trained VGG19 network. Features were extracted after each max-pooling layer. A stepwise feature selection method was used, and the most frequently selected features were used to train SVM models to predict the likelihood of malignancy. SVM model using DBT images yielded significantly higher classification accuracy than SVM model trained using mammograms, demonstrating that the features extracted from the DBT images may carry more clinically relevant tumor classification information than mammograms (85).

While deep CNN based models have seen tremendous success, traditional ML-based models that use handcrafted radiomic features benefit from prior knowledge of useful feature extraction methods making the handcrafted features more interpretable than automated features produced by deep learning models. Recently, fusion of traditional handcrafted features and deep learning-based features has been a hot topic and several studies report superior performance of the fusion approach over using either method alone. For example, Caballo et al. developed a CADx scheme for 3D breast computed tomography (bCT) images. The 3D mass classification problem was collapsed into a 2D classification problem by extracting nine 2D square boxes from each mass that mirror one of the nine symmetry planes of a 3D cube. The developed CADx scheme was then designed to take nine-2D images as an input. A U-Net based CNN model was used to segment the tumor from each of the nine 2D images. Then, 1,354 radiomic features were extracted from each image patch. The architecture of the rest of the proposed CADx scheme had two branches that work in parallel. The first arm of the system was a multilayer perceptron (MLP) composed of four fully connected layers that takes the radiomic features as an input. The second arm of the system was a CNN that processes the 2D image patch as is, meaning without the U-Net segmentation of the mass. The results of the last fully connected layer of both arms of the system were concatenated and processed by two more fully connected layers before tumor classification result is produced. The proposed model yielded AUC = 0.947 that outperforms three radiologists with AUC ranging from 0.814 – 0.902. This study demonstrates the utility of combining handcrafted features and CNN generated features in a singular CADx scheme (86).

Last, since original deep learning (CNN) models have been pretrained on a natural image data set like ImageNet, the models have three input channels to accept color images, yet medical images are typically gray scale images that only occupy a single input channel of the deep learning model. Thus, some studies directly copy the original grayscale image into three channels, while other studies added additional images into the other two input channels (28). Antropova et al. conducted a study that developed a classification model that fuses radiomics and deep transfer learning generated image features using a mammogram dataset, a DCE-MRI dataset, and an US dataset (87). The mammograms and ultrasound images were stacked in three input channels and fed to a pretrained VGG19 model, while the DCE-MRI pre-contrast (t0), first time-point (t1), and post-contrast (t2) were stacked in three input channels to form the input image of another VGG19 model. The deep CNN based features were extracted after each max pooling layer, average pooled in the spatial dimension, and concatenated into a final CNN feature vector. A semi-automated tumor segmentation method was used to segment the suspicious tumors before radiomic feature extraction. The radiomic and deep CNN feature set were used to train non-linear SVM with an RBF kernel using 5-fold cross validation. To build the fusion classifier the outputs of each SVM were averaged. Classifiers trained using the fusion of the two types of features outperformed all classifiers that used either feature set alone, demonstrating that traditional radiomic features and features extracted from transfer learning may provide complimentary information that can increase the performance of CADx schemes to help radiologist better make decisions. In addition to developing this CADx scheme for three independent imaging modalities, this study also demonstrated that features extracted from each max pooling layer of a pretrained CNN outperformed features extracted from the fully connected layers. This is significant as authors claim this is the first study using a hierarchical deep feature extraction technique for CADx of breast tumor classification. Similarly, Moon et al. developed a CADx scheme using multiple US image representations to train multiple CNNs which were then combined using an ensemble method (91). Four different US image representations were used: an ROI surrounding the whole tumor and tumor boundary that was manually annotated by an expert, the segmented tumor region, the tumor shape image which is a binary mask of the segmented tumor region, and a fused RGB image of the three prior image types. Multiple CNNs were then trained on each of the four image types and the best models were combined *via* an ensemble method. All models were evaluated using one private and one public dataset involving 1,687 and 697 tumors, respectively. Results of this study further demonstrate that the more information used in the input image, the better the model performs. Future work to automate the segmentation steps will improve the robustness of this model.

The above studies demonstrate that tumor segmentation remains one of the most difficult challenges that traditional ML based CADx schemes encounter and a major hurdle to clinical implementation. The shift from manual to semi-automated to fully automated lesion segmentation has decreased the inherent

bias associated with human intervention, but elimination of the segmentation step in its entirety through either feature extraction from whole breast images or CNNs will be more generalizable than models involving a segmentation step when a large and diverse image database is available. Additionally, there remains no consensus on whether conventional ML models or new CNN-based DL models are better for breast lesion diagnosis as both methods have unique strengths and limitations. However, fusion of the two types of models has been shown to produce the best results as meaning these models may provide complementary information.

Prediction of tumor response to treatment

Monitoring response to treatment is one of the most crucial aspects of breast cancer treatment and management. This must be done continuously through a combination of physical examinations, imaging techniques, surgical interventions, and pathological analyses. Molecular subtyping of each cancer based on histopathology into either luminal A, luminal B, human epidermal growth factor 2 (HER2) enriched, and basal-like subtypes is an important first step before deciding on the optimal treatment plan as each group has shown different responses to treatments and has varying survival outcomes (132, 133). Discovery of additional molecular signatures such as presence or absence of Ki67, expression of estrogen receptors (ER) and progesterone receptor (PR), cyclin-dependent kinases (CDKs), PIK3CA mutation, and others has opened the door for new targeted therapies that aim to inhibit cancer growth rather than shrink solid tumors (134, 135).

Neoadjuvant chemotherapy (NACT) is often used as a first line treatment with the goal of decreasing the size of the tumor. Evaluation of the efficacy of NACT is traditionally done through clinical evaluation using the Response Evaluation Criteria in Solid Tumors (RECIST), a size-based guideline (136, 137). The goal of the RECIST criteria is to categorize the response as either complete response (CR), partial response (PR), progressive disease (PD), or stable disease (SD). However, changes in the size of tumors will often not be detectable until 6-8 weeks in the treatment course therefore patients may continue experiencing the toxicity affects from chemotherapy or radiation therapy while not actually treating the cancer (138). In addition, the invention of many molecularly targeted therapies may be successful without showing a decrease in the size of the tumors, other factors such as change in vasculature or molecular composition may be better indicators of treatment response (139). Immunohistochemical (IHC) analysis can also be conducted before and after therapies to uncover molecular signatures and information about the vascular density of the tumor microenvironment (140–142). However, IHC analysis is an invasive procedure that is limited by the heterogeneity of the tumor since the biopsy sample is not necessarily reflective of the entire tumor (140, 143). The heterogeneity of tumors is a major hallmark of cancer, yet it is difficult to capture in a clinical setting making it difficult to predict response to therapy without knowing the entire molecular composition of the tumor. The need for non-invasive imaging markers that can quickly and accurately predict response to therapies has never been greater.

In current clinical practice, breast MRI is the most accurate imaging modality for monitoring tumor response to treatment as confirmed by The American College of Radiology Imaging Network (ACRIN) 6657 study performed in combination with the multi-institutional Investigation of Serial Studies to Predict Your Therapeutic Response with Imaging And molecular Analysis (I-SPY TRIAL) (144). In these clinical trials, radiologists read MR images and predict tumor response to treatment based on RECIST guidelines. In order to predict tumor response or cancer prognosis more accurately and effectively, many researchers have tried to develop AI-based prediction models of breast MR images acquired before, during or post therapy to predict tumor response to chemotherapy at an early stage.

In one study, Giannini et al. extracted 27 texture features from pre-NACT MRI and trained a Bayesian classifier to predict pathological complete response (pCR) post-NACT (92). In another study, Michoux et al. extracted texture, kinetic, and BI-RADS features from pre-NACT MRI to try and differentiate between individuals who would have no response (NR) and those who had either a partial response (PR) or complete response (CR) (93). Predictive capabilities of the features were analyzed independently and in combination through supervised and unsupervised ML models. Results showed that texture and kinetic features helped differentiate responders vs. non-responders, but BI-RADS features did not significantly contribute to the differentiation.

Aghaei et al. reported two studies that identified two new imaging markers by training two ANN models using kinetic image features extracted from DCE-MRI acquired prior to NACT to predict complete response (CR) to NACT (94). In the first study, an existing CAD scheme was applied to segment tumors depicting on DCE-MRI. Thirty-nine contrast enhanced kinetic features were then extracted from five groups: the whole tumor area, the contrast-enhanced tumor area, the necrotic tumor area, the entire background parenchymal region of both breasts, and the absolute value of bilateral BPE between the left and right breast. Using a leave-one-case-out cross validation method embedded with a feature selection algorithm, the trained ANN yielded prediction performance with an AUC = 0.96 ± 0.03 when 10 kinetic features were used. When comparing some of the common MRI features between the CR and NR groups using

DeLong's Method, no significant differences were seen between the two groups which demonstrates that conventional MR features alone may not have enough discriminatory power to predict whether a patient will respond to NACT or not. This study demonstrates that extracting more complex MRI features will yield greater performance in predicting the likelihood of a patient responding to NACT. As with many CAD studies, inclusion of the segmentation step often limits the robustness of the scheme. Thus, Aghaei et al. conducted a follow-up study using an increased image dataset and a new scheme that only computes 10 global kinetic features from the whole breast volume including average enhancement value (EV), standard deviation (STD) of EV, skewness of EV, maximum EV, average EV of top 10%, average EV of 5%, bilateral average EV difference, bilateral STD EV difference, bilateral difference of average EV of top 10%, and bilateral difference of average EV of top 5% without tumor segmentation. Then, by using the same ANN training and testing method, the ANN trained using 4 features yielded an AUC = 0.83 ± 0.04. Three of these four features were computed to characterize the bilateral asymmetry between left and right breasts, highlighting the key role that breast asymmetry may play in predicting whether a patient will respond well to chemotherapy (95).

CNNs provide another tool that can overcome the limitations intrinsic to tumor segmentation steps. Ravichandran et al. used a CNN with six convolutional blocks trained over 30 epochs to extract features from pre-NACT DCE-MRI to predict the likelihood of a pathological CR (pCR) (96). This study looked at the pre-contrast and post-contrast images separately and together and found that the CNN performed best when using 3-channel images that contained the pre-contrast images in the red and green channel and the post-contrast images in the blue channel. The addition of clinical variables such as age, largest diameter, and hormone receptor status increased the AUC values from 0.77 to 0.85, demonstrating how the addition of AI can streamline imaging and clinical data into a single workflow for the increased prediction accuracy. Additionally, regions in the images that contain the most valuable information for predicting response to NACT can often be displayed in a heatmap (Figure 4). This may be an important step to reveal rationale of DL model prediction as few existing DL models are very interpretable which hinders their clinical translation.

Traditionally, pathological assessment of a representative tissue sample from the original tumor mass is used to identify the molecular subtype and develop a treatment plan. This is a sub-optimal technique as this representative tissue sample cannot capture the molecular composition of the whole tumor as cancer is often extremely heterogenous. Imaging modalities have the unique advantage of being able to capture information relating to an entire tumor which can help to overcome the limitations intrinsic to tissue biopsies. Additionally, the mechanism of many therapies is dependent on tumor vasculature which is not often probed before deciding on a treatment plan. Modalities that can image tumor vasculature such as DCE-MRI continue to be the most accurate and useful modalities in AI-based models for predicting response to treatment as valuable information pertaining to treatment response is contained in the tumor vasculature. Despite pre-clinical research progress, there are currently no image-based markers clinically used to predict response to any cancer therapies. Thus, more research efforts are needed to continue making progress to identify and validate robust image-based biomarkers that can predict response to therapy before the therapy is administered.

Discussion – outlook and challenges

Breast cancer remains an extremely deadly disease with incidence on the rise. Early detection through routine

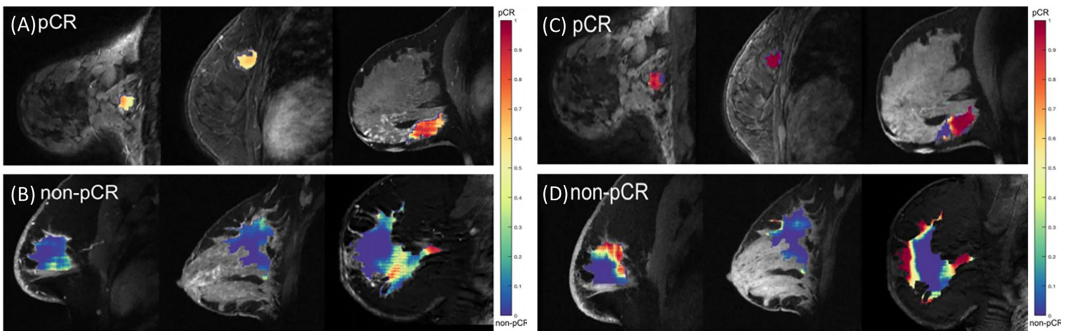


FIGURE 4
Illustration of heatmaps displaying the regions within a tumor that were used to predict the probability of pathological complete response. **(A, B)** show the results when using the CNNs trained on only the pre-contrast images. **(C, D)** show the results when using the CNN trained using a combination of pre-contrast and post-contrast images. **(A, C)** display cases that were correctly identified as pCR, while **(B, D)** are cases that were correctly identified as non-pCR. Modified from (96).

screening exams remains the best method for reducing the mortality associated with the disease. However, the efficacy including both sensitivity and specificity of current breast screening must be improved. The increase in the number of breast imaging modalities coupled with a large amount of clinical, pathological, and genetic information has made it more difficult and time consuming for clinicians to digest all available information and make an accurate diagnosis and appropriate personalized treatment plan. Recent advances in radiomics and DL-based AI technology provide promising opportunities to extract more clinically relevant image features as well as to streamline many different types of diagnostic information to build novel decision-making support tools that aim to help clinicians make more accurate and robust cancer diagnosis and treatment decisions. In this review paper, we reviewed recent studies of developing AI-based models of breast images in three application realms.

In recent years, many "omics" topics including genomics, transcriptomics, proteomics, metabolomics, and others have attracted broad research interest in order to improve early diagnosis of breast cancer, better characterize the molecular biology of tumors, and establish an optimal personalized cancer treatment paradigm. However, these "omics" studies often require additionally invasive procedures and expensive tests generating high-throughput data that is difficult to do robust data analysis. Radiomics is advantageous in that it is non-invasive and low cost (because it only uses existing image data and does not require additional tests). Thus, the reported studies that directly apply radiomics concept and software to medical images has grown exponentially in recent years. In breast imaging, a large number of radiomics features can be extracted and computed such as from mammograms and DCE-MRI. Despite great research effort and progress, the association between radiomics and other "omics" is still not very clear and more in-depth research is needed. Thus, in this paper, we review several recent studies that investigated the relationship between radiomics features and the tumor microenvironment or tumor subtypes, which may provide researchers valuable references to continue in-depth research.

In addition, AI-based prediction models have expanded from the traditional task of detecting and diagnosing suspicious breast lesions in CAD schemes to much broader applications in breast cancer research. In this paper, we select and review application of AI-based prediction models to predict risk of having or developing breast cancer, the likelihood of the detected lesion being malignant, and cancer prognosis or response to treatment. These studies demonstrate that by applying either radiomics concepts through ML methods or deep transfer learning methods, clinically relevant image features can be extracted to build new quantitative image markers or prediction models for different breast cancer research tasks. If successful, the role of AI in breast cancer is paving the way for developing personalized medicine as detecting and diagnosing cancer are no longer driven by generic qualitative markers but now driven by quantitative patient specific data.

Despite the extensive research efforts dedicated to the development and testing of new AI-based models in the laboratory environment, very few of these studies or models have made into clinical practice. This can be attributed to several obstacles or challenges. First, currently, most of the studies reported in the literature trained AI-based models using small datasets (i.e., <500 images). Training a model using a small dataset often results in poor generalizability and poor performance due to unavoidable bias and model overfitting. Thus, one important obstacle is lack of large and high-quality image databases for many different application tasks. Although several breast image databases are publicly available including DDSM, INbreast, MIAS, and BCDR (87), these databases mainly contain easy cases and lack subtle cases, which substantially reduces the diversity and heterogeneity of these image databases. Many existing databases reported in previous research papers are also either obsolete (i.e., DDSM and MIAS used the digitized screen-film based mammograms) or have a lack of biopsy-approved ground-truth (i.e., INbreast). Thus, AI-models developed using these "easy" databases have lower performance in applying to real diverse images acquired in clinical practice. By recognizing such limitations or challenges, more research efforts continue to build better public image databases. For example, The Cancer Imaging Archive (TCIA) was created in 2011 with the aim of developing a large, de-identified, open-access archive of medical images from a wide variety of cancers and imaging modalities (145). New significant progress is expected in future studies to build this important infrastructure in help develop robust AI-based models in medical imaging field.

Second, medical images acquired using different machines made by different companies and different image acquisition or scanning protocols in different medical centers or hospitals may have different image characteristics (i.e., image contrast or contrast-to-noise ratio). CAD schemes or AI-models are often quite sensitive to the small variations of image characteristics due to the risk of overtraining. Thus, AI-models developed in this manner are not easily translatable to independent test images acquired by different imaging machines at different clinical sites. Compared to mammograms and MRI, developing AI-models of ultrasound images faces additional challenges because the quality of US images (particularly US images acquired using handheld US devices) heavily depends on the operators. Thus, establishment of TCIA allows researchers to train and validate their prediction models on imaging data acquired from other clinical sites to help researchers develop

more accurate and robust models that can eventually be translated to the clinic. Additionally, developing and implementing image pre-processing algorithms to effectively standardize or normalize images acquired from different machines or clinic sites (146, 147) have also attracted research interest and effort, which may also need before AI-based models can be adopted on a widescale clinical level.

Third, another common limitation of traditional ML or radiomics based AI-based models is that they often require a lesion segmentation step prior to feature extraction. Whether lesion segmentation is done semi-automatically based on an initial seed or automatically without human intervention, accurate and robust segmentation of breast lesions from the highly heterogeneous background tissue remains difficult (148). The lesion segmentation error introduces uncertainty or bias to the model due to the variation of the computed image features and hinders the translation of the AI-based models to clinical applications. Recent attention to DL technology provides a way to overcome this limitation as the deep CNNs will extract features directly from the images themselves, bypassing the need for a lesion segmentation step. However, the lack of big and diverse datasets is a major challenge in developing robust DL-based AI models. Although transfer learning has emerged as a mainstream in the medical imaging field, its advantages and limitations are still under investigation. While there is a huge focus on using pre-trained CNNs as feature extractors as it is computationally inexpensive and generalizable since these models avoid having to train or re-train the CNN at different centers with different imaging parameters, fine tuning the models has showed better results (129). Additionally, no CNN-based transfer learning models have made it to clinical use since the models are still not robust as investigated in a recent comprehensive AI-model evaluation study (31). Therefore, more development and validation studies are needed to address and overcome this challenge.

Fourth, currently most AI-based models use a "black-box" type approach and lack explainability. As a result, it reduces the confidence or willingness of clinicians to consider or accept AI-generated prediction results (149). Understanding how an AI-based CAD scheme or prediction model can make reliable prediction is non-trivial to most individuals because it is very difficult to explain the clinical or physical meanings of the features automatically extracted by a CNN-based deep transfer learning model. Thus, developing explainable AI models in medical image analysis has emerged as a hot research topic (150). Among these efforts, visualization tools with interactive capability or functions have been developed that aim to show the user what regions in an image or image patterns (i.e., "heat maps") contribute the most to the decision made by AI models (151, 152). In general, new explainable AI models must be able to provide sound interpretation of how the features extracted result in the output produced. Ideally this should be done in ways that directly tie to the medical condition in question. Since this is an emerging research field and important research direction, more research efforts should dedicate to extensive development of new technologies to make AI-based CAD schemes and/or prediction models more transparent, interpretable, and explainable before AI-based models or decision-making supporting tools can be fully accepted by the clinicians and then integrated into the clinical workflow.

Fifth, performance of AI-based models reported in the literature based on laboratory studies may not be directly applicable to clinical practice. For example, researchers have found that higher sensitivity of AI-based models may not help radiologists in reading and interpreting images in clinical practice. One previous observer performance study reported that radiologists failed to recognize correct prompts of CADe scheme in 71% of missed cancer cases due to higher false-positive prompts (153). By retrospectively analyzing a large cohort of clinical data before and after implementing CADe schemes in multiple community hospitals, one study reported that the current method of using CADe schemes in mammography reduced radiologists' performance as seen by decreased specificity and positive predictive values (21). In order to overcome this issue, researchers have investigated several new approaches of using CADe schemes. One study reported that using an interactive prompt method to replace a conventional "second reader" prompt method significantly improves radiologists' performance in detecting malignant masses from mammograms (154). However, this interactive prompting method has not been accepted in clinical practice. Thus, the lessons learned from CADe schemes used in clinical practice indicate that more research efforts are needed to investigate and develop new methods, including FDA clearance processes, to evaluate the potential clinical utility of all new AI-based models for many different clinical medical imaging applications (155).

In conclusion, besides CADe schemes that have been commercially available, advances in new technologies including data analysis of high throughput radiomics features and AI-based deep transfer learning have led to the development of large number of new CAD schemes or prediction models for different research tasks in breast cancer including prediction of cancer risk, likelihood of tumor being malignant, tumor subtypes or staging, tumor response to chemotherapies or radiation therapies, and patient progression-free survival (PFS) or overall survival (OS). However, before each of the new AI-based CAD schemes can be accepted in clinic practice, more work still needs to be done to overcome the remaining obstacles and validate its scientific rigor using large and diverse image databases acquired from multiple clinical sites. The overarching goal of this review paper is to provide readers with a better understanding of state-of-the-art status of developing new AI-based prediction models of breast images and the promising potential of using these models to help improve efficacy of breast cancer screening, diagnosis, and treatment. Additionally, by better understanding the remaining obstacles or challenges, we

expect more progress and future breakthroughs will be made by continuing research efforts in the future.

Author contributions

MJ writing of original manuscript preparation, revisions, and editing. WI, RF, XC writing, revisions, and editing. BZ. writing, revisions, editing, and funding acquisition All authors contributed to the article and approved the submitted version.

Funding

This work was funded in part by the National Institutes of Health, USA, under grant number P20GM135009.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA: A Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708

2. DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, et al. Breast cancer statistics, 2019. *CA: A Cancer J Clin* (2019) 69(6):438–51. doi: 10.3322/caac.21583

3. Berlin L, Hall FM. More mammography muddle: emotions, politics, science, costs, and polarization. *Radiology* (2010) 255(2):311–6. doi: 10.1148/radiol.10100056

4. McCann J, Stockton D, Godward S. Impact of false-positive mammography on subsequent screening attendance and risk of cancer. *Breast Cancer Res* (2002) 4 (5):1–9. doi: 10.1186/bcr455

5. Gøtzsche PC. Mammography screening is harmful and should be abandoned. *J R Soc Med* (2015) 108(9):341–5. doi: 10.1177/0141076815602452

6. Brennan M, Houssami N. Discussing the benefits and harms of screening mammography. *Maturitas* (2016) 92:150–3. doi: 10.1016/j.maturitas.2016.08.003

7. Wilkinson L, Gathani T. Understanding breast cancer as a global health concern. *Br J Radiol* (2022) 95(1130):20211033–. doi: 10.1259/bjr.20211033

8. Schaffter T, Buist DSM, Lee CI, Nikulin Y, Ribli D, Guan Y, et al. Evaluation of combined artificial intelligence and radiologist assessment to interpret screening mammograms. *JAMA Netw Open* (2020) 3(3):e200265. doi: 10.1001/jamanetworkopen.2020.0265

9. Berg WA, Zhang Z, Lehrer D, Jong RA, Pisano ED, Barr RG, et al. Detection of breast cancer with addition of annual screening ultrasound or a single screening MRI to mammography in women with elevated breast cancer risk. *Jama* (2012) 307 (13):1394–404. doi: 10.1001/jama.2012.388

10. Patel BK, Lobbes M, Lewin J. Contrast enhanced spectral mammography: a review. *Semin Ultrasound CT MRI* (2018) 39(1):70–79. doi: 10.1053/j.sult.2017.08.005

11. Vedantham S, Karellas A, Vijayaraghavan GR, Kopans DB. Digital breast tomosynthesis: state of the art. *Radiology* (2015) 277(3):663. doi: 10.1148/radiol.2015141303

12. Taba ST, Gureyev TE, Alakhras M, Lewis S, Lockie D, Brennan PC. X-Ray phase-contrast technology in breast imaging: principles, options, and clinical application. *Am J Roentgenology* (2018) 211(1):133–45. doi: 10.2214/AJR.17.19179

13. Berger N, Marcon M, Saltybaeva N, Kalender WA, Alkadhi H, Frauenfelder T, et al. Dedicated breast computed tomography with a photon-counting detector: initial results of clinical *in vivo* imaging. *Invest Radiology* (2019) 54(7):409–18. doi: 10.1097/RLI.0000000000000552

14. Zuluaga-Gomez J, Zerhouni N, Al Masry Z, Devalland C, Varnier C. A survey of breast cancer screening techniques: thermography and electrical impedance tomography. *J Med Eng Technol* (2019) 43(5):305–22. doi: 10.1080/03091902.2019.1664672

15. Covington MF, Parent EE, Dibble EH, Rauch GM, Fowler AM. Advances and future directions in molecular breast imaging. *J Nucl Med* (2022) 63(1):17–21. doi: 10.2967/jnumed.121.261988

16. Katzen J, Dodelzon K. A review of computer aided detection in mammography. *Clin Imaging* (2018) 52:305–9. doi: 10.1016/j.clinimag.2018.08.014

17. Dorrius MD, der Weide MC, van Ooijen P, Pijnappel RM, Oudkerk M. Computer-aided detection in breast MRI: a systematic review and meta-analysis. *Eur Radiol* (2011) 21(8):1600–8. doi: 10.1007/s00330-011-2091-9

18. Freer TW, Ulissey MJ. Screening mammography with computer-aided detection: prospective study of 12,860 patients in a community breast center. *Radiol* (2001) 220(3):781–6. doi: 10.1148/radiol.2203001282

19. Keen JD, Keen JM, Keen JE. Utilization of computer-aided detection for digital screening mammography in the united states, 2008 to 2016. *J Am Coll Radiology* (2018) 15(1):44–8. doi: 10.1016/j.jacr.2017.08.033

20. Rodríguez-Ruiz A, Krupinski E, Mordang J-J, Schilling K, Heywang-Köbrunner SH, Sechopoulos I, et al. Detection of breast cancer with mammography: Effect of an artificial intelligence support system. *Radiology* (2018) 290(2):305–14. doi: 10.1148/radiol.2018181371

21. Fenton JJ, Taplin SH, Carney PA, Abraham L, Sickles EA, D'Orsi C, et al. Influence of computer-aided detection on performance of screening mammography. *N Engl J Med* (2007) 356(14):1399–409. doi: 10.1056/NEJMoa066099

22. Henriksen EL, Carlsen JF, Vejborg IM, Nielsen MB, Lauridsen CA. The efficacy of using computer-aided detection (CAD) for detection of breast cancer in mammography screening: a systematic review. *Acta Radiol* (2019) 60(1):13–8. doi: 10.1177/0284185118770917

23. Jiang Y, Edwards AV, Newstead GM. Artificial intelligence applied to breast MRI for improved diagnosis. *Radiology* (2021) 298(1):38–46. doi: 10.1148/radiol.2020200292

24. Nishikawa RM, Gur D. CADe for early detection of breast cancer–current status and why we need to continue to explore new approaches. *Acad Radiol* (2014) 21(10):1320–1. doi: 10.1016/j.acra.2014.05.018

25. Rizzo S, Botta F, Raimondi S, Origgi D, Fanciullo C, Morganti AG, et al. Radiomics: the facts and the challenges of image analysis. *Eur Radiol Exp* (2018) 2 (1):1–8. doi: 10.1186/s41747-018-0068-z

26. Lambin P, Leijenaar RT, Deist TM, Peerlings J, De Jong EE, Van Timmeren J, et al. Radiomics: the bridge between medical imaging and personalized medicine. *Nat Rev Clin Oncol* (2017) 14(12):749–62. doi: 10.1038/nrclinonc.2017.141

27. Chan H-P, Samala RK, Hadjiiski LM. CAD And AI for breast cancer–recent development and challenges. *Br J Radiol* (2019) 93(1108):20190580. doi: 10.1259/bjr.20190580

28. Jones MA, Faiz R, Qiu Y, Zheng B. Improving mammography lesion classification by optimal fusion of handcrafted and deep transfer learning features. *Phys Med Biol* (2022) 67(5):054001. doi: 10.1088/1361-6560/ac5297

29. Danala G, Maryada SK, Islam W, Faiz R, Jones M, Qiu Y, et al. Comparison of computer-aided diagnosis schemes optimized using radiomics and deep transfer learning methods. *Bioengineering (Basel)* (2022) 9(6):256. doi: 10.3390/bioengineering9060256.

30. Tran KA, Kondrashova O, Bradley A, Williams ED, Pearson JV, Waddell N. Deep learning in cancer diagnosis, prognosis and treatment selection. *Genome Med* (2021) 13(1):152. doi: 10.1186/s13073-021-00968-x

31. Roberts M, Driggs D, Thorpe M, Gilbey J, Yeung M, Ursprung S, et al. Common pitfalls and recommendations for using machine learning to detect and prognosticate for COVID-19 using chest radiographs and CT scans. *Nat Mach Intelligence* (2021) 3(3):199–217. doi: 10.1038/s42256-021-00307-0

32. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144(5):646–74. doi: 10.1016/j.cell.2011.02.013

33. Li T, Kang G, Wang T, Huang H. Tumor angiogenesis and anti-angiogenic gene therapy for cancer. *Oncol Lett* (2018) 16(1):687–702. doi: 10.3892/ol.2018.8733

34. Li L, Wang K, Sun X, Wang K, Sun Y, Zhang G, et al. Parameters of dynamic contrast-enhanced MRI as imaging markers for angiogenesis and proliferation in human breast cancer. *Med Sci Monit* (2015) 21:376–82. doi: 10.12659/MSM.892534

35. Xiao J, Rahbar H, Hippe DS, Rendi MH, Parker EU, Shekar N, et al. Dynamic contrast-enhanced breast MRI features correlate with invasive breast cancer angiogenesis. *NPJ Breast Cancer* (2021) 7(1):42. doi: 10.1038/s41523-021-00247-3

36. Mori N, Abe H, Mugikura S, Takasawa C, Sato S, Miyashita M, et al. Ultrafast dynamic contrast-enhanced breast MRI: Kinetic curve assessment using empirical mathematical model validated with histological microvessel density. *Acad Radiol* (2019) 26(7):e141–e9. doi: 10.1016/j.acra.2018.08.016

37. Kim SH, Lee HS, Kang BJ, Song BJ, Kim H-B, Lee H, et al. Dynamic contrast-enhanced MRI perfusion parameters as imaging biomarkers of angiogenesis. *PloS One* (2016) 11(12):e0168632–e. doi: 10.1371/journal.pone.0168632

38. Li X, Arlinghaus LR, Ayers GD, Chakravarthy AB, Abramson RG, Abramson VG, et al. DCE-MRI analysis methods for predicting the response of breast cancer to neoadjuvant chemotherapy: Pilot study findings. *Magnetic Resonance Med* (2014) 71(4):1592–602. doi: 10.1002/mrm.24782

39. Yu HJ, Chen J-H, Mehta RS, Nalcioglu O, Su M-Y. MRI Measurements of tumor size and pharmacokinetic parameters as early predictors of response in breast cancer patients undergoing neoadjuvant anthracycline chemotherapy. *J Magnetic Resonance Imaging* (2007) 26(3):615–23. doi: 10.1002/jmri.21060

40. Kang SR, Kim HW, Kim HS. Evaluating the relationship between dynamic contrast-enhanced MRI (DCE-MRI) parameters and pathological characteristics in breast cancer. *J Magnetic Resonance Imaging* (2020) 52(5):1360–73. doi: 10.1002/jmri.27241

41. Braman N, Prasanna P, Whitney J, Singh S, Beig N, Etesami M, et al. Association of peritumoral radiomics with tumor biology and pathologic response to preoperative targeted therapy for HER2 (ERBB2)–positive breast cancer. *JAMA Netw Open* (2019) 2(4):e192561–e. doi: 10.1001/jamanetworkopen.2019.2561

42. da Rocha SV, Braz Junior G, Silva AC, de Paiva AC, Gattass M. Texture analysis of masses malignant in mammograms images using a combined approach of diversity index and local binary patterns distribution. *Expert Syst Applications* (2016) 66:7–19. doi: 10.1016/j.eswa.2016.08.070

43. Zhu Y, Li H, Guo W, Drukker K, Lan L, Giger ML, et al. Deciphering genomic underpinnings of quantitative MRI-based radiomic phenotypes of invasive breast carcinoma. *Sci Rep* (2015) 5(1):17787. doi: 10.1038/srep17787

44. Drukker K, Li H, Antropova N, Edwards A, Papaioannou J, Giger ML. Most-enhancing tumor volume by MRI radiomics predicts recurrence-free survival "early on" in neoadjuvant treatment of breast cancer. *Cancer Imaging* (2018) 18(1):12–. doi: 10.1186/s40644-018-0145-9

45. Varela C, Timp S, Karssemeijer N. Use of border information in the classification of mammographic masses. *Phys Med Biol* (2006) 51(2):425–41. doi: 10.1088/0031-9155/51/2/016

46. La Forgia D, Fanizzi A, Campobasso F, Bellotti R, Didonna V, Lorusso V, et al. Radiomic analysis in contrast-enhanced spectral mammography for predicting breast cancer histological outcome. *Diagnostics* (2020) 10(9):708. doi: 10.3390/diagnostics10090708

47. Wu J, Sun X, Wang J, Cui Y, Kato F, Shirato H, et al. Identifying relations between imaging phenotypes and molecular subtypes of breast cancer: model discovery and external validation. *J Magnetic Resonance Imaging* (2017) 46(4):1017–27. doi: 10.1002/jmri.25661

48. Madu CO, Wang S, Madu CO, Lu Y. Angiogenesis in breast cancer progression, diagnosis, and treatment. *J Cancer* (2020) 11(15):4474–94. doi: 10.7150/jca.44313

49. Horak ER, Klenk N, Leek R, LeJeune S, Smith K, Stuart N, et al. Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer. *Lancet* (1992) 340(8828):1120–4. doi: 10.1016/0140-6736(92)93150-L

50. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis–correlation in invasive breast carcinoma. *New Engl J Med* (1991) 324(1):1–8. doi: 10.1056/NEJM199101033240101

51. Shrivastav S, Bal A, Singh G, Joshi K. Tumor angiogenesis in breast cancer: Pericytes and maturation does not correlate with lymph node metastasis and molecular subtypes. *Clin Breast Cancer* (2016) 16(2):131–8. doi: 10.1016/j.clbc.2015.09.002

52. Gelao L, Criscitiello C, Fumagalli L, Locatelli M, Manunta S, Esposito A, et al. Tumour dormancy and clinical implications in breast cancer. *Ecancermedicalscience* (2013) 7:320. doi: 10.3332/ecancer.2013.320

53. Uzzan B, Nicolas P, Cucherat M, Perret GY. Microvessel density as a prognostic factor in women with breast cancer: a systematic review of the literature and meta-analysis. *Cancer Res* (2004) 64(9):2941–55. doi: 10.1158/0008-5472.CAN-03-1957

54. Schneider BP, Miller KD. Angiogenesis of breast cancer. *J Clin Oncol* (2005) 23(8):1782–90. doi: 10.1200/JCO.2005.12.017

55. Moon M, Cornfeld D, Weinreb J. Dynamic contrast-enhanced breast MR imaging. *Magn Reson Imaging Clin N Am* (2009) 17(2):351–62. doi: 10.1016/j.mric.2009.01.010

56. Paldino MJ, Barboriak DP. Fundamentals of quantitative dynamic contrast-enhanced MR imaging. *Magn Reson Imaging Clin N Am* (2009) 17(2):277–89. doi: 10.1016/j.mric.2009.01.007

57. Ye D-M, Wang H-T, Yu T. The application of radiomics in breast MRI: a review. *Technol Cancer Res Treat* (2020) 19:1533033820916191. doi: 10.1177/1533033820916191

58. Cui Y, Li Y, Xing D, Bai T, Dong J, Zhu J. Improving the prediction of benign or malignant breast masses using a combination of image biomarkers and clinical parameters. *Front Oncol* (2021) 11:629321–. doi: 10.3389/fonc.2021.629321

59. Goto M, Ito H, Akazawa K, Kubota T, Kizu O, Yamada K, et al. Diagnosis of breast tumors by contrast-enhanced MR imaging: comparison between the diagnostic performance of dynamic enhancement patterns and morphologic features. *J Magn Reson Imaging* (2007) 25(1):104–12. doi: 10.1002/jmri.20812

60. Rezaei Z. A review on image-based approaches for breast cancer detection, segmentation, and classification. *Expert Syst Appl* (2021) 182:115204. doi: 10.1016/j.eswa.2021.115204

61. Wang T, Gong J, Duan HH, Wang LJ, Ye XD, Nie SD. Correlation between CT based radiomics features and gene expression data in non-small cell lung cancer. *J Xray Sci Technol* (2019) 27(5):773–803. doi: 10.3233/XST-190526

62. Haralick RM, Shanmugam K, Dinstein IH. Textural features for image classification. *IEEE Trans Systems Man Cybernetics* (1973) 3(6):610–21. doi: 10.1109/TSMC.1973.4309314

63. Nailon WH. Texture analysis methods for medical image characterisation. *Biomed Imaging* (2010) 75:100. doi: 10.5772/8912

64. Ashraf AB, Daye D, Gavenonis S, Mies C, Feldman M, Rosen M, et al. Identification of intrinsic imaging phenotypes for breast cancer tumors: preliminary associations with gene expression profiles. *Radiology* (2014) 272(2):374–84. doi: 10.1148/radiol.14131375

65. Savaridas SL, Tennant SL. Quantifying lesion enhancement on contrast-enhanced mammography: a review of published data. *Clin Radiology* (2022) 77(4):e313–e20. doi: 10.1016/j.crad.2021.12.010

66. Xiang W, Rao H, Zhou L. A meta-analysis of contrast-enhanced spectral mammography versus MRI in the diagnosis of breast cancer. *Thorac Cancer* (2020) 11(6):1423–32. doi: 10.1111/1759-7714.13400

67. Lobbes MBI, Heuts EM, Moossdorff M, van Nijnatten TJA. Contrast enhanced mammography (CEM) versus magnetic resonance imaging (MRI) for staging of breast cancer: The pro CEM perspective. *Eur J Radiol* (2021) 142:109883. doi: 10.1016/j.ejrad.2021.109883

68. Patel BK, Hilal T, Covington M, Zhang N, Kosiorek HE, Lobbes M, et al. Contrast-enhanced spectral mammography is comparable to MRI in the assessment of residual breast cancer following neoadjuvant systemic therapy. *Ann Surg Oncol* (2018) 25(5):1350–6. doi: 10.1245/s10434-018-6413-x

69. Patel BK, Ranjbar S, Wu T, Pockaj BA, Li J, Zhang N, et al. Computer-aided diagnosis of contrast-enhanced spectral mammography: A feasibility study. *Eur J Radiol* (2018) 98:207–13. doi: 10.1016/j.ejrad.2017.11.024

70. Heidari M, Khuzani AZ, Danala G, Qiu Y, Zheng B. Improving performance of breast cancer risk prediction using a new CAD-based region segmentation scheme. In: *Medical Imaging 2018: Computer-Aided Diagnosis*. SPIE (2018) 10575:166–171.

71. Sun W, Tseng T-LB, Qian W, Zhang J, Saltzstein EC, Zheng B, et al. Using multiscale texture and density features for near-term breast cancer risk analysis. *Med Physics* (2015) 42(6):2853–62. doi: 10.1118/1.4919772

72. Mirniaharikandehei S, Hollingsworth AB, Patel B, Heidari M, Liu H, Zheng B. Applying a new computer-aided detection scheme generated imaging marker to predict short-term breast cancer risk. *Phys Med Biol* (2018) 63(10):105005–. doi: 10.1088/1361-6560/aabefe

73. Tan M, Pu J, Cheng S, Liu H, Zheng B. Assessment of a four-view mammographic image feature based fusion model to predict near-term breast cancer risk. *Ann Biomed Engineering* (2015) 43(10):2416–28. doi: 10.1007/s10439-015-1316-5

74. Gierach GL, Li H, Loud JT, Greene MH, Chow CK, Lan L, et al. Relationships between computer-extracted mammographic texture pattern features and BRCA1/2 mutation status: a cross-sectional study. *Breast Cancer Res* (2014) 16(4):424. doi: 10.1186/s13058-014-0424-8

75. Li H, Giger ML, Huynh BQ, Antropova NO. Deep learning in breast cancer risk assessment: evaluation of convolutional neural networks on a clinical dataset of full-field digital mammograms. *J Med Imaging (Bellingham)* (2017) 4(4):041304. doi: 10.1117/1.JMI.4.4.041304

76. Saha A, Grimm LJ, Ghate SV, Kim CE, Soo MS, Yoon SC, et al. Machine learning-based prediction of future breast cancer using algorithmically measured background parenchymal enhancement on high-risk screening MRI. *J Magn Reson Imaging* (2019) 50(2):456–64. doi: 10.1002/jmri.26636

77. Portnoi T, Yala A, Schuster T, Barzilay R, Dontchos B, Lamb L, et al. Deep learning model to assess cancer risk on the basis of a breast MR image alone. *Am J Roentgenology* (2019) 213(1):227–33. doi: 10.2214/AJR.18.20813

78. Yala A, Lehman C, Schuster T, Portnoi T, Barzilay R. A deep learning mammography-based model for improved breast cancer risk prediction. *Radiology* (2019) 292(1):60–6. doi: 10.1148/radiol.2019182716

79. Yala A, Mikhael PG, Strand F, Lin G, Smith K, Wan YL, et al. Toward robust mammography-based models for breast cancer risk. *Sci Transl Med* (2021) 13(578). doi: 10.1126/scitranslmed.aba4373

80. El-Sokkary N, Arafa AA, Asad AH, Hefny HA. (2019). Machine learning algorithms for breast cancer CADx system in the mammography, *2019 15th International Computer Engineering Conference (ICENCO)*, (2019) 2019:210–215.

81. Dalmış MU, Gubern-Mérida A, Vreemann S, Karssemeijer N, Mann R, Platel B. A computer-aided diagnosis system for breast DCE-MRI at high spatiotemporal resolution. *Med Phys* (2016) 43(1):84–94. doi: 10.1118/1.4937787

82. Qiu Y, Yan S, Gundreddy RR, Wang Y, Cheng S, Liu H, et al. A new approach to develop computer-aided diagnosis scheme of breast mass classification using deep learning technology. *J X-ray Sci Technology* (2017) 25(5):751–63. doi: 10.3233/XST-16226

83. Yurttakal AH, Erbay H, İkizceli T, Karaçavuş S. Detection of breast cancer *via* deep convolution neural networks using MRI images. *Multimedia Tools Applications* (2020) 79(21):15555–73. doi: 10.1007/s11042-019-7479-6

84. Hassan S, Sayed MS, Abdalla MI, Rashwan MA. Breast cancer masses classification using deep convolutional neural networks and transfer learning. *Multimedia Tools Applications* (2020) 79(41):30735–68. doi: 10.1007/s11042-020-09518-w

85. Mendel K, Li H, Sheth D, Giger M. Transfer learning from convolutional neural networks for computer-aided diagnosis: a comparison of digital breast tomosynthesis and full-field digital mammography. *Acad Radiol* (2019) 26(6):735–43. doi: 10.1016/j.acra.2018.06.019

86. Caballo M, Hernandez AM, Lyu SH, Teuwen J, Mann RM, van Ginneken B, et al. Computer-aided diagnosis of masses in breast computed tomography imaging: deep learning model with combined handcrafted and convolutional radiomic features. *J Med Imaging (Bellingham)* (2021) 8(2):024501. doi: 10.1117/1.JMI.8.2.024501

87. Antropova N, Huynh BQ, Giger ML. A deep feature fusion methodology for breast cancer diagnosis demonstrated on three imaging modality datasets. *Med Phys* (2017) 44(10):5162–71. doi: 10.1002/mp.12453

88. Tan M, Qian W, Pu J, Liu H, Zheng B. A new approach to develop computer-aided detection schemes of digital mammograms. *Phys Med Biol* (2015) 60(11):4413. doi: 10.1088/0031-9155/60/11/4413

89. Li H, Mendel KR, Lan L, Sheth D, Giger ML. Digital mammography in breast cancer: additive value of radiomics of breast parenchyma. *Radiology* (2019) 291(1):15–20. doi: 10.1148/radiol.2019181113

90. Heidari M, Mirniaharikandehei S, Danala G, Qiu Y, Zheng B. A new case-based CAD scheme using a hierarchical SSIM feature extraction method to classify between malignant and benign cases, in: *SPIE Medical Imaging 2020: Imaging Informatics for Healthcare, Research, and Applications*; (2020) doi: 10.1117/12.2549130.

91. Moon WK, Lee YW, Ke HH, Lee SH, Huang CS, Chang RF. Computer-aided diagnosis of breast ultrasound images using ensemble learning from convolutional neural networks. *Comput Methods Programs Biomed* (2020) 190:105361. doi: 10.1016/j.cmpb.2020.105361

92. Giannini V, Mazzetti S, Marmo A, Montemurro F, Regge D, Martincich L. A computer-aided diagnosis (CAD) scheme for pretreatment prediction of pathological response to neoadjuvant therapy using dynamic contrast-enhanced MRI texture features. *Br J Radiol* (2017) 90(1077):20170269. doi: 10.1259/bjr.20170269

93. Michoux N, Van den Broeck S, Lacoste L, Fellah L, Galant C, Berlière M, et al. Texture analysis on MR images helps predicting non-response to NAC in breast cancer. *BMC Cancer* (2015) 15:574–. doi: 10.1186/s12885-015-1563-8

94. Aghaei F, Tan M, Hollingsworth AB, Qian W, Liu H, Zheng B. Computer-aided breast MR image feature analysis for prediction of tumor response to chemotherapy. *Med Phys* (2015) 42(11):6520–8. doi: 10.1118/1.4933198

95. Aghaei F, Tan M, Hollingsworth AB, Zheng B. Applying a new quantitative global breast MRI feature analysis scheme to assess tumor response to chemotherapy. *J Magn Reson Imaging* (2016) 44(5):1099–106. doi: 10.1002/jmri.25276

96. Ravichandran K, Braman N, Janowczyk A, Madabhushi A. A deep learning classifier for prediction of pathological complete response to neoadjuvant chemotherapy from baseline breast DCE-MRI. In: *Medical imaging 2018: computer-aided diagnosis*. SPIE (2018) 10575:79–88.

97. Wang L. Early diagnosis of breast cancer. *Sensors (Basel)* (2017) 17(7). doi: 10.3390/s17071572

98. Amir E, Freedman OC, Seruga B, Evans DG. Assessing women at high risk of breast cancer: a review of risk assessment models. *J Natl Cancer Inst* (2010) 102 (10):680–91. doi: 10.1093/jnci/djq088

99. Tice JA, Cummings SR, Ziv E, Kerlikowske K. Mammographic breast density and the Gail model for breast cancer risk prediction in a screening population. *Breast Cancer Res Treat* (2005) 94(2):115–22. doi: 10.1007/s10549-005-5152-4

100. Hollingsworth AB, Stough RG. An alternative approach to selecting patients for high-risk screening with breast MRI. *Breast J* (2014) 20(2):192–7. doi: 10.1111/tbj.12242

101. Madigan MP, Ziegler RG, Benichou J, Byrne C, Hoover RN. Proportion of breast cancer cases in the united states explained by well-established risk factors. *JNCI* (1995) 87(22):1681–5. doi: 10.1093/jnci/87.22.1681

102. Harvey JA, Bovbjerg VE. Quantitative assessment of mammographic breast density: relationship with breast cancer risk. *Radiology* (2004) 230(1):29–41. doi: 10.1148/radiol.2301020870

103. Kolb TM, Lichy J, Newhouse JH. Comparison of the performance of screening mammography, physical examination, and breast US and evaluation of factors that influence them: an analysis of 27,825 patient evaluations. *Radiology* (2002) 225(1):165–75. doi: 10.1148/radiol.2251011667

104. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. *Cancer Epidemiol Prev Biomarkers* (2006) 15(6):1159–69. doi: 10.1158/1055-9965.EPI-06-0034

105. Wolfe JN. Risk for breast cancer development determined by mammographic parenchymal pattern. *Cancer* (1976) 37(5):2486–92. doi: 10.1002/1097-0142(197605)37:5<2486::AID-CNCR2820370542>3.0.CO;2-8

106. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, et al. Mammographic density and the risk and detection of breast cancer. *N Engl J Med* (2007) 356 (3):227–36. doi: 10.1056/NEJMoa062790

107. Manduca A, Carston MJ, Heine JJ, Scott CG, Pankratz VS, Brandt KR, et al. Texture features from mammographic images and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* (2009) 18(3):837–45. doi: 10.1158/1055-9965.EPI-08-0631

108. Vachon CM, Brandt KR, Ghosh K, Scott CG, Maloney SD, Carston MJ, et al. Mammographic breast density as a general marker of breast cancer risk. *Cancer Epidemiol Prev Biomarkers* (2007) 16(1):43–9. doi: 10.1158/1055-9965.EPI-06-0738

109. Tan M, Zheng B, Ramalingam P, Gur D. Prediction of near-term breast cancer risk based on bilateral mammographic feature asymmetry. *Acad Radiology* (2013) 20(12):1542–50. doi: 10.1016/j.acra.2013.08.020

110. Mohamed AA, Berg WA, Peng H, Luo Y, Jankowitz RC, Wu S. A deep learning method for classifying mammographic breast density categories. *Med Phys* (2018) 45(1):314–21. doi: 10.1002/mp.12683

111. Chang Y-H, Wang X-H, Hardesty LA, Chang TS, Poller WR, Good WF, et al. Computerized assessment of tissue composition on digitized mammograms. *Acad Radiol* (2002) 9(8):899–905. doi: 10.1016/S1076-6332(03)80459-2

112. Byng JW, Yaffe MJ, Lockwood GA, Little LE, Tritchler DL, Boyd NF. Automated analysis of mammographic densities and breast carcinoma risk. *Cancer* (1997) 80(1):66–74. doi: 10.1002/(SICI)1097-0142(19970701)80:1<66::AID-CNCR9>3.0.CO;2-D

113. Glide-Hurst CK, Duric N, Littrup P. A new method for quantitative analysis of mammographic density. *Med Phys* (2007) 34(11):4491–8. doi: 10.1118/1.2789407

114. Van Gils CH, Otten JD, Verbeek AL, Hendriks JH. Mammographic breast density and risk of breast cancer: masking bias or causality? *Eur J Epidemiol* (1998) 14(4):315–20. doi: 10.1023/a:1007423824675

115. Nielsen M, Karemore G, Loog M, Raundahl J, Karssemeijer N, Otten JD, et al. A novel and automatic mammographic texture resemblance marker is an independent risk factor for breast cancer. *Cancer Epidemiol* (2011) 35(4):381–7. doi: 10.1016/j.canep.2010.10.011

116. Li H, Giger ML, Huo Z, Olopade OI, Lan L, Weber BL, et al. Computerized analysis of mammographic parenchymal patterns for assessing breast cancer risk: effect of ROI size and location. *Med Phys* (2004) 31(3):549–55. doi: 10.1118/1.1644514

117. Sutton EJ, Huang EP, Drukker K, Burnside ES, Li H, Net JM, et al. Breast MRI radiomics: comparison of computer- and human-extracted imaging phenotypes. *Eur Radiol Exp* (2017) 1(1):22. doi: 10.1186/s41747-017-0025-2

118. Birdwell RL, Ikeda DM, O'Shaughnessy KF, Sickles EA. Mammographic characteristics of 115 missed cancers later detected with screening mammography and the potential utility of computer-aided detection. *Radiology* (2001) 219(1):192–202. doi: 10.1148/radiology.219.1.r01ap16192

119. Zheng B, Good WF, Armfield DR, Cohen C, Hertzberg T, Sumkin JH, et al. Performance change of mammographic CAD schemes optimized with most-recent and prior image databases. *Acad Radiol* (2003) 10(3):283–8. doi: 10.1016/S1076-6332(03)80102-2

120. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, et al. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. *Jama* (2017) 317(23):2402–16. doi: 10.1001/jama.2017.7112

121. Wei J, Chan HP, Wu YT, Zhou C, Helvie MA, Tsodikov A, et al. Association of computerized mammographic parenchymal pattern measure with breast cancer risk: a pilot case-control study. *Radiology* (2011) 260(1):42–9. doi: 10.1148/radiol.11101266

122. Zheng Y, Keller BM, Ray S, Wang Y, Conant EF, Gee JC, et al. Parenchymal texture analysis in digital mammography: A fully automated pipeline for breast cancer risk assessment. *Med Phys* (2015) 42(7):4149–60. doi: 10.1118/1.4921996

123. Arasu VA, Miglioretti DL, Sprague BL, Alsheik NH, Buist DSM, Henderson LM, et al. Population-based assessment of the association between magnetic resonance imaging background parenchymal enhancement and future primary breast cancer risk. *J Clin Oncol* (2019) 37(12):954–63. doi: 10.1200/JCO.18.00378

124. Bauer E, Levy MS, Domachevsky L, Anaby D, Nissan N. Background parenchymal enhancement and uptake as breast cancer imaging biomarkers: A state-of-the-art review. *Clin Imaging* (2022) 83:41–50. doi: 10.1016/j.clinimag.2021.11.021

125. Dontchos BN, Rahbar H, Partridge SC, Korde LA, Lam DL, Scheel JR, et al. Are qualitative assessments of background parenchymal enhancement, amount of fibroglandular tissue on MR images, and mammographic density associated with breast cancer risk? *Radiology* (2015) 276(2):371–80. doi: 10.1148/radiol.2015142304

126. Niell BL, Abdalah M, Stringfield O, Raghunand N, Ataya D, Gillies R, et al. Quantitative measures of background parenchymal enhancement predict breast cancer risk. *AJR Am J Roentgenol* (2021) 217(1):64–75. doi: 10.2214/AJR.20.23804

127. Gao F, Wu T, Li J, Zheng B, Ruan L, Shang D, et al. SD-CNN: A shallow-deep CNN for improved breast cancer diagnosis. *Computerized Med Imaging Graphics* (2018) 70:53–62. doi: 10.1016/j.compmedimag.2018.09.004

128. Alzubaidi L, Fadhel MA, Al-Shamma O, Zhang J, Santamaría J, Duan Y, et al. Towards a better understanding of transfer learning for medical imaging: A case study. *Appl Sci* (2020) 10(13):4523. doi: 10.3390/app10134523

129. Shin HC, Roth HR, Gao M, Lu L, Xu Z, Nogues I, et al. Deep convolutional neural networks for computer-aided detection: CNN architectures, dataset characteristics and transfer learning. *IEEE Trans Med Imaging* (2016) 35(5):1285–98. doi: 10.1109/TMI.2016.2528162

130. Deng J, Dong W, Socher R, Li L-J, Li K, Fei-Fei L. (2009). Imagenet: A large-scale hierarchical image database, in: *2009 IEEE conference on computer vision and pattern recognition* (pp. 248–255)

131. Kim HE, Cosa-Linan A, Santhanam N, Jannesari M, Maros ME, Ganslandt T. Transfer learning for medical image classification: a literature review. *BMC Med imaging* (2022) 22(1):1–13. doi: 10.1186/s12880-022-00793-7

132. Omranipour R, Jalili R, Yazdankhahkenary A, Assarian A, Mirzania M, Eslami B. Evaluation of pathologic complete response (pCR) to neoadjuvant chemotherapy in Iranian breast cancer patients with estrogen receptor positive and HER2 negative and impact of predicting variables on pCR. *Eur J Breast Health* (2020) 16(3):213–8. doi: 10.5152/ejbh.2020.5487

133. Haque W, Verma V, Hatch S, Suzanne Klimberg V, Brian Butler E, Teh BS. Response rates and pathologic complete response by breast cancer molecular subtype following neoadjuvant chemotherapy. *Breast Cancer Res Treat* (2018) 170(3):559–67. doi: 10.1007/s10549-018-4801-3

134. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. *Nature* (2012) 490(7418):61–70. doi: 10.1038/nature11412

135. Nwabo Kamdje AH, Seke Etet PF, Vecchio L, Muller JM, Krampera M, Lukong KE. Signaling pathways in breast cancer: therapeutic targeting of the microenvironment. *Cell Signal* (2014) 26(12):2843–56. doi: 10.1016/j.cellsig.2014.07.034

136. Wang H, Mao X. Evaluation of the efficacy of neoadjuvant chemotherapy for breast cancer. *Drug Des Devel Ther* (2020) 14:2423–33. doi: 10.2147/DDDT.S253961

137. Graham LJ, Shupe MP, Schneble EJ, Flynt FL, Clemenshaw MN, Kirkpatrick AD, et al. Current approaches and challenges in monitoring treatment responses in breast cancer. *J Cancer* (2014) 5(1):58–68. doi: 10.7150/jca.7047

138. Thoeny HC, Ross BD. Predicting and monitoring cancer treatment response with diffusion-weighted MRI. *J Magn Reson* (2010) 32(1):2–16. doi: 10.1002/jmri.22167

139. Gerwing M, Herrmann K, Helfen A, Schliemann C, Berdel WE, Eisenblätter M, et al. The beginning of the end for conventional RECIST — novel therapies require novel imaging approaches. *Nat Rev Clin Oncol* (2019) 16(7):442–58. doi: 10.1038/s41571-019-0169-5

140. Choi M, Park YH, Ahn JS, Im Y-H, Nam SJ, Cho SY, et al. Evaluation of pathologic complete response in breast cancer patients treated with neoadjuvant chemotherapy: Experience in a single institution over a 10-year period. *J Pathol Transl Med* (2017) 51(1):69–78. doi: 10.4132/jptm.2016.10.05

141. Zaha DC. Significance of immunohistochemistry in breast cancer. *World J Clin Oncol* (2014) 5(3):382–92. doi: 10.5306/wjco.v5.i3.382

142. Bergin A, Loi S. Triple-negative breast cancer: recent treatment advances [version 1; peer review: 2 approved]. *F1000Res.* (2019) 8(F1000 Faculty Rev-1342). doi: 10.12688/f1000research.18888.1

143. Arunachalam HB, Mishra R, Daescu O, Cederberg K, Rakheja D, Sengupta A, et al. Viable and necrotic tumor assessment from whole slide images of osteosarcoma using machine-learning and deep-learning models. *PloS One* (2019) 14(4):e0210706–e. doi: 10.1371/journal.pone.0210706

144. Hylton NM, Blume JD, Bernreuter WK, Pisano ED, Rosen MA, Morris EA, et al. Locally advanced breast cancer: MR imaging for prediction of response to neoadjuvant chemotherapy–results from ACRIN 6657/I-SPY TRIAL. *Radiology* (2012) 263(3):663. doi: 10.1148/radiol.12110748

145. Clark K, Vendt B, Smith K, Freymann J, Kirby J, Koppel P, et al. The cancer imaging archive (TCIA): Maintaining and operating a public information repository. *J Digit Imaging* (2013) 26(6):1045–57. doi: 10.1007/s10278-013-9622-7

146. Thrall JH, Li X, Li Q, Cruz C, Do S, Dreyer K, et al. Artificial intelligence and machine learning in radiology: Opportunities, challenges, pitfalls, and criteria for success. *J Am Coll Radiol* (2018) 15(3 Pt B):504–8. doi: 10.1016/j.jacr.2017.12.026

147. Li XT, Huang RY. Standardization of imaging methods for machine learning in neuro-oncology. *Neurooncol Adv* (2020) 2(Suppl 4):iv49–55. doi: 10.1093/noajnl/vdaa054

148. Sala E, Mema E, Himoto Y, Veeraraghavan H, Brenton JD, Snyder A, et al. Unravelling tumour heterogeneity using next-generation imaging: radiomics, radiogenomics, and habitat imaging. *Clin Radiol* (2017) 72(1):3–10. doi: 10.1016/j.crad.2016.09.013

149. Kelly CJ, Karthikesalingam A, Suleyman M, Corrado G, King D. Key challenges for delivering clinical impact with artificial intelligence. *BMC Med* (2019) 17(1):195. doi: 10.1186/s12916-019-1426-2

150. van der Velden BHM, Kuijf HJ, Gilhuijs KGA, Viergever MA. Explainable artificial intelligence (XAI) in deep learning-based medical image analysis. *Med Image Anal* (2022) 79:102470. doi: 10.1016/j.media.2022.102470

151. Linardatos P, Papastefanopoulos V, Kotsiantis S. Explainable ai: A review of machine learning interpretability methods. *Entropy* (2020) 23(1):18. doi: 10.3390/e23010018

152. Holzinger A, Biemann C, Pattichis CS, Kell DB. What do we need to build explainable AI systems for the medical domain? *arXiv* (2017) arXiv:1712.09923.

153. Nishikawa RM, Schmidt RA, Linver MN, Edwards AV, Papaioannou J, Stull MA. Clinically missed cancer: how effectively can radiologists use computer-aided detection? *AJR Am J Roentgenol* (2012) 198(3):708–16. doi: 10.2214/AJR.11.6423

154. Hupse R, Samulski M, Lobbes MB, Mann RM, Mus R, den Heeten GJ, et al. Computer-aided detection of masses at mammography: interactive decision support versus prompts. *Radiology* (2013) 266(1):123–9. doi: 10.1148/radiol.12120218

155. Elmore JG, Lee CI. Artificial intelligence in medical imaging–learning from past mistakes in mammography. *JAMA Health Forum* (2022) 3(2):e215207–e. doi: 10.1001/jamahealthforum.2021.5207

frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 15 September 2022
DOI 10.3389/fonc.2022.961970

OPEN ACCESS

EDITED BY
Claudia Mello-Thoms,
The University of Iowa, United States

REVIEWED BY
Ernest Ekpo,
The University of Sydney, Australia
Sadaf Alipour,
Tehran University of Medical Sciences, Iran

*CORRESPONDENCE
Ning He
bcsmoking@163.com

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 05 June 2022
ACCEPTED 24 August 2022
PUBLISHED 15 September 2022

CITATION
He Y, Si Y, Li X, Hong J, Yu C and He N (2022) The relationship between tobacco and breast cancer incidence: A systematic review and meta-analysis of observational studies.
Front. Oncol. 12:961970.
doi: 10.3389/fonc.2022.961970

The relationship between tobacco and breast cancer incidence: A systematic review and meta-analysis of observational studies

Yujing He[1], Yuexiu Si[2], Xiangyuan Li[1], Jiaze Hong[1], Chiyuan Yu[1] and Ning He[3]*

[1]The Second Clinical Medical College, Zhejiang Chinese Medical University, Hangzhou, China, [2]School of Basic Medical Sciences, Zhejiang Chinese Medical University, Hangzhou, China, [3]Department of Tumor High-intensity focused ultrasound (HIFU) Therapy, HwaMei Hospital, University of Chinese Academy of Sciences, Ningbo, China

Background: The effect of tobacco on breast cancer (BC) is controversial. The purpose of this study was to investigate the relationship between tobacco and BC.

Methods: A search was conducted in PubMed, EBSCO, Web of Science and Cochrane Library databases before February 2022. The adjusted odd ratio (OR) and corresponding 95% confidence interval (CI) were used to examine the relationship between active or passive smoking and BC risk.

Results: A total of 77 articles composed of 2,326,987 participants were included for this meta-analysis. Active (OR=1.15, 95% CI=1.11-1.20, p<0.001) and passive (OR=1.17, 95% CI=1.09-1.24, p<0.001) smoking increased the risk of BC in the female population, especially premenopausal BC (active smoking: OR=1.24, p<0.001; passive smoking: OR=1.29, p<0.001), but had no effect on postmenopausal BC (active smoking: OR=1.03, p=0.314; passive smoking: OR=1.13, p=0.218). Active smoking increased the risk of estrogen receptor-positive (ER+) BC risk (OR=1.13, p<0.001), but had no effect on estrogen receptor-negative (ER-) BC (OR=1.08, p=0.155). The risk of BC was positively associated with the duration and intensity of smoking, negatively associated with the duration of smoking cessation. Active smoking increased the risk of BC in the multiparous population (OR=1.13, p<0.001), but had no effect on the nulliparous population (OR=1.05, p=0.432), and smoking before the first birth (OR=1.22, 95% CI=1.17-1.27) had a greater impact on the risk of BC than smoking after the first birth (OR=1.08, 95% CI=1.04-1.12).

Conclusion: Smoking (active and passive) increased the risk of BC in women. The effect of smoking on BC was influenced by smoking-related factors (duration, intensity, years of quitting), population-related factors (fertility status), and BC subtypes.

Systematic Review Registration: identifier CRD42022322699.

KEYWORDS

breast cancer, active smoking, passive smoking, incidence, meta-analysis selection criteria

Introduction

Breast cancer (BC) is the most common cancer in women worldwide (1). As a heterogeneous disease, its occurrence is influenced by both endogenous factors (such as heredity (2, 3), gene mutation (4, 5)) and exogenous factors (such as reproduction (6, 7), environment (8)). It is estimated that only 5-10% of BC cases are induced by genetic factors, while the remaining 90-95% are highly related to environmental factors or specific lifestyle (9, 10). Therefore, researchers are trying to provide better preventional strategies by adjusting exposure to BC protective or risky factors (1, 11). Evidence has shown that unhealthy lifestyle and some environmental factors are harmful to women (12–14), and eliminating these factors may help reduce the morbidity and mortality rate (15, 16).

The potential role of smoking in BC risk has been under intense discussion (17, 18). Although BC is not initially thought to be a tobacco-related cancer, over the past few decades, many chemicals contained in tobacco have been investigated to be a trigger of BC, such as 4-aminobiphenyl (19, 20) and benzopyrene (21, 22). In addition, evidence of the role of active smoking (23, 24) and secondhand smoke (25, 26) in the etiology of BC is accumulating, based on adequate animal trials (27, 28) and relevant epidemiological evidence (29). Recent trends have discovered smoking as one of the potential risk factors for BC (30).

Although many studies have shown that smoking may increase the risk of BC, a review of studies over the past 30 years has found that opinions among clinical researchers are still widely divided (17, 31). Firstly, some studies [e.g. Yingsong Lin et al. (32) and Chelsea Catsburg et al. (23)] failed to observe any association between smoking and BC incidence. Secondly, the results of subgroup analyses among different studies were high inconsistent (33, 34), or even reversed, such as subgroup analyses on menstrual status and BC subtypes. Third, published meta-analyses on the topic have also not reached consistent conclusions. Although most meta-analyses on active smoking suggest that smoking increases the incidence of BC, the conclusions of subgroup analyses are inconsistent (35, 36), and the meta-analyses on passive smoking are more inconsistent (37, 38). The last relevant meta-analysis was conducted and published in 2018. As of 2021, there are 153 million adult female smokers (including smoking, secondhand, and chewing) worldwide, accounting for 12% (39) of global smokers. Therefore, based on the inconsistency of previous studies, the large smoking population and the significant disease burden caused by tobacco (40), this study aimed to investigate the relationship between smoking and BC by conducting a systematic review and meta-analysis by searching for relevant observational studies. Therefore, it can provide a preventive reference for the female group and create greater value for the society.

Abbreviations: BC, breast cancer; MOOSE, the meta-analysis of observational studies in epidemiology; PICOS, the population, intervention, comparison, outcome and setting criteria; NOS, the newcastle-ottawa quality assessment scale checklist; ER+, estrogen receptor-positive; ER-, estrogen receptor-negative; CI, confidence interval; OR, odd ratio; BMI, body mass index; WHO, world health organization.

Materials and methods

Search strategy

A comprehensive search of studies investigating the association between smoking and BC was carried out before February 2022 in electronic databases of PubMed, Web of science, EBSCO, and the Cochrane Library. The complete retrieval formula that was used to identify the related studies includes: ("breast cancer" OR "breast neoplasms" OR "BC") AND ("smoking" OR "tobacco smoke pollution" OR "tobacco use" OR "tobacco products" OR "active smoking" OR "passive smoking" OR "secondhand smoking" OR "tobacco"). The reference lists of retrieved studies and conference records were also reviewed for potentially inclusive studies. When referring to duplicate literature, the original article was included if the study was published as an abstract or an original article. Also, if a study was continuously updated and reported, only the most recent or comprehensive articles were included. This meta-analysis was conducted according to the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines (41). The population, intervention, comparison, outcome, and setting (PICOS) criteria were used to describe the research question. Participants in this study were people who had not previously been diagnosed with BC, the intervention was exposure to

tobacco environments, including active and passive smoking, the comparison was a non-smoker, the outcome was the incidence of BC, and the setting was observational research. This meta-analysis's prospero registration number was CRD42022322699.

Selection criteria

An eligible criterion was formulated. The specific criteria were as follows. Inclusion criteria: (1) all included studies are observational studies. (2) The main exposure of study was smoking including active and passive smoking, and the outcome was BC risk. (3) All studies included available data which reported the relationship between smoking and BC. Exclusion criteria: (1) the study was conducted on BC population and used mortality or recovery rate as the outcome. (2) The study was published in duplicate. (3) The study was not published in English.

Data collection and quality assessment

A jointly agreed data collection form was used to extract all data. Information was extracted as follows: the author's name, year of publication, study type, age, exposure assessment, number of participants, number of BC cases, number of smokers, number of non-smokers, variables adjusted in the statistical analyses, and outcomes. To ensure the objectivity and accuracy of the data, two researchers independently extracted data from each study. Disagreements were resolved by consensus or consultation with a third researcher.

The quality of each included study was evaluated by the Newcastle-Ottawa Quality Assessment Scale (NOS) checklist, a tool used for quality assessment of non-randomized studies. NOS checklist is composed of eight items classified into three aspects, including selection, comparability, and outcome. The maximum scores of this checklist were nine, and scores between seven and nine were identified to be of higher study quality.

Objectives and endpoints

The primary objective was to explore the relationship between smoking and the incidence of BC. Secondary objectives were to explore the relationship between the incidence of BC and smoking subgroups (e.g. smoking pattern, smoking time, smoking frequency, smoking place, smoking cessation time, age of starting smoking), the relationship between smoking and BC in different populations (e.g. fertility status, menopausal status, race), and the association between smoking and different BC subtypes (e.g. estrogen receptor-positive (ER+) BC, estrogen receptor-negative (ER-) BC). The results after adjusting for relevant confounding factors were used consistently for the processing of relevant data from the included articles.

Statistical analysis

The Stata software version 12 (StataCorp, College Station, Texas, USA) was used to analyze the data. The confidence interval (CI) of odd ratio (OR) was set at 95% to examine the relationship between smoking and BC risk. Heterogeneity of included studies was tested by Q statistic and I^2 statistic to quantitatively assess inconsistency. For statistical results, values of $p<0.10$ and $I^2>50\%$ were considered to be representative of having statistically significant heterogeneity. Based on the heterogeneity of smoking intensity, smoking duration, race, BC subtype, etc. in different studies, in order to improve the reliability of the results, the random effects model was uniformly used in this study. When more than ten studies were included, sensitivity analysis and publication bias test were performed to evaluate the stability and reliability of their results. Publication bias was evaluated by the Begg's test. Results with P-values less than 0.05 were considered to be statistically significant.

Results

Literature search

A total of 19,746 relevant articles were identified based on retrieval formula described in the methods section by initial search in PubMed, EBSCO, Web of Science, and Cochrane Library database. No additional records were identified through other sources. A total of 8,463 duplicate articles were deleted, and 11,283 articles were excluded due to the title or abstract. The remaining 932 articles were reviewed through full-text. Among them, 855 articles were eliminated because of being non-observational study (n=339), duplicate publication (n=218), not exploring the risk of BC (n=176), no relevant results reported (n=85), and not published in English (n=37). Eventually, 77 articles (13, 32–34, 42–115) composed of 2,326,987 participants were selected for this meta-analysis. The detailed search and study selection process was shown in Figure 1.

Characteristic of studies

Of the 77 included studies, 24 were cohort studies (2,138,338 participants and 55,703 BC cases), 53 were case-control studies (188,649 controls and 58,859 BC cases). The participants in the two studies included men and women, and the rest were women. All studies were published between 1988 and 2022, with follow-up periods ranging from 6 to 24.6 years. Regarding age at recruitment, eight studies did not set the upper age limit, four studies did not set a lower age limit and four studies did not report the requirement for age. Among them, 30 studies were conducted in America, 24 were in Asia, 22 were in Europe, and 1 was in Oceania. Fifty-six studies investigated the association

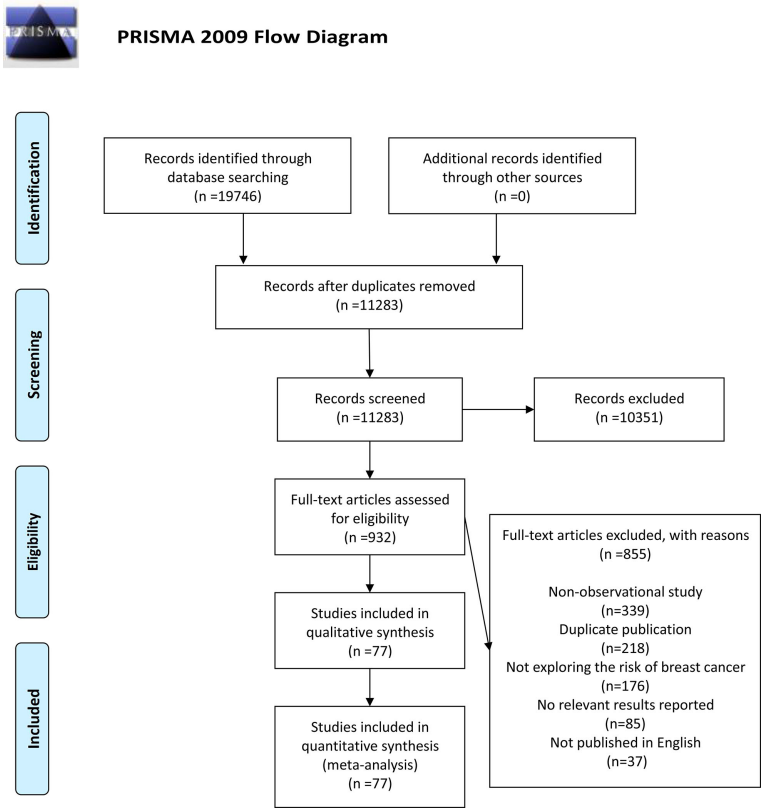


FIGURE 1
A schematic flow for the selection of articles included in this meta-analysis.

between active smoking and BC risk, 39 investigated the association between passive smoking and BC risk. The number of smokers (included active and passive smokers) was 1,326,603 in cohort studies and 108,175 in case-control studies. In order to collect data and evaluate relevant exposure factors, 59 studies chose questionnaire, 9 studies chose interview, and 9 studies chose questionnaire combined with interviews. In addition, the adjustment of potential confounding factors varied in different studies. Most of the adjustment parameters were age, body mass index (BMI), family history of BC, total energy intake, alcohol consumption, number of births, and physical activity. The characteristics of the included studies were shown in Table 1 and Supplementary Table 1.

Overall effect of active smoking

Fifty-six studies recorded data about active smoking in female population that was inducing BC. Studies had shown that women who actively smoked had a significantly higher incidence of BC than those who had never actively smoked (OR=1.15, 95% CI=1.11-1.20, p<0.001, I^2 = 54.9%). Among them, current active smoking (OR=1.12, 95% CI=1.08-1.16, p=0.007, I^2 = 40.1%) and former active smoking (OR=1.09, 95% CI=1.06-1.12, p<0.001, I^2 = 33.3%) had a significantly increase on the incidence of BC, but current active smoking increased the incidence of BC more than former active smoking. In other words, active smoking is a risk factor for women, and the population who is still active smoking is under more risk than the population who quit smoking after active smoking. In addition, cohort studies (OR=1.13, p<0.001) and case-control studies (OR=1.19, p<0.001) had consistently concluded that active smoking increases the risk of BC in women. The detailed data was contained in Table 2.

Menopausal status

The correlation between smoking and BC is affected by menopausal status. Related data were available in 23 studies with premenopausal BC and 25 with postmenopausal BC. The analysis showed that active smoking increases the incidence of premenopausal BC (OR=1.24, 95% CI=1.17-1.32, p<0.001, I^2 = 6.2%), but had no effect on postmenopausal BC (OR=1.03, 95%

10.3389/fonc.2022.961970

TABLE 1 Characteristics of included observational studies in the meta-analysis.

Author, year	Country	Median follow-up time (years)	Age at recruitment (year)	Median age at time of analysis (years)	No. of BC cases	No. of participants	Study Type
Vatten LJ, 1990	Norway	12	35-51	NA	242	24,617	Cohort study
Bennicke K, 1995	Denmark	NA	15-92	45.0	230	3,240	Cohort study
Calle EE, 1994	America	6	30-75	56.0	880	604,412	Cohort study
Goodman MT, 1997	Japan	8.31	30-85	64.5	161	22,200	Cohort study
Nishino Y, 2001	Japan	9	>40	56.6	67	9,675	Cohort study
Hanaoka T, 2005	Japan	9	40-59	49.0	180	21,805	Cohort study
Olson JE, 2005	America	14	55-69	62.0	2,017	37,105	Cohort study
Lin Y, 2005	Japan	7.8	40-79	57.0	208	34,410	Cohort study
Pirie K, 2008	United Kingdom	6.3	50-64	57.0	2,518	210,647	Cohort study
Reynolds P, 2009	America	8	>35	53.0	1,754	57,523	Cohort study
Xue F, 2010	America	24.6	30-55	58.0	8,772	121,700	Cohort study
Luo J, 2011	America	10.3	50-79	62.0	3,520	79,900	Cohort study
Rosenberg L, 2013	America	14	21-69	37.0	1,377	59,000	Cohort study
Dossus L, 2014	France	11	35-65	58.0	9,822	322,988	Cohort study
Catsburg C, 2015	Canada	22.1	40-59	52.0	6,549	89,835	Cohort study
Wada K, 2015	Japan	10	>35	53.0	543	15,719	Cohort study
White AJ, 2017	America	6.4	35-74	54.9	1,843	50,884	Cohort study
van den Brandt PA, 2017	Netherlands	NA	55-69	59.0	2,526	62,573	Cohort study
Jones ME, 2017	United Kingdom	7.7	>16	47.0	1,815	102,927	Cohort study
Gram IT, 2019	America	16.7	45-75	62.0	4,230	67,313	Cohort study
Heberg J, 2019	Denmark	18.8	>44	56.0	1,407	16,106	Cohort study
Zeinomar N, 2019	America	10.4	18-79	46.7	1,009	17,435	Cohort study
Botteri E, 2021	Sweden	9.5	30-49	40.0	1,848	29,930	Cohort study
Gram IT, 2022	Norway	19.8	34-70	49.8	2,185	76,394	Cohort study
Kato I, 1992	Japan	NA	20-75	48.0	908	1,816	Case-control study
Field NA, 1992	America	NA	20-79	NA	1,617	3,234	Case-control study

(Continued)

10.3389/fonc.2022.961970

TABLE 1 Continued

Author, year	Country	Median follow-up time (years)	Age at recruitment (year)	Median age at time of analysis (years)	No. of BC cases	No. of participants	Study Type
Pawlega J, 1992	Poland	NA	35-75	52.0	127	377	Case-control study
Chu SY, 1990	America	NA	20-54	45.0	4,134	8,351	Case-control study
Schechter MT, 1989	Canada	NA	40-59	NA	254	1,061	Case-control study
Adami HO, 1988	Sweden, Norway	NA	<45	37.0	422	949	Case-control study
Hirose K, 1995	Japan	NA	20-80	49.0	1,186	24,349	Case-control study
Smith SJ, 1994	United Kingdom	NA	<36	NA	755	1,502	Case-control study
Braga C, 1996	Italy	NA	20-74	56.0	2,569	5,157	Case-control study
Ranstam J, 1955	United Kingdom	NA	25-59	NA	998	1,996	Case-control study
Morabia A, 1998	Switzerland	NA	30-74	53.0	242	1,301	Case-control study
Tung HT, 1999	Japan	NA	29-85	51.6	376	806	Case-control study
Johnson KC, 2000	Canada	NA	25-74	43.0	2,317	4,755	Case-control study
Marcus PM, 2000	America	NA	20-74	NA	864	1,654	Case-control study
Ueji M, 1998	Japan	NA	26-69	48.0	145	385	Case-control study
Lash TL, 2002	America	NA	40-85	65.0	615	1,281	Case-control study
Kropp S, 2002	Germany	NA	<50	43.0	468	1,561	Case-control study
Liu L, 2000	China	NA	24-55	41.0	186	372	Case-control study
Shrubsole MJ, 2004	China	NA	25-64	47.0	1,013	2,130	Case-control study
Alberg AJ, 2004	America	NA	NA	NA	110	223	Case-control study
Gammon MD, 2004	America	NA	24-98	56.0	1,356	2,739	Case-control study
Manjer J, 2004	Sweden	NA	NA	59.0	260	801	Case-control study
Bonner MR, 2005	America	NA	35-79	51.0	1,166	3,271	Case-control study
Metsola K, 2005	Finland	NA	44-77	55.0	483	965	Case-control study
Mechanic LE, 2006	America	NA	NA	NA	2,311	4,333	Case-control study
Ha M,2007	America	NA	22-92	37.5	906	12,372	Case-control study
Roddam AW, 2007	United Kingdom	NA	36-45	41.0	639	1,279	Case-control study
Slattery ML,2008	America	NA	>50	NA	1,183	2,266	Case-control study

(Continued)

10.3389/fonc.2022.961970

TABLE 1 Continued

Author, year	Country	Median follow-up time (years)	Age at recruitment (year)	Median age at time of analysis (years)	No. of BC cases	No. of participants	Study Type
Rollison DE, 2008	America	NA	40-79	63.0	287	598	Case-control study
Young E, 2009	America, Canada	NA	25-75	55.0	6,235	12,768	Case-control study
Ahern TP, 2009	America	NA	<75	59.0	557	989	Case-control study
Conlon MS, 2010	Canada	NA	25-75	55.9	347	1,122	Case-control study
De Silva M,2010	Sri Lanka	NA	30-64	48.0	100	303	Case-control study
Sezer H, 2011	Turkey	NA	35-60	54.0	172	555	Case-control study
Hu M, 2013	China	NA	25-75	46.7	196	407	Case-control study
Gao CM, 2013	China	NA	30-65	50.0	669	1,351	Case-control study
McKenzie F, 2013	New Zealand	NA	NA	NA	1,799	4,339	Case-control study
Ilic M, 2013	Serbia	NA	30-75	60.0	191	382	Case-control study
Kawai M, 2014	America	NA	20-44	35.0	1,920	2,858	Case-control study
Tong JH, 2014	China	NA	>18	49.0	312	624	Case-control study
Pimhanam C, 2014	Thailand	NA	17-76	45.0	444	888	Case-control study
Li B, 2015	China	NA	25-70	46.0	877	1,767	Case-control study
Connor AE, 2015	Spain	NA	25-70	7026.0	2,889	7,917	Case-control study
Hara A, 2017	Japan	NA	35-85	55.0	511	1,038	Case-control study
Butler EN, 2016	America	NA	20-64	51.0	1,808	3,372	Case-control study
Park SY, 2016	America	NA	20-75	43.0	5,791	23,167	Case-control study
Strumylaite L, 2017	Lithuania	NA	28-90	60.0	449	1,379	Case-control study
Dianatinasab M, 2017	Iran	NA	35-65	49.0	526	1,052	Case-control study
Ellingjord-Dale M, 2017	Norway	NA	50-69	58.0	4,420	28,700	Case-control study
Regev-Avraham Z, 2018	Israel	NA	30-70	52.8	137	411	Case-control study
Godinho-Mota JCM, 2019	Brazil	NA	30-80	41.0	197	542	Case-control study
Alsolami FJ, 2019	Saudi Arabia	NA	45-75	57.0	214	432	Case-control study
Baset Z, 2021	Afghanistan	NA	>30	45.8	201	402	Case-control study

NA, not available; BC, breast cancer.

10.3389/fonc.2022.961970

TABLE 2 Effects of active smoking on breast cancer incidence.

Subgroup analysis	No. ofstudies	OR	95%CI	*p*	Heterogeneity (I^2) (%)
Ever active smoking	56	1.15	1.11-1.20	*<0.001*	54.9
Current	39	1.12	1.08-1.16	*0.007*	40.1
Former	42	1.09	1.06-1.12	*<0.001*	33.3
Cohort study	17	1.13	1.07-1.18	*<0.001*	72.6
Case-control study	39	1.19	1.12-1.26	*<0.001*	31.9
Premenopausal BC	23	1.24	1.17-1.32	*<0.001*	6.2
Postmenopausal BC	25	1.03	0.97-1.10	0.314	30.8
Smoking duration					
<20 years	38	1.06	1.03-1.09	*<0.001*	0
20-30 years	36	1.15	1.10-1.19	*<0.001*	27.8
30-40 years	20	1.15	1.10-1.20	*<0.001*	5.7
>40 years	13	1.22	1.13-1.31	*<0.001*	40.8
Smoking intensity					
<10 cigarettes per day	35	1.06	1.03-1.10	*0.001*	13.3
10-20 cigarettes per day	38	1.19	1.14-1.25	*<0.001*	30.4
20-30 cigarettes per day	29	1.16	1.11-1.22	*<0.001*	30.2
>30 cigarettes per day	4	1.18	1.07-1.31	*0.001*	9.4
Pack-years smoked					
<10 years	31	1.05	1.01-1.08	*0.005*	5.5
10-20 yeasr	36	1.11	1.08-1.15	*<0.001*	0.9
20-40 yeasr	29	1.21	1.17-1.27	*<0.001*	17.8
>40 yeasr	12	1.17	1.11-1.23	*<0.001*	0
Age started smoking					
< 16 years	25	1.11	1.07-1.15	*<0.001*	0
17-19 years	34	1.16	1.12-1.20	*<0.001*	9.2
>20 years	33	1.08	1.04-1.11	*<0.001*	16.5
Years since quitting					
<10 years	18	1.27	1.15-1.41	*<0.001*	74.2
10-20 yeasr	18	1.05	1.00-1.09	*0.046*	5.0
>20 yeasr	11	1.01	0.97-1.06	0.552	0
Fertility status					
Multiparous population	6	1.13	1.07-1.20	*<0.001*	0
Nulliparous population	6	1.05	0.92-1.20	0.432	0
Active smoking before first birth	24	1.22	1.17-1.27	*<0.001*	9.4
<5 years before first birth	13	1.06	1.01-1.11	*0.023*	0
>5 years before first birth	21	1.24	1.14-1.35	*<0.001*	49.9
Active smoking after first birth	22	1.08	1.04-1.12	*<0.001*	0
<10 years after first birth	7	1.00	0.93-1.09	0.922	19.1
>10 years after first birth	10	1.06	0.99-1.14	0.077	48.8
BC subtypes					
ER+ BC	6	1.13	1.08-1.18	*<0.001*	0
<10 years smoking	5	0.99	0.90-1.09	0.870	30.0
>10 years smoking	13	1.14	1.04-1.25	*0.007*	49.6
<10 cigarettes per day	7	1.08	1.00-1.17	*0.041*	25.9
>10 cigarettes per day	7	1.18	1.06-1.32	*0.002*	62.7
ER- BC	6	1.08	0.97-1.19	0.155	0
<10 years smoking	5	1.02	0.91-1.16	0.699	0

(Continued)

TABLE 2 Continued

Subgroup analysis	No. ofstudies	OR	95%CI	*p*	Heterogeneity (I^2) (%)
>10 years smoking	13	1.08	0.98-1.18	0.105	0
<10 cigarettes per day	13	0.97	0.87-1.08	0.603	0
>10 cigarettes per day	13	1.18	1.00-1.39	*0.049*	53.5

OR, odd ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; BC, breast cancer.

CI=0.97-1.10, p=0.314, I^2 = 30.8%) with slight heterogeneity. The detailed data was contained in Table 2.

Smoking duration

Years were used to measure smoking duration in this study. The related data were divided into '<20 years group', '20-30 years group', '30-40 years group', and '>40 years group' according to the most studies. The results showed that women who smoked for less than 20 years (OR=1.06, p<0.001), 20-30 years (OR=1.15, p<0.001), 30-40 years (OR=1.15, p<0.001), and more than 40 years (OR=1.22, p<0.001) had a higher incidence of BC than those without smoking history. The incidence of BC was positively correlated with smoking duration. The detailed data was contained in Table 2.

Smoking intensity

Cigarettes per day were used to measure smoking intensity in this study. The data is grouped by 10 cigarettes per day, 20 cigarettes per day, and 30 cigarettes per day. Subgroup analysis showed smoking which less than 10 cigarettes per day (OR=1.06, p=0.001), between 10-20 cigarettes per day (OR=1.19, p<0.001), between 20-30 cigarettes per day (OR=1.16, p<0.001), and more than 30 cigarettes per day (OR=1.18, p=0.001) increased the incidence of BC with statistical significance. The incidence of BC increased with the increase of smoking intensity. The detailed data was contained in Table 2.

Pack-years smoked

Pack-years were used to simultaneously assess smoking duration and smoking intensity. Pack-years were defined as the product of the number of cigarettes smoked per day and the number of years of smoking. According to the grouping criteria of the included studies, this study divided the relevant data into '<10 pack-years group', '10-20 pack-years group', '20-40 pack-years group', and '>40 pack-years group'. The analysis showed that women who smoke with less than 10 pack-years (OR=1.05, p=0.005), 10-20 pack-years (OR=1.11, p<0.001), 20-40 pack-years (OR=1.21, p<0.001), and >40 pack-years (OR=1.17, p<0.001) had a higher incidence of BC than those who had never smoked. The detailed data was contained in Table 2.

Age started smoking

In this study, smoking initiation age was divided into '<16 years group', '17-19 years group', and '>20 years group'. The results suggested that active smoking, regardless of the age at which smoking started is younger than 16 years old (OR=1.11, 95% CI=1.07-1.15), between 17-19 years old (OR=1.06, 95% CI=1.12-1.20), or older than 20 years old (OR=1.08, 95% CI=1.04-1.11), would significantly increase the incidence of BC in women with slight heterogeneity. The detailed data was contained in Table 2.

Years since quitting

Years of quitting smoking were used to measure the effect of smoking cessation in the participants. Data were grouped by 10- and 20-year cessation years. Subgroup analysis showed that previous smoking history remained a risk factor for BC among women who had quit smoking for less than 20 years. Among them, the harm of previous smoking history to women who quit smoking for less than 10 years (OR=1.27, 95% CI=1.15-41, p<0.001) is significantly greater than that to those who quit smoking for 10-20 years (OR=1.05, 95% CI=1.00-1.09, p=0.046). With increased time to quit smoking comes a reduction in the harm caused by previous smoking history. Previous smoking history was no longer an observable risk factor for BC in women who had quit smoking for more than 20 years (OR=1.01, 95% CI=0.97-1.06, p=0.552). The detailed data was contained in Table 2.

Fertility status

Six studies explored the association between active smoking and BC in different fertility statuses. The analysis showed that active smoking can increase the risk of BC in the multiparous population (OR=1.13, 95% CI=1.07-1.20, p<0.001), but had no effect on BC in the nulliparous population (OR=1.05, 95%

CI=0.92-1.20, p=0.432) without heterogeneity. The detailed data was contained in Table 2.

Active smoking before/after the first birth

Regarding the relationship between active smoking and BC risk before/after the first birth, 24 studies contained data before the first birth and 22 studies contained data after the first birth. The results of the analysis showed that active smoking significantly increased the incidence of BC, regardless of whether the mother was smoking before the first birth (OR=1.22, 95% CI=1.17-1.27, p<0.001) or smoking after the first birth (OR=1.08, 95% CI=1.04-1.12, p<0.001), with slight heterogeneity. Furthermore, active smoking before the first birth had a greater impact on inducing BC than active smoking after the first birth. The detailed data was contained in Table 2.

Among those who actively smoked before the first birth, data were grouped by 5 years of smoking. Subgroup analysis showed that active smoking before the first birth increased the risk of BC whether the duration of smoking less than 5 years (OR=1.06, p=0.023) or more than 5 years (OR=1.24, p<0.001). There was a positive correlation between the smoking duration before the first birth and the risk of BC. Among those who have actively smoked after the first born, data were grouped by 10 years of smoking. Subgroup analysis showed that active smoking after the first birth had no effect on BC whether the duration of smoking less than 10 years (OR=1.00, p=0.922) or more than 10 years (OR=1.06, p=0.077). However, with the increase of smoking duration, active smoking had a tendency to harm the female population after the first birth by inducing BC. The detailed data was contained in Table 2.

BC subtypes

Six studies examined the association between active smoking and BC subtypes. The results showed that active smoking increased the incidence of ER+ BC (OR=1.13, 95% CI=1.08-1.18, p<0.001), but had no effect on ER- BC (OR=1.08, 95% CI=0.97-1.19, p=0.155), without heterogeneity. The detailed data was contained in Table 2.

BC subtypes and smoking duration

This study grouped data by 10-year active smoking aimed to investigate the correlation between different smoking duration and BC subtype. The analysis showed that active smoking for less than 10 years did not increase the incidence of BC, regardless of whether it was ER+ BC (OR=0.99, p=0.870) or ER- BC (OR=1.02, p=0.699). Active smoking for more than 10 years had no effect on ER- BC (OR=1.08, p=0.105), but could increase the incidence of ER+ BC (OR=1.14, p=0.007). The detailed data was contained in Table 2.

BC subtypes and smoking intensity

This study investigated the effect of smoking on BC subtypes at different smoking intensities by grouping data at 10 cigarettes per day boundaries. Subgroup analysis showed that smoking less than 10 cigarettes per day (OR=1.08, p=0.041) and more than 10 cigarettes per day (OR=1.18, p=0.002) could increase the risk of ER+ BC, and the risk was positively related to smoking intensity. For ER- BC, smoking less than 10 cigarettes per day had not been discovered as being effective (OR=0.97, p=0.603), However, smoking more than 10 cigarettes per day could increase the risk of suffering from ER- BC (OR=1.18, p=0.049). The results suggested that the occurrence of ER+ BC was more likely to be affected by active smoking than ER- BC. The detailed data was contained in Table 2.

Overall effect of passive smoking

Thirty-nine studies documented BC risk data from passive smoking in women. The analysis showed that the risk of BC was significantly higher among women who passively smoked than those without passive smoking episode (OR=1.17, 95% CI=1.09-1.24, p<0.001, I^2 = 59.2%). Among them, current passive smoking had a significant effect on BC (OR=1.31, 95% CI=1.08-1.60, p=0.007, I^2 = 27.6%), but such history had no effect on BC (OR=1.18, 95% CI=0.97-1.43, p=0.107, I^2 = 42.5%). This suggests that passive smoking, especially current passive smoking would increase the risk of BC. Furthermore, cohort studies (OR=1.08, 95% CI=1.03-1.13) and case-control studies (OR=1.15, 95% CI=1.14-1.39) had consistently concluded that passive smoking increases the risk of BC in women. The detailed data was shown in Table 3.

Menopausal status

Eleven studies included data on the relationship between passive smoking and BC in different menopausal states. The analysis showed that passive smoking increased the risk of premenopausal BC (OR=1.29, 95% CI=1.13-1.49, p<0.001, I^2 = 37.3%), but had no effect on the incidence of postmenopausal BC (OR=1.13, 95% CI=0.93-1.36, p=0.218, I^2 = 73.5%). The detailed data was contained in Table 3.

Places exposed to passive smoking

Regarding the relationship of passive smoking and BC in different exposure places, 11 studies had data on home exposure,

TABLE 3 Effects of passive smoking on breast cancer incidence.

Subgroup analysis	No. ofstudies	OR	95%CI	*p*	Heterogeneity (I^2) (%)
Ever passive smoking	39	1.17	1.09-1.24	*<0.001*	59.2
Current	4	1.31	1.08-1.60	*0.007*	27.6
Former	4	1.18	0.97-1.43	0.107	42.5
Cohort study	11	1.08	1.03-1.13	*0.002*	0
Case-control study	28	1.26	1.14-1.39	*<0.001*	66.5
Premenopausal BC	11	1.29	1.13-1.49	*<0.001*	37.3
Postmenopausal BC	11	1.13	0.93-1.36	0.218	73.5
Places exposed to passive smoking					
Home	11	1.07	0.95-1.21	0.269	63.2
Work	11	1.09	1.00-1.20	0.051	46.7
Home and work	5	1.40	1.00-1.97	0.051	88.3
Age stage exposure to passive smoking					
Childhood	16	1.15	1.05-1.25	*0.002*	63.7
Adult	15	1.21	1.04-1.40	*0.014*	79.1
Childhood and adult	8	1.49	1.15-1.93	*0.003*	72.2
Years passive smoked					
<10 years	15	0.99	0.89-1.10	0.876	8.4
10-20 years	19	1.13	1.03-1.25	*0.011*	41.2
20-30 years	17	1.38	1.18-1.61	*<0.001*	76.2
>30 years	9	1.35	1.10-1.65	*0.004*	74.4

OR, odd ratio; CI, confidence interval; BC, breast cancer.

11 studies had data on work exposure, and 5 studies had data on both home and work exposure. Subgroup analysis showed no relationship between passive smoking and BC incidence in different passive smoking exposure settings. However, passive smoking exposure at work (OR=1.09, p=0.051) and exposure at both home and work (OR=1.40, p=0.051) had a trend of harm to female population. The detailed data was contained in Table 3.

Age stage exposure to passive smoking

In terms of the association between passive smoking and BC at different exposure ages, 16 studies had data on exposure in childhood, 15 studies had data on exposure in adult, and 8 studies had data on exposure in children and adult. Subgroup analyses showed that passive smoking increased BC risk regardless of exposure to childhood (OR=1.15, p=0.002), adult (OR=1.21, p=0.014), or both childhood and adult (OR=1.49, p=0.003). Among them, the increased risk of BC in those with simultaneous exposure in childhood and adult was significantly greater than that in those only with a single age group. The detailed data was contained in Table 3.

Years passive smoked

Years were used to measure the duration of passive smoking exposure in this study. The relevant data were divided into 'less than 10 years group', '10-20 years group', '20-30 years group', and 'more than 30 years group', in the way most studies were segmented. This study showed that passive smoking which duration was less than 10 years in female population had no effect on BC (OR=0.99, p=0.876), while passive smoking exposure for 10-20 years (OR=1.13, p=0.011), 20-30 years (OR=1.38, p<0.001) and more than 30 years (OR=1.35, p=0.004) had a significant impact on the incidence of BC, compared to women who had never smoked. In all, increased incidence was positively correlated with longer duration of passive smoking exposure. The detailed data was contained in Table 3.

Study quality

The NOS checklist was adopted to objectively evaluate the quality of included observational studies in this meta-study. 95.83% of the cohort studies were of high quality (NOS score >7), while 94.33% case-control studies were of high quality (NOS score >7). The quality ratings of cohort and case-control studies were listed in Supplementary Tables 2 and 3.

Publication bias and sensitivity analysis

Publication bias was evaluated by the Begg's test. The results of Begg's test indicated the absence of publication bias among

included articles (p>0.05). Sensitivity analysis was used to assess whether the individual studies affected the overall results or not. The results indicated that the analysis was relatively stable.

Discussion

Through data analysis, this study found that smoking (active and passive) increases the risk of BC in women, with cohort and case-control studies showing consistent conclusions. Subgroup analysis of smoking-related factors showed that the effect of smoking on BC was positively correlated with smoking intensity and smoking duration. Among active smokers, current active smoking is more harmful to women than previous active smoking. With the increase of smoking cessation time, the harm of previous smoking history to the female population decreased. No differences were observed in the effect of smoking on BC at different starting ages. Among passive smokers, current passive smoking increases the incidence of BC, but past passive smoking does not. No differences in the effects of smoking on BC were observed between different passive smoking exposure sites and exposure age groups.

Subgroup analyses of population-related factors showed that smoking significantly increased the risk of BC in the multiparous population, but not in the nulliparous population. Smoking before the first birth has a greater effect on BC risk than smoking after the first birth. The risk of BC increases in women of different reproductive statuses with increasing duration of smoking.

Subgroup analysis of BC-related factors showed that smoking increases the risk of premenopausal BC, but has no effect on postmenopausal BC. At the same time, it can be clearly observed that smoking increases the risk of ER+ BC, and it is positively correlated with smoking duration and smoking intensity. For ER-BC, there was a trend of harm to women from smoking with increasing duration and intensity of smoking, but the difference did not reach statistical significance.

There is no consensus on the mechanism by which smoking increasing the risk of BC in women. The mainstream view is that smoking-specific DNA adducts (116, 117) (chemical carcinogens are activated by enzymes into electrophile and covalent combined with DNA, which are used to show DNA damage of specific carcinogens in human tissues (118)), mutations, and mal-regulated signaling pathways (119) represented by p53 [genes that inhibit cells from turning into cancer cells (75)] are the most important factors in BC (120). Animal and *in vitro* studies have shown that fat-soluble mutagenic compounds (121) in tobacco smoke, such as polycyclic hydrocarbons (122), aromatic amines (20) and N-nitrosamines (123), are major components of DNA adducts that can induce breast tumors (117) and have been detected in human milk (116). Compared with nonsmokers, detectable increases in cancer-causing DNA adducts were found in BC tissues and normal tissue adjacent to tumors in smokers (34, 124). In addition, studies have found that tobacco alters the incidence and spectrum of p53 mutations in breast cells, making smokers significantly more likely to carry p53 mutations (125). The potentially increased mutations affect related signaling pathways in smokers' breast cells, hinder damage DNA repair and apoptosis, cause the body to be unable to respond to oncogenic signals, and ultimately induce tumors (126). The longer the exposure and the greater the intensity, the greater the effect (127). The starting point for these mechanisms is the compounds in tobacco smoke, which are present both in the smoke inhaled by smokers (mainly active smokers) and in the smoke exhaled by smokers and the end of lit cigarettes (mainly passive smokers) (128). This supports the conclusion in this study that both active smoking and passive smoking can induce BC in women, and confirms the biological plausibility of the positive correlation between BC risk and smoking intensity and duration. In addition, smoking status was correlated with the levels of carcinogenic DNA adducts in normal tissues adjacent to tumors, with a significant linear trend in the levels of carcinogenic DNA adducts in never-smokers, former smokers, and current smokers (19). When tobacco exposure was stopped, cancer cells became less active and the mutant gene was partially restored (34, 129). This supports our findings that the risk of current smoking is greater for women than previous smoking, and that the risk of BC from previous smoking decreases as the duration of cessation increases.

A relatively new view is that the harmful effects of smoking on BC depend on the antagonism of the estrogen-like and anti-estrogen-like effects of tobacco. According to previous studies, the health of the female breast is affected by the level and proportion of estrogen and progesterone (130, 131). Long-term exposure to estrogen or increased cell response to estrogen is an important risk factor for BC development (132, 133). On the one hand, carcinogenic metal-like metals in tobacco (106, 134), such as cadmium, chromium and arsenic, can induce estrogen receptor activation through hormone-binding domains and play estrogen-like roles in cell culture and animals (134). On the other hand, polycyclic aromatic hydrocarbons substances in tobacco play an anti-estrogen-like effect by competing with estrogen receptors or inducing hormone metabolism to reduce the level of active estrogen in the body (135, 136). At present, researchers tend to believe that the estrogen-like effect of tobacco and its carcinogenic effect are far superior to the breast protective effect brought by the anti-estrogen effect (114, 137). The anti-estrogen effect may cause breast cells to increase the number of estrogen receptors and enhance the sensitivity to estrogen, thus leading to the occurrence of hormone-sensitive tumors (138). There is accumulating evidence that ER+ and lobular BCs are more sensitive to ovarian hormones than are ER- and ductal cancers (139, 140). This may explain why smoking increases the incidence of ER+ BC, and the risk is positively correlated with the duration and intensity of smoking,

but had no effect on ER- BC. In addition, premenopausal women have active gonadal function and secrete more estrogen (12, 129), which further aggravates the imbalance between estrogen and anti-estrogen effect on the basis of estrogen-like effect caused by tobacco, thus more likely to lead to the higher occurrence of BC (141). This supports the conclusion in this study that smoking increases the risk in premenopausal BC development, but not in postmenopausal BC development.

Based on the above two theories, tobacco exposure during the critical period is also considered to be an important factor affecting the occurrence of BC (100, 142). Animal models show that breast tissue is highly differentiated from puberty to the first full-term pregnancy, during which time the rapidly dividing cells are susceptible to malignant transformation due to carcinogens (143, 144). This period is therefore considered to be the period when tobacco smoke causes the greatest carcinogenic damage to breast tissue (145). During or after pregnancy, the second stage of BC carcinogenic damage is considered to be due to the onset of lactation, when breast cells are again active proliferation and vulnerable to tobacco smoke (146, 147). This may explain why smoking before the first birth had a greater impact on BC risk than smoking after the first birth. Unfortunately, no significant difference was observed in the subgroup analysis of the effect of smoking initiation on BC at different age in this study. In addition, increased exposure to estrogen (148), progesterone (149), and insulin-like growth factor (increased by growth hormone) (150) during pregnancy has been associated with promoting BC cell proliferation, which can trigger and/or promote tumors during continued tobacco exposure, known as "pregnancy-associated BC" (151–153). Epidemiological studies have found a higher incidence of BC in all multiparous women with, compared to all multiparous women regardless of their age (154–157). The higher incidence rate of BC in the multiparous population and the impact of tobacco exposure on estrogen levels in pregnant people may explain why smoking significantly increases the risk of BC in the multiparous population, but had no impact in the nulliparous population.

According to the above mechanisms and the characteristics of different included studies, we believe that the reasons for the differences between different studies may be as follows: First, each study has different assessment methods for exposure factors. Questionnaires and interviews both produce recall bias. The rigor of questionnaire design and the professionalism of interviewers will affect the validity of data collection, which makes researchers inevitably biased when exploring the relationship between smoking and BC; second, The duration of follow-up in the included studies varied considerably. The occurrence of BC often takes years to decades, and there is no exact number of years, but a longer follow-up period can often find more cases of BC, which can provide more abundant research data, conversely, a shorter follow-up period Time, not only limited the researchers' discovery of the association between smoking and BC, but also prevented subgroup analyses; third, different studies defined smoking differently. According to World Health Organization (WHO) regulations, people who smoke continuously or cumulatively for 6 months or more are smokers in some studies, some studies extend the duration to 1 year, and some studies define smokers as long as they smoke. Different criteria make the baseline status of the control population different, and although the concentration of carcinogens in tobacco is not high, it may still have an impact on the final results with long-term follow-up. Therefore, we believe that the results of the study can be improved by shortening the time between two follow-up visits, increasing the number of follow-up visits, and updating them in a timely manner. In addition, large-scale cohort studies are still a feasible way to verify the conclusions of this study and narrow the differences between different studies.

Reviewing the same type of studies, A-sol Kim et al.'s study (158) reached a similar conclusion to the present study that passive smoking increases the risk of BC in women (OR=1.23, 95%CI=1.10-1.38). However, they did not perform subgroup analysis on population and smoking factors, thus could not provide reference to the female population from multiple aspects. Moreover, they only included those who had never smoked, did not consider those who had previously smoked and had successfully gone through smoking cessation. These may have led to their findings being overestimated and lacking reliability. The study by Lisa A DeRoo et al (159) did not find any association between smoking and BC. This may be due to the limited number of studies they included, or it may be that the low concentration of carcinogens in tobacco with a long latency to harm the breast make the relationship between smoking and BC not easily observed.

While this meta-analysis yielded comprehensive and objective conclusions, there were still some potential limitations to consider. Firstly, the design, study population, sample size, risk assessment, and adjustment for related confounding factors varied among the included studies, which may bias the results and reduce the confidence of the conclusions. Therefore, this study used a random-effects model to evaluate the effect of smoking on BC. Secondly, most studies used questionnaires to assess smoking exposure, and a few used the form of interviews or a combination of interviews and questionnaires, therefore inevitably led to evaluation bias or recall bias during the evaluation, especially the case-control studies nested in the cohort, which may bias the findings. Therefore, this study selected relevant data adjusted for the largest number of potential confounders for statistical analysis to improve the accuracy of the conclusions. Thirdly, some trials did not report more adequate subgroup data, such as BC type subgroup data, fertility status subgroup data, etc., which made it very difficult to conduct some subgroup analyses in this study.

Apart from its limitations, this meta-analysis had its own strengths. Firstly, this study included a large number of observational studies including more than 2.3 million

participants in Asia, Europe, America, and Oceania. The larger observational population increases the reliability and authenticity of the conclusions of this study. Additionally, this study grouped the extracted data (by smoking related factors, population related factors, BC-related factors) and performed subgroup analysis to comprehensively explore the possibility of the effect of different kinds of smoking on different populations, different BC types from different aspects. Overall, this meta-analysis led to some meaningful conclusions that may provide a new reference for BC prevention in the female population.

Conclusion

This meta-analysis found that smoking (active and passive smoking) increases the risk of BC in the female population, especially premenopausal BC and ER+ BC, but had no effect on postmenopausal BC and ER- BC. The risk of BC was positively associated with the longer duration and stronger intensity of smoking, negatively associated with the duration of smoking cessation. Smoking increases BC risk in the multiparous population, but had no effect in the nulliparous population, where smoking before the first birth had a larger effect on BC risk than smoking after the first birth.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

All authors helped to perform the research. YH and XL writing manuscript; YH and YS performing procedures and data analysis; JH and CY contribution to writing the manuscript; NH contribution to drafting conception and design. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.961970/full#supplementary-material

References

1. Nagini S. Breast cancer: Current molecular therapeutic targets and new players. *Anticancer Agents Med Chem* (2017) 17(2):152–63. doi: 10.2174/1871520616666160502122724

2. Brignoni L, Cappetta M, Colistro V, Sans M, Artagaveytia N, Bonilla C, et al. Genomic diversity in sporadic breast cancer in a Latin American population. *Genes (Basel)* (2020) 11(11):1272. doi: 10.3390/genes11111272

3. Lee A, Moon BI, Kim TH. BRCA1/BRCA2 pathogenic variant breast cancer: Treatment and prevention strategies. *Ann Lab Med* (2020) 40(2):114–21. doi: 10.3343/alm.2020.40.2.114

4. Ambrosone CB, Hong CC, Goodwin PJ. Host factors and risk of breast cancer recurrence: Genetic, epigenetic and biologic factors and breast cancer outcomes. *Adv Exp Med Biol* (2015) 862:143–53. doi: 10.1007/978-3-319-16366-6_10

5. Kwong A, Shin VY, Ho JC, Kang E, Nakamura S, Teo SH, et al. Comprehensive spectrum of BRCA1 and BRCA2 deleterious mutations in breast cancer in Asian countries. *J Med Genet* (2016) 53(1):15–23. doi: 10.1136/jmedgenet-2015-103132

6. Rieder V, Salama M, Glockner L, Muhr D, Berger A, Tea MK, et al. Effect of lifestyle and reproductive factors on the onset of breast cancer in female BRCA 1 and 2 mutation carriers. *Mol Genet Genomic Med* (2016) 4(2):172–7. doi: 10.1002/mgg3.191

7. Zbuk K, Anand SS. Declining incidence of breast cancer after decreased use of hormone-replacement therapy: Magnitude and time lags in different countries. *J Epidemiol Community Health* (2012) 66(1):1–7. doi: 10.1136/jech.2008.083774

8. Park SY, Kolonel LN, Lim U, White KK, Henderson BE, Wilkens LR. Alcohol consumption and breast cancer risk among women from five ethnic groups with light to moderate intakes: The multiethnic cohort study. *Int J Cancer* (2014) 134 (6):1504–10. doi: 10.1002/ijc.28476

9. Castello A, Martin M, Ruiz A, Casas AM, Baena-Canada JM, Lope V, et al. Lower breast cancer risk among women following the world cancer research fund and American institute for cancer research lifestyle recommendations: EpiGEICAM case-control study. *PloS One* (2015) 10(5):e0126096. doi: 10.1371/journal.pone.0126096

10. Ferrini K, Ghelfi F, Mannucci R, Titta L. Lifestyle, nutrition and breast cancer: facts and presumptions for consideration. *Ecancermedicalscience* (2015) 9:557. doi: 10.3332/ecancer.2015.557

11. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, et al. Risk factors and preventions of breast cancer. *Int J Biol Sci* (2017) 13(11):1387–97. doi: 10.7150/ijbs.21635

12. Cordina-Duverger E, Koudou Y, Truong T, Arveux P, Kerbrat P, Menegaux F, et al. Night work and breast cancer risk defined by human epidermal growth factor receptor-2 (HER2) and hormone receptor status: A population-based case-control study in France. *Chronobiol Int* (2016) 33(6):783–7. doi: 10.3109/07420528.2016.1167709

13. Godinho-Mota JCM, Goncalves LV, Mota JF, Soares LR, Schincaglia RM, Martins KA, et al. Sedentary behavior and alcohol consumption increase breast cancer risk regardless of menopausal status: A case-control study. *Nutrients* (2019) 11(8):1871. doi: 10.3390/nu11081871

14. Lee J, Lee J, Lee DW, Kim HR, Kang MY. Sedentary work and breast cancer risk: A systematic review and meta-analysis. *J Occup Health* (2021) 63(1):e12239. doi: 10.1002/1348-9585.12239

15. Chlebowski RT, Aragaki AK, Anderson GL, Thomson CA, Manson JE, Simon MS, et al. Low-fat dietary pattern and breast cancer mortality in the women's health initiative randomized controlled trial. *J Clin Oncol* (2017) 35(25):2919–26. doi: 10.1200/JCO.2016.72.0326

16. Goncalves RM, Delgobo M, Agnes JP, Neves das RN, Falchetti M, Casagrande T, et al. COX-2 promotes mammary adipose tissue inflammation, local estrogen biosynthesis, and carcinogenesis in high-sugar/fat diet treated mice. *Cancer Lett* (2021) 502:44–57. doi: 10.1016/j.canlet.2021.01.003

17. Johnson KC, Miller AB, Collishaw NE, Palmer JR, Hammond SK, Salmon AG, et al. Active smoking and secondhand smoke increase breast cancer risk: the report of the Canadian expert panel on tobacco smoke and breast cancer risk (2009). *Tob Control* (2011) 20(1):e2. doi: 10.1136/tc.2010.035931

18. Reynolds P. Smoking and breast cancer. *J Mammary Gland Biol Neoplasia* (2013) 18(1):15–23. doi: 10.1007/s10911-012-9269-x

19. Faraglia B, Chen SY, Gammon MD, Zhang Y, Teitelbaum SL, Neugut AI, et al. Evaluation of 4-aminobiphenyl-DNA adducts in human breast cancer: The influence of tobacco smoke. *Carcinogenesis* (2003) 24(4):719–25. doi: 10.1093/carcin/bgg013

20. Sheikh IA, Beg MA, Yasir M. Molecular interactions of carcinogenic aromatic amines, 4-aminobiphenyl and 4,4'-diaminobiphenyl, with lactoperoxidase - insight to breast cancer. *Anticancer Res* (2017) 37(11):6245–9. doi: 10.21873/anticanres.12075

21. Amadou A, Praud D, Coudon T, Deygas F, Grassot L, Faure E, et al. Risk of breast cancer associated with long-term exposure to benzo[a]pyrene (BaP) air pollution: Evidence from the French E3N cohort study. *Environ Int* (2021) 149:106399. doi: 10.1016/j.envint.2021.106399

22. Sadikovic B, Rodenhiser DI. Benzopyrene exposure disrupts DNA methylation and growth dynamics in breast cancer cells. *Toxicol Appl Pharmacol* (2006) 216(3):458–68. doi: 10.1016/j.taap.2006.06.012

23. Catsburg C, Kirsh VA, Soskolne CL, Kreiger N, Rohan TE. Active cigarette smoking and the risk of breast cancer: A cohort study. *Cancer Epidemiol* (2014) 38(4):376–81. doi: 10.1016/j.canep.2014.05.007

24. Ghasemian A, Rezaei N, Saeedi Moghaddam S, Mansouri A, Parsaeian M, Delavari A, et al. Tobacco smoking status and the contribution to burden of diseases in Iran, 1990-2010: Findings from the global burden of disease study 2010. *Arch Iran Med* (2015) 18(8):493–501. doi: 10.015188/AIM.006

25. Carreras G, Lachi A, Boffi R, Clancy L, Gallus S, Fernandez E, et al. Burden of disease from breast cancer attributable to smoking and second-hand smoke exposure in Europe. *Int J Cancer* (2020) 147(9):2387–93. doi: 10.1002/ijc.33021

26. Malik A, Jeyaraj PA, Shankar A, Rath GK, Mukhopadhyay S, Kamal VK. Passive smoking and breast cancer - a suspicious link. *Asian Pac J Cancer Prev* (2015) 16(14):5715–9. doi: 10.7314/APJCP.2015.16.14.5715

27. Thomas RD, Vigerstad TJ. Use of laboratory animal models in investigating emphysema and cigarette smoking in humans. *Regul Toxicol Pharmacol* (1989) 10(3):264–71. doi: 10.1016/0273-2300(89)90053-6

28. Wehner AP, Dagle GE, Milliman EM, Phelps DW, Carr DB, Decker JR, et al. Inhalation bioassay of cigarette smoke in rats. *Toxicol Appl Pharmacol* (1981) 61(1):1–17. doi: 10.1016/0041-008X(81)90002-8

29. Hecht SS. Tobacco smoke carcinogens and breast cancer. *Environ Mol Mutagen* (2002) 39(2-3):119–26. doi: 10.1002/em.10071

30. Izano M, Satariano WA, Hiatt RA, Braithwaite D. Smoking and mortality after breast cancer diagnosis: The health and functioning in women study. *Cancer Med* (2015) 4(2):315–24. doi: 10.1002/cam4.359

31. Castillo-Sanchez R, Villegas-Comonfort S, Galindo-Hernandez O, Gomez R, Salazar EP. Benzo-[a]-pyrene induces FAK activation and cell migration in MDA-MB-231 breast cancer cells. *Cell Biol Toxicol* (2013) 29(4):303–19. doi: 10.1007/s10565-013-9254-1

32. Zeinomar N, Knight JA, Genkinger JM, Phillips KA, Daly MB, Milne RL, et al. Alcohol consumption, cigarette smoking, and familial breast cancer risk: findings from the prospective family study cohort (ProF-SC). *Breast Cancer Res* (2019) 21(1):128. doi: 10.1186/s13058-019-1213-1

33. Hanaoka T, Yamamoto S, Sobue T, Sasaki S, Tsugane SC Japan Public Health Center-Based Prospective Study on, et al. Active and passive smoking and breast cancer risk in middle-aged Japanese women. *Int J Cancer* (2005) 114(2):317–22. doi: 10.1002/ijc.20709

34. Luo J, Margolis KL, Wactawski-Wende J, Horn K, Messina C, Stefanick ML, et al. Association of active and passive smoking with risk of breast cancer among postmenopausal women: A prospective cohort study. *BMJ* (2011) 342:d1016. doi: 10.1136/bmj.d1016

35. Gaudet MM, Gapstur SM, Sun J, Diver WR, Hannan LM, Thun MJ. Active smoking and breast cancer risk: Original cohort data and meta-analysis. *J Natl Cancer Inst* (2013) 105(8):515–25. doi: 10.1093/jnci/djt023

36. Macacu A, Autier P, Boniol M, Boyle P. Active and passive smoking and risk of breast cancer: A meta-analysis. *Breast Cancer Res Treat* (2015) 154(2):213–24. doi: 10.1007/s10549-015-3628-4

37. Chen Z, Shao J, Gao X, Li X. Effect of passive smoking on female breast cancer in China: A meta-analysis. *Asia Pac J Public Health* (2015) 27(2):NP58–64. doi: 10.1177/1010539513481493

38. Yang Y, Zhang F, Skrip L, Wang Y, Liu S. Lack of an association between passive smoking and incidence of female breast cancer in non-smokers: Evidence from 10 prospective cohort studies. *PloS One* (2013) 8(10):e77029. doi: 10.1371/journal.pone.0077029

39. Burki TK. WHO releases latest report on the global tobacco epidemic. *Lancet Oncol* (2021) 22(9):1217. doi: 10.1016/S1470-2045(21)00464-2

40. Zhang K, Tartarone A, Perez-Rios M, Novello S, Mariniello A, Roviello G, et al. Smoking burden, MPOWER, future tobacco control and real-world challenges in China: Reflections on the WHO report on the global tobacco epidemic 2021. *Transl Lung Cancer Res* (2022) 11(1):117–21. doi: 10.21037/tlcr-22-27

41. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: A proposal for reporting. meta-analysis of observational studies in epidemiology (MOOSE) group. *JAMA* (2000) 283(15):2008–12. doi: 10.1001/jama.283.15.2008

42. Adami HO, Lund E, Bergstrom R, Meirik O. Cigarette smoking, alcohol consumption and risk of breast cancer in young women. *Br J Cancer* (1988) 58(6):832–7. doi: 10.1038/bjc.1988.320

43. Ahern TP, Lash TL, Egan KM, Baron JA. Lifetime tobacco smoke exposure and breast cancer incidence. *Cancer Causes Control* (2009) 20(10):1837–44. doi: 10.1007/s10552-009-9376-1

44. Alberg AJ, Daudt A, Huang HY, Hoffman SC, Comstock GW, Helzlsouer KJ, et al. N-acetyltransferase 2 (NAT2) genotypes, cigarette smoking, and the risk of breast cancer. *Cancer Detect Prev* (2004) 28(3):187–93. doi: 10.1016/j.cdp.2004.04.001

45. Alsolami FJ, Azzeh FS, Ghafouri KJ, Ghaith MM, Almaimani RA, Almasmoum HA, et al. Determinants of breast cancer in Saudi women from makkah region: A case-control study (breast cancer risk factors among Saudi women). *BMC Public Health* (2019) 19(1):1554. doi: 10.1186/s12889-019-7942-3

46. Baset Z, Abdul-Ghafar J, Parpio YN, Haidary AM. Risk factors of breast cancer among patients in a tertiary care hospitals in Afghanistan: A case control study. *BMC Cancer* (2021) 21(1):71. doi: 10.1186/s12885-021-07798-5

47. Bennicke K, Conrad C, Sabroe S, Sorensen HT. Cigarette smoking and breast cancer. *BMJ* (1995) 310(6992):1431–3. doi: 10.1136/bmj.310.6992.1431

48. Bonner MR, Nie J, Han D, Vena JE, Rogerson P, Muti P, et al. Secondhand smoke exposure in early life and the risk of breast cancer among never smokers (United states). *Cancer Causes Control* (2005) 16(6):683–9. doi: 10.1007/s10552-005-1906-x

49. Botteri E, Berstad P, Sandin S, Weiderpass E. Lifestyle changes and risk of cancer: experience from the Swedish women's lifestyle and health cohort study. *Acta Oncol* (2021) 60(7):827–34. doi: 10.1080/0284186X.2021.1919756

50. Braga C, Negri E, La Vecchia C, Filiberti R, Franceschi S. Cigarette smoking and the risk of breast cancer. *Eur J Cancer Prev* (1996) 5(3):159–64. doi: 10.1097/00008469-199606000-00003

51. Butler EN, Tse CK, Bell ME, Conway K, Olshan AF, Troester MA. Active smoking and risk of luminal and basal-like breast cancer subtypes in the Carolina breast cancer study. *Cancer Causes Control* (2016) 27(6):775–86. doi: 10.1007/s10552-016-0754-1

52. Calle EE, Miracle-McMahill HL, Thun MJ, Heath CW. Cigarette smoking and risk of fatal breast cancer. *Am J Epidemiol* (1994) 139(10):1001–7. doi: 10.1093/oxfordjournals.aje.a116939

53. Catsburg C, Miller AB, Rohan TE. Active cigarette smoking and risk of breast cancer. *Int J Cancer* (2015) 136(9):2204–9. doi: 10.1002/ijc.29266

54. Chu SY, Stroup NE, Wingo PA, Lee NC, Peterson HB, Gwinn ML. Cigarette smoking and the risk of breast cancer. *Am J Epidemiol* (1990) 131(2):244–53. doi: 10.1093/oxfordjournals.aje.a115494

55. Conlon MS, Johnson KC, Bewick MA, Lafrenie RM, Donner A. Smoking (active and passive), n-acetyltransferase 2, and risk of breast cancer. *Cancer Epidemiol* (2010) 34(2):142–9. doi: 10.1016/j.canep.2010.02.001

56. Connor AE, Baumgartner KB, Baumgartner RN, Pinkston CM, Boone SD, John EM, et al. Cigarette smoking and breast cancer risk in Hispanic and non-Hispanic white women: The breast cancer health disparities study. *J Womens Health (Larchmt)* (2016) 25(3):299–310. doi: 10.1089/jwh.2015.5502

57. De Silva M, Senarath U, Gunatilake M, Lokuhetty D. Prolonged breastfeeding reduces risk of breast cancer in Sri Lankan women: A case-control study. *Cancer Epidemiol* (2010) 34(3):267–73. doi: 10.1016/j.canep.2010.02.012

58. Dianatinasab M, Fararouei M, Mohammadianpanah M, Zare-Bandamiri M, Rezaianzadeh A. Hair coloring, stress, and smoking increase the risk of breast cancer: A case-control study. *Clin Breast Cancer* (2017) 17(8):650–9. doi: 10.1016/j.clbc.2017.04.012

59. Dossus L, Boutron-Ruault MC, Kaaks R, Gram IT, Vilier A, Fervers B, et al. Active and passive cigarette smoking and breast cancer risk: Results from the EPIC cohort. *Int J Cancer* (2014) 134(8):1871–88. doi: 10.1002/ijc.28508

60. Ellingjord-Dale M, Vos L, Hjerkind KV, Hjartaker A, Russnes HG, Tretli S, et al. Alcohol, physical activity, smoking, and breast cancer subtypes in a Large, nested case-control study from the Norwegian breast cancer screening program. *Cancer Epidemiol Biomarkers Prev* (2017) 26(12):1736–44. doi: 10.1158/1055-9965.EPI-17-0611

61. Field NA, Baptiste MS, Nasca PC, Metzger BB. Cigarette smoking and breast cancer. *Int J Epidemiol* (1992) 21(5):842–8. doi: 10.1093/ije/21.5.842

62. Gammon MD, Eng SM, Teitelbaum SL, Britton JA, Kabat GC, Hatch M, et al. Environmental tobacco smoke and breast cancer incidence. *Environ Res* (2004) 96(2):176–85. doi: 10.1016/j.envres.2003.08.009

63. Gao CM, Ding JH, Li SP, Liu YT, Qian Y, Chang J, et al. Active and passive smoking, and alcohol drinking and breast cancer risk in chinese women. *Asian Pac J Cancer Prev* (2013) 14(2):993–6. doi: 10.7314/apjcp.2013.14.2.993

64. Goodman MT, Cologne JB, Moriwaki H, Vaeth M, Mabuchi K. Risk factors for primary breast cancer in Japan: 8-year follow-up of atomic bomb survivors. *Prev Med* (1997) 26(1):144–53. doi: 10.1006/pmed.1996.9979

65. Gram IT, Park SY, Maskarinec G, Wilkens LR, Haiman CA, Marchand Le L. Smoking and breast cancer risk by race/ethnicity and oestrogen and progesterone receptor status: The multiethnic cohort (MEC) study. *Int J Epidemiol* (2019) 48(2):501–11. doi: 10.1093/ije/dyy290

66. Gram IT, Wiik AB, Lund E, Licaj I, Braaten T. Never-smokers and the fraction of breast cancer attributable to second-hand smoke from parents during childhood: The Norwegian women and cancer study 1991–2018. *Int J Epidemiol* (2021) 50(6):1927–35. doi: 10.1093/ije/dyab153

67. Ha M, Mabuchi K, Sigurdson AJ, Freedman DM, Linet MS, Doody MM, et al. Smoking cigarettes before first childbirth and risk of breast cancer. *Am J Epidemiol* (2007) 166(1):55–61. doi: 10.1093/aje/kwm045

68. . !!! INVALID CITATION!!!.

69. Hara A, Taira N, Mizoo T, et al. N-acetyltransferase 2 polymorphism and breast cancer risk with smoking: A case control study in Japanese women. *Breast Cancer* (2017) 24(2):254–62. doi: 10.1007/s12282-016-0696-1

70. Heberg J, Simonsen MK, Danielsen AK, Klausen TW, Zoffmann V, Thomsen T. Joint tobacco smoking and alcohol intake exacerbates cancer risk in women- the Danish nurse cohort. *Eur J Oncol Nurs* (2019) 43:101675. doi: 10.1016/j.ejon.2019.101675

71. Hirose K, Tajima K, Hamajima N, Inoue M, Takezaki T, Kuroishi T, et al. A large-scale, hospital-based case-control study of risk factors of breast cancer according to menopausal status. *Jpn J Cancer Res* (1995) 86(2):146–54. doi: 10.1111/j.1349-7006.1995.tb03032.x

72. Hu M, Han D, Sun S, Yan Y, Zhang J, Zhou Y. Bleomycin-induced mutagen sensitivity, passive smoking, and risk of breast cancer in Chinese women: A case-control study. *Cancer Causes Control* (2013) 24(4):629–36. doi: 10.1007/s10552-012-0137-1

73. Ilic M, Vlajinac H, Marinkovic J. Cigarette smoking and breast cancer: A case-control study in Serbia. *Asian Pac J Cancer Prev* (2014) 14(11):6643–7. doi: 10.7314/apjcp.2013.14.11.6643

74. Johnson KC, Hu J, Mao Y, et al. Passive and active smoking and breast cancer risk in Canada, 1994-97. *Cancer Causes Control* (2000) 11(3):211–21. doi: 10.1023/A:1008906105790

75. Jones ME, Schoemaker MJ, Wright LB, Ashworth A, Swerdlow AJ. Smoking and risk of breast cancer in the generations study cohort. *Breast Cancer Res* (2017) 19(1):118. doi: 10.1186/s13058-017-0908-4

76. Kato I, Miura S, Kasumi F, Iwase T, Tashiro H, Fujita Y, et al. A case-control study of breast cancer among Japanese women: With special reference to family history and reproductive and dietary factors. *Breast Cancer Res Treat* (1992) 24(1):51–9. doi: 10.1007/BF01832358

77. Kawai M, Malone KE, Tang MT, Li CI. Active smoking and the risk of estrogen receptor-positive and triple-negative breast cancer among women ages 20 to 44 years. *Cancer* (2014) 120(7):1026–34. doi: 10.1002/cncr.28402

78. Kropp S, Chang-Claude J. Active and passive smoking and risk of breast cancer by age 50 years among German women. *Am J Epidemiol* (2002) 156(7):616–26. doi: 10.1093/aje/kwf093

79. Lash TL, Aschengrau A. A null association between active or passive cigarette smoking and breast cancer risk. *Breast Cancer Res Treat* (2002) 75(2):181–4. doi: 10.1023/A:1019625102365

80. Li B, Wang L, Lu MS, et al. Passive smoking and breast cancer risk among non-smoking women: A case-control study in China. *PloS One* (2015) 10(4):e0125894. doi: 10.1371/journal.pone.0125894

81. Lin Y, Kikuchi S, Tamakoshi K, Wakai K, Kondo T, Niwa Y, et al. Active smoking, passive smoking, and breast cancer risk: findings from the Japan collaborative cohort study for evaluation of cancer risk. *J Epidemiol* (2008) 18(2):77–83. doi: 10.2188/jea.18.77

82. Liu L, Wu K, Lin X, Yin W, Zheng X, Tang X, et al. Passive smoking and other factors at different periods of life and breast cancer risk in Chinese women who have never smoked - a case-control study in chongqing, people's republic of China. *Asian Pac J Cancer Prev* (2000) 1(2):131–7.

83. Manjer J, Johansson R, Lenner P. Smoking is associated with postmenopausal breast cancer in women with high levels of estrogens. *Int J Cancer* (2004) 112(2):324–8. doi: 10.1002/ijc.20409

84. Marcus PM, Newman B, Millikan RC, et al. The associations of adolescent cigarette smoking, alcoholic beverage consumption, environmental tobacco smoke, and ionizing radiation with subsequent breast cancer risk (United states). *Cancer Causes Control* (2000) 11(3):271–8. doi: 10.1023/A:1008911902994

85. McKenzie F, Ellison-Loschmann L, Jeffreys M, Firestone R, Pearce N, Romieu I. Cigarette smoking and risk of breast cancer in a new Zealand multi-ethnic case-control study. *PloS One* (2013) 8(4):e63132. doi: 10.1371/journal.pone.0063132

86. Mechanic LE, Millikan RC, Player J, de Cotret AR, Winkel S, Worley K, et al. Polymorphisms in nucleotide excision repair genes, smoking and breast cancer in African americans and whites: A population-based case-control study. *Carcinogenesis* (2006) 27(7):1377–85. doi: 10.1093/carcin/bgi330

87. Metsola K, Kataja V, Sillanpaa P, Siivola P, Heikinheimo L, Eskelinen M, et al. XRCC1 and XPD genetic polymorphisms, smoking and breast cancer risk in a Finnish case-control study. *Breast Cancer Res* (2005) 7(6):R987–97. doi: 10.1186/bcr1333

88. Morabia A, Bernstein M, Ruiz J, Heritier S, Berger Diebold S, Borisch B. Relation of smoking to breast cancer by estrogen receptor status. *Int J Cancer* (1998) 75(3):339–42. doi: 10.1002/(SICI)1097-0215(19980130)75:3<339::AID-IJC2>3.0.CO;2-3

89. Nishino Y, Tsubono Y, Tsuji I, Komatsu S, Kanemura S, Nakatsuka H, et al. Passive smoking at home and cancer risk: A population-based prospective study in Japanese nonsmoking women. *Cancer Causes Control* (2001) 12(9):797–802. doi: 10.1023/A:1012273806199

90. Olson JE, Vachon CM, Vierkant RA, Sweeney C, Limburg PJ, Cerhan JR, et al. Prepregnancy exposure to cigarette smoking and subsequent risk of postmenopausal breast cancer. *Mayo Clin Proc* (2005) 80(11):1423–8. doi: 10.4065/80.11.1423

91. Park SY, Palmer JR, Rosenberg L, Haiman CA, Bandera EV, Bethea TN, et al. A case-control analysis of smoking and breast cancer in African American women: findings from the AMBER consortium. *Carcinogenesis* (2016) 37(6):607–15. doi: 10.1093/carcin/bgw040

92. Pawlega J. Breast cancer and smoking, vodka drinking and dietary habits. a case-control study. *Acta Oncol* (1992) 31(4):387–92. doi: 10.3109/02841869209088276

93. Pimhanam C, Sangrajrang S, Ekpanyaskul C. Tobacco smoke exposure and breast cancer risk in Thai urban females. *Asian Pac J Cancer Prev* (2014) 15(17):7407–11. doi: 10.7314/APJCP.2014.15.17.7407

94. Pirie K, Beral V, Peto R, et al. Passive smoking and breast cancer in never smokers: Prospective study and meta-analysis. *Int J Epidemiol* (2008) 37(5):1069–79. doi: 10.1093/ije/dyn110

95. Ranstam J, Olsson H. Alcohol, cigarette smoking, and the risk of breast cancer. *Cancer Detect Prev* (1995) 19(6):487–93.

96. Regev-Avraham Z, Baron-Epel O, Hammond SK, et al. Passive smoking, NAT2 polymorphism, and breast cancer risk in Israeli Arab women: A case-control study. *Breast Cancer* (2018) 25(2):176–84. doi: 10.1007/s12282-017-0809-5

97. Reynolds P, Goldberg D, Hurley S, Nelson DO, Largent J, Henderson KD, et al. Passive smoking and risk of breast cancer in the California teachers study. *Cancer Epidemiol Biomarkers Prev* (2009) 18(12):3389–98. doi: 10.1158/1055-9965.EPI-09-0936

98. Roddam AW, Pirie K, Pike MC, Chilvers C, Crossley B, Hermon C, et al. Active and passive smoking and the risk of breast cancer in women aged 36-45 years: A population based case-control study in the UK. *Br J Cancer* (2007) 97 (3):434–9. doi: 10.1038/sj.bjc.6603859

99. Rollison DE, Brownson RC, Hathcock HL, Newschaffer CJ. Case-control study of tobacco smoke exposure and breast cancer risk in Delaware. *BMC Cancer* (2008) 8:157. doi: 10.1186/1471-2407-8-157

100. Rosenberg L, Boggs DA, Bethea TN, Wise LA, Adams-Campbell LL, Palmer JR. A prospective study of smoking and breast cancer risk among African-American women. *Cancer Causes Control* (2013) 24(12):2207–15. doi: 10.1007/s10552-013-0298-6

101. Schechter MT, Miller AB, Howe GR, Baines CJ, Craib KJ, Wall C. Cigarette smoking and breast cancer: case-control studies of prevalent and incident cancer in the Canadian national breast screening study. *Am J Epidemiol* (1989) 130(2):213–20. doi: 10.1093/oxfordjournals.aje.a115327

102. Sezer H, Yilmaz M, Gurler H, Koyuncu A. Breast cancer risk factors in Turkey: a hospital-based case-control study. *Asian Pac J Cancer Prev* (2011) 12 (9):2317–22.

103. Shrubsole MJ, Gao YT, Dai Q, Shu XO, Ruan ZX, Jin F, et al. Passive smoking and breast cancer risk among non-smoking Chinese women. *Int J Cancer* (2004) 110(4):605–9. doi: 10.1002/ijc.20168

104. Slattery ML, Curtin K, Giuliano AR, Sweeney C, Baumgartner R, Edwards S, et al. Active and passive smoking, IL6, ESR1, and breast cancer risk. *Breast Cancer Res Treat* (2008) 109(1):101–11. doi: 10.1007/s10549-007-9629-1

105. Smith SJ, Deacon JM, Chilvers CE. Alcohol, smoking, passive smoking and caffeine in relation to breast cancer risk in young women. UK national case-control study group. *Br J Cancer* (1994) 70(1):112–9. doi: 10.1038/bjc.1994.258

106. Strumylaite L, Kregzdyte R, Poskiene L, et al. Association between lifetime exposure to passive smoking and risk of breast cancer subtypes defined by hormone receptor status among non-smoking Caucasian women. *PloS One* (2017) 12(2): e0171198. doi: 10.1371/journal.pone.0171198

107. Tong JH, Li Z, Shi J, Li HM, Wang Y, Fu LY, et al. Passive smoking exposure from partners as a risk factor for ER+/PR+ double positive breast cancer in never-smoking Chinese urban women: A hospital-based matched case control study. *PloS One* (2014) 9(5):e97498. doi: 10.1371/journal.pone.0097498

108. Tung HT, Tsukuma H, Tanaka H, Kinoshita N, Koyama Y, Ajiki W, et al. Risk factors for breast cancer in Japan, with special attention to anthropometric measurements and reproductive history. *Jpn J Clin Oncol* (1999) 29(3):137–46. doi: 10.1093/jjco/29.3.137

109. Ueji M, Ueno E, Hyiaman DO, Saito T, Takahashi H, Kano K. Risk factors for breast cancer among Japanese women: A case-control study in ibaraki, Japan. *Breast Cancer* (1998) 5(4):351–8. doi: 10.1007/BF02967431

110. van den Brandt PA. A possible dual effect of cigarette smoking on the risk of postmenopausal breast cancer. *Eur J Epidemiol* (2017) 32(8):683–90. doi: 10.1007/s10654-017-0282-7

111. Vatten LJ, Kvinnsland S. Cigarette smoking and risk of breast cancer: A prospective study of 24,329 Norwegian women. *Eur J Cancer* (1990) 26(7):830–3. doi: 10.1016/0277-5379(90)90164-O

112. Wada K, Kawachi T, Hori A, et al. Husband's smoking status and breast cancer risk in Japan: From the takayama study. *Cancer Sci* (2015) 106(4):455–60. doi: 10.1111/cas.12619

113. White AJ, D'Aloisio AA, Nichols HB, DeRoo LA, Sandler DP. Breast cancer and exposure to tobacco smoke during potential windows of susceptibility. *Cancer Causes Control* (2017) 28(7):667–75. doi: 10.1007/s10552-017-0903-1

114. Xue F, Willett WC, Rosner BA, Hankinson SE, Michels KB. Cigarette smoking and the incidence of breast cancer. *Arch Intern Med* (2011) 171(2):125–33. doi: 10.1001/archinternmed.2010.503

115. Young E, Leatherdale S, Sloan M, Kreiger N, Barisic A. Age of smoking initiation and risk of breast cancer in a sample of Ontario women. *Tob Induc Dis* (2009) 5(1):4. doi: 10.1186/1617-9625-5-4

116. Lissowska J, Brinton LA, Zatonski W, Blair A, Bardin-Mikolajczak A, Peplonska B, et al. Tobacco smoking, NAT2 acetylation genotype and breast cancer risk. *Int J Cancer* (2006) 119(8):1961–9. doi: 10.1002/ijc.22044

117. Terry PD, Rohan TE. Cigarette smoking and the risk of breast cancer in women: A review of the literature. *Cancer Epidemiol Biomarkers Prev* (2002) 11(10 Pt 1):953–71.

118. Hwa Yun B, Guo J, Bellamri M, et al. DNA Adducts: Formation, biological effects, and new biospecimens for mass spectrometric measurements in humans. *Mass Spectrom Rev* (2020) 39(1-2):55–82. doi: 10.1002/mas.21570

119. Ko KP, Kim SJ, Huzarski T, Gronwald J, Lubinski J, Lynch HT, et al. The association between smoking and cancer incidence in BRCA1 and BRCA2 mutation carriers. *Int J Cancer* (2018) 142(11):2263–72. doi: 10.1002/ijc.31257

120. Li D, Zhang W, Sahin AA, Hittelman WN. DNA Adducts in normal tissue adjacent to breast cancer: A review. *Cancer Detect Prev* (1999) 23(6):454–62. doi: 10.1046/j.1525-1500.1999.99059.x

121. Petrakis NL. Nipple aspirate fluid in epidemiologic studies of breast disease. *Epidemiol Rev* (1993) 15(1):188–95. doi: 10.1093/oxfordjournals.epirev.a036104

122. Pedersen JE, Strandberg-Larsen K, Andersson M, et al. Breast cancer among Danish women occupationally exposed to diesel exhaust and polycyclic aromatic hydrocarbons, 1964-2016. *Scand J Work Environ Health* (2021) 47 (2):154–62. doi: 10.5271/sjweh.3923

123. Risch HA. Etiology of pancreatic cancer, with a hypothesis concerning the role of n-nitroso compounds and excess gastric acidity. *J Natl Cancer Inst* (2003) 95 (13):948–60. doi: 10.1093/jnci/95.13.948

124. Perera FP, Estabrook A, Hewer A, et al. Carcinogen-DNA adducts in human breast tissue. *Cancer Epidemiol Biomarkers Prev* (1995) 4(3):233–8.

125. Conway K, Edmiston SN, Cui L, Drouin SS, Pang J, He M, et al. Prevalence and spectrum of p53 mutations associated with smoking in breast cancer. *Cancer Res* (2002) 62(7):1987–95.

126. Pilley S, Rodriguez TA, Vousden KH. Mutant p53 in cell-cell interactions. *Genes Dev* (2021) 35(7-8):433–48. doi: 10.1101/gad.347542.120

127. Firozi PF, Bondy ML, Sahin AA, et al. Aromatic DNA adducts and polymorphisms of CYP1A1, NAT2, and GSTM1 in breast cancer. *Carcinogenesis* (2002) 23(2):301–6. doi: 10.1093/carcin/23.2.301

128. Hoffmann D, Hoffmann I, El-Bayoumy K. The less harmful cigarette: a controversial issue. A tribute to Ernst l. wynder. *Chem Res Toxicol* (2001) 14 (7):767–90. doi: 10.1021/tx000260u

129. Daly AA, Rolph R, Cutress RI, et al. A review of modifiable risk factors in young women for the prevention of breast cancer. *Breast Cancer (Dove Med Press)* (2021) 13:241–57. doi: 10.2147/BCTT.S268401

130. Mascarenhas M, Kamath MS, Chandy A, Kunjummen AT. Progesterone/Estradiol ratio as a predictor in the ART cycles with premature progesterone elevation on the day of hCG trigger. *J Reprod Infertil* (2015) 16(3):155–61.

131. Shalom-Paz E, Aslih N, Samara N, Michaeli M, Ellenbogen A. Late follicular progesterone to estradiol ratio is not influenced by protocols or gonadotropins used. *Reprod Biol Endocrinol* (2015) 13:119. doi: 10.1186/s12958-015-0116-y

132. Anisimov VN, Alimova IN, Baturin DA, Popovich IG, Zabezhinski MA, Manton KG, et al. The effect of melatonin treatment regimen on mammary adenocarcinoma development in HER-2/neu transgenic mice. *Int J Cancer* (2003) 103(3):300–5. doi: 10.1002/ijc.10827

133. Girgert R, Bartsch C, Hill SM, Kreienberg R, Hanf V. Tracking the elusive antiestrogenic effect of melatonin: A new methodological approach. *Neuro Endocrinol Lett* (2003) 24(6):440–4.

134. Martin MB, Reiter R, Johnson M, Shah MS, Iann MC, Singh B, et al. Effects of tobacco smoke condensate on estrogen receptor-alpha gene expression and activity. *Endocrinology* (2007) 148(10):4676–86. doi: 10.1210/en.2007-0208

135. Letters: PERT: A tool for nurse administrators. *J Nurs Adm* (1975) 5(2):4–5.

136. Baron JA, La Vecchia C, Levi F. The antiestrogenic effect of cigarette smoking in women. *Am J Obstet Gynecol* (1990) 162(2):502–14. doi: 10.1016/0002-9378(90)90420-C

137. Gram IT, Braaten T, Terry PD, et al. Breast cancer risk among women who start smoking as teenagers. *Cancer Epidemiol Biomarkers Prev* (2005) 14(1):61–6. doi: 10.1158/1055-9965.61.14.1

138. Chen C, Wang X, Wang L, Yang F, Tang G, Xing H, et al. Effect of environmental tobacco smoke on levels of urinary hormone markers. *Environ Health Perspect* (2005) 113(4):412–7. doi: 10.1289/ehp.7436

139. Beral V, Reeves G, Bull D, Green JC Million Women Study. Breast cancer risk in relation to the interval between menopause and starting hormone therapy. *J Natl Cancer Inst* (2011) 103(4):296–305. doi: 10.1093/jnci/djq527

140. Reeves GK, Beral V, Green J, Gathani T, Bull DC Million Women Study. Hormonal therapy for menopause and breast-cancer risk by histological type: A cohort study and meta-analysis. *Lancet Oncol* (2006) 7(11):910–8. doi: 10.1016/S1470-2045(06)70911-1

141. Pesch B, Harth V, Rabstein S, Baisch C, Schiffermann M, Pallapies D, et al. Night work and breast cancer - results from the German GENICA study. *Scand J Work Environ Health* (2010) 36(2):134–41. doi: 10.5271/sjweh.2890

142. Humans and IWGotEoCRt. Tobacco smoke and involuntary smoking. *IARC Monogr Eval Carcinog Risks Hum* (2004) 83:1–1438. doi: 10.5271/sjweh.2890

143. Althuis MD, Fergenbaum JH, Garcia-Closas M, et al. Etiology of hormone receptor-defined breast cancer: A systematic review of the literature. *Cancer Epidemiol Biomarkers Prev* (2004) 13(10):1558–68. doi: 10.1158/1055-9965.1558.13.10

144. Habel LA, Stanford JL. Hormone receptors and breast cancer. *Epidemiol Rev* (1993) 15(1):209–19. doi: 10.1093/oxfordjournals.epirev.a036107

145. Morabia A. Smoking (active and passive) and breast cancer: Epidemiologic evidence up to June 2001. *Environ Mol Mutagen* (2002) 39(2-3):89–95. doi: 10.1002/em.10046

146. Palmer JR, Rosenberg L, Clarke EA, et al. Breast cancer and cigarette smoking: A hypothesis. *Am J Epidemiol* (1991) 134(1):1–13. doi: 10.1093/oxfordjournals.aje.a115984

147. Tredaniel J, Boffetta P, Little J, Saracci R, Hirsch A. Exposure to passive smoking during pregnancy and childhood, and cancer risk: The epidemiological evidence. *Paediatr Perinat Epidemiol* (1994) 8(3):233–55. doi: 10.1111/j.1365-3016.1994.tb00455.x

148. Schedin P. Pregnancy-associated breast cancer and metastasis. *Nat Rev Cancer* (2006) 6(4):281–91. doi: 10.1038/nrc1839

149. Pike MC, Krailo MD, Henderson BE, et al. 'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer. *Nature* (1983) 303(5920):767–70. doi: 10.1038/303767a0

150. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the women's health initiative randomized controlled trial. *JAMA* (2002) 288(3):321–33. doi: 10.1001/jama.288.3.321

151. Lyons TR, Schedin PJ, Borges VF. Pregnancy and breast cancer: When they collide. *J Mammary Gland Biol Neoplasia* (2009) 14(2):87–98. doi: 10.1007/s10911-009-9119-7

152. Schedin P, O'Brien J, Rudolph M, et al. Microenvironment of the involuting mammary gland mediates mammary cancer progression. *J Mammary Gland Biol Neoplasia* (2007) 12(1):71–82. doi: 10.1007/s10911-007-9039-3

153. Tiede B, Kang Y. From milk to malignancy: The role of mammary stem cells in development, pregnancy and breast cancer. *Cell Res* (2011) 21(2):245–57. doi: 10.1038/cr.2011.11

154. Albrektsen G, Heuch I, Hansen S, et al. Breast cancer risk by age at birth, time since birth and time intervals between births: Exploring interaction effects. *Br J Cancer* (2005) 92(1):167–75. doi: 10.1038/sj.bjc.6602302

155. Chie WC, Hsieh C, Newcomb PA, Longnecker MP, Mittendorf R, Greenberg ER, et al. Age at any full-term pregnancy and breast cancer risk. *Am J Epidemiol* (2000) 151(7):715–22. doi: 10.1093/oxfordjournals.aje.a010266

156. Lambe M, Hsieh C, Trichopoulos D, Ekbom A, Pavia M, Adami HO. Transient increase in the risk of breast cancer after giving birth. *N Engl J Med* (1994) 331(1):5–9. doi: 10.1056/NEJM199407073310102

157. Liu Q, Wuu J, Lambe M, Hsieh SF, Ekbom A, Hsieh CC. Transient increase in breast cancer risk after giving birth: Postpartum period with the highest risk (Sweden). *Cancer Causes Control* (2002) 13(4):299–305. doi: 10.1023/A:1015287208222

158. Kim AS, Ko HJ, Kwon JH, Lee JM. Exposure to secondhand smoke and risk of cancer in never smokers: A meta-analysis of epidemiologic studies. *Int J Environ Res Public Health* (2018) 15(9):1981. doi: 10.3390/ijerph15091981

159. DeRoo LA, Cummings P, Mueller BA. Smoking before the first pregnancy and the risk of breast cancer: A meta-analysis. *Am J Epidemiol* (2011) 174(4):390–402. doi: 10.1093/aje/kwr090

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 23 September 2022
DOI 10.3389/fonc.2022.998964

OPEN ACCESS

EDITED BY
Maria Rosaria De Miglio,
University of Sassari, Italy

REVIEWED BY
Yusuke Yoshioka,
Tokyo Medical University, Japan
Antonio Giordano,
Temple University, United States

*CORRESPONDENCE
Yueyuan Zhou
yueyuanzhou16@hotmail.com

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 20 July 2022
ACCEPTED 24 August 2022
PUBLISHED 23 September 2022

CITATION
Zhou Y, Xiao Z and Zhu W (2022) The roles of small extracellular vesicles as prognostic biomarkers and treatment approaches in triple-negative breast cancer. *Front. Oncol.* 12:998964. doi: 10.3389/fonc.2022.998964

The roles of small extracellular vesicles as prognostic biomarkers and treatment approaches in triple-negative breast cancer

Yueyuan Zhou[1,2]*, Zhongdang Xiao[2] and Wei Zhu[3]

[1]Department of Clinical Medical Engineering, First Affiliated Hospital of Nanjing Medical University, Nanjing, China, [2]State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing, China, [3]Department of Oncology, First Affiliated Hospital of Nanjing Medical University, Nanjing, China

Triple-negative breast cancer (TNBC) is a particularly aggressive and invasive breast cancer subtype and is associated with poor clinical outcomes. Treatment approaches for TNBC remain limited partly due to the lack of expression of well-known molecular targets. Small extracellular vesicles (sEVs) carrying a variety of bioactive contents play an important role in intercellular communications. The biomolecules including nucleic acids, proteins, and metabolites can be transferred locally or systematically to recipient cells and regulate their biological states and are involved in physiological and pathological processes. Recently, despite the extensive attraction to the physiological functions of sEVs, few studies focus on the roles of sEVs in TNBC. In this review, we will summarize the involvement of sEVs in the tumor microenvironment of TNBC. Moreover, we will discuss the potential roles of sEVs as diagnostic markers and treatment therapy in this heterogeneous breast cancer subtype. We finally summarize the clinical application of sEVs in TNBC.

KEYWORDS

small extracellular vesicles, exosomes, triple-negative breast cancer, prognosis, therapeutics, tumor microenvironment

Introduction

Breast cancer has been globally the most frequent cancer affecting women. Triple-negative breast cancer (TNBC) accounts for approximately 15%–20% of all breast cancer cases and generally demonstrates more aggressive biology with higher grades, more advanced stages at diagnosis, and poorer long-term clinical outcomes compared to other

breast cancer subtypes (1–3). It is defined by the absence of expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2-receptor (HER2), which are molecular markers to guide treatment and predict prognosis (4–6). Hence, TNBC does not respond to endocrine therapy or other available targeted drugs. Traditional therapeutic approaches such as surgery and systemic chemotherapy are still the first-line treatment for TNBC. However, recurrence and metastases frequently occur in the first 3 years, and the 5-year survival rate is lower than that of other subtypes (7). Therefore, it is urgent to understand the biological profiles of TNBC to develop novel effective therapeutic strategies.

Extracellular vesicles (EVs) can be secreted by nearly all cell types and are found in all biological fluids, including blood, urine, saliva, tears, breast milk, cerebrospinal fluid, amniotic fluid, seminal fluid, and lymphatic fluid (8, 9). They encompass various bioactive molecules such as nucleic acids (mRNA, miRNA, DNA, etc.), lipids, proteins, and even pharmacological compounds (10, 11). Based on particular biogenesis pathways, EVs are classified into three subgroups: endosome-origin exosomes, plasma membrane-derived microvesicles (MVs), and apoptotic bodies (12). Exosomes are secreted and released into the extracellular milieu after the multivesicular body (MVB) fuses with the plasma membrane and released the intraluminal vesicles inside (13–15). MVs are shed from the outward protrusion of the plasma membrane, and apoptotic bodies are released *via* blebbing of the plasma membrane during the late stages of cell death (16–18). Although exosomes are endowed with exquisite activities, they are still lacking experimental support, and there is no consensus on specific markers of EV subpopulations. It was suggested in the MISEV2018 guideline that EVs are defined considering a certain size range as small EVs (<200 nm) and medium/large EVs (>200 nm) (19). Hence, we use the term sEVs to refer to endosome-origin exosomes. Recently, sEVs have emerged as critical mediators of intercellular communication through local and systemic transfer of biological molecules, thereby involved in a variety of physiological and pathological processes. It is suggested that further analysis of sEV contents can unveil the molecular mechanisms involved in tumor progression. Despite limited knowledge of the composition, categories, and functions of sEVs, they still have immense potential as diagnostic biomarkers and therapeutic targets in cancer treatment. In this review, we will briefly report recent studies on sEV communication with the tumor microenvironment in TNBC and summarize the clinical application of sEVs in diagnosis and treatment in TNBC.

Fields of unsolved problems in triple-negative breast cancer

Although the characterization of TNBC results in the phenotypic absence of ER, PR, and lack of overexpression of HER2, TNBC is a heterogeneous disease comprising various breast cancer subtypes according to the receptor expression profiles. Pathologic and molecular studies revealed that TNBCs correspond to basal-like breast cancers. It has been reported that basal-like markers, including keratin 5, EGFR, and laminin, could be used to classify TNBC (20, 21). However, TNBC is not completely equal to basal-like tumors since 21% of TNBCs are not basal-like, whereas 31% of basal-like are not triple-negative (22). It is necessary to further study the genomic, molecular, and biological bases of TNBC, leading to the identification of novel therapeutic targets. According to gene expression profiles, TNBC was classified into six subtypes, including basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), and mesenchymal stem-like (MSL) groups and luminal androgen receptor (LAR) (23). It was demonstrated that the BL1 and BL2 subtypes displayed higher expression of cell cycle and DNA damage response genes, and M and MSL were enriched for epithelial–mesenchymal transition and growth factor signals. The IM subtype was enriched for gene ontologies in immune cell processes, including immune cell signaling (TH1/TH2 pathway, NK cell pathway, B-cell receptor signaling, DC pathway, and T-cell signaling), cytokine signaling (IL-12 and IL-7 pathways), antigen processing, presentation, and key immune signal transduction pathways (such as NF-κB, TNF, and JAK-STAT signaling). The LAR subtype was characterized by androgen receptor (AR) signaling and was associated with decreased relapse-free survival. In addition, it identified four stable TNBC subtypes—LAR, mesenchymal (MES), basal-like immune suppressed (BLIS), and basal-like immune activated (BLIA)—based on mRNA and DNA profiles (24). BLIS tumors have the worst prognoses, while BLIA tumors have the best prognoses. It was revealed that the LAR, MES, BLIS, and BLIA subtypes displayed amplification of specific genes CCND1, EGFR, FGFR2, and CDK1, respectively. These results promote the development of TNBC subtype-specific molecularly targeted therapy and immune treatment.

Biogenesis and contents of small extracellular vesicles

Biogenesis and secretion of small extracellular vesicles

sEVs are nano-sized (30–150 nm) vesicles released by almost all cell types and widely present in biological liquids. It was first discovered by the Johnstone team in 1983 that these small particles were associated with the release of transferrin receptors during the maturation of sheep reticulocytes (25) (26). Later, these functional vesicles were defined as exosomes by Johnstone in 1989 (27). sEVs were initially thought to act as the transporter for cells to get rid of metabolic waste (28). It has been recently proved that the secretion of exosomes was an

alternative approach to eliminating cellular metabolic products to maintain cellular homeostasis (29, 30). Moreover, growing studies have revealed that sEVs play a critical role in cell-to-cell communication and get involved in both physiological and pathological processes (31–33). Significantly, accumulating evidence demonstrates that tumor-derived sEVs help prepare a suitable microenvironment for cancer cell colonization and distal metastasis (34, 35).

The release of sEVs requires several cellular steps, including the generation of intraluminal vesicles (ILVs) from MVBs, fusion of MVBs with the plasma membrane, and sorting of distinct sEV cargoes (36–38). As shown in Figure 1, sEVs originate from the endosomal pathway by the formation of early endosomes and late endosomes/MVBs. Extracellular fluids and constitutions enter the cells through endocytosis, and the plasma membrane invaginates. Then, internalized contents are sorted into early endosomes. Subsequently, late endosomes/MVBs are formed from early endosomes mediated by endosomal sorting complexes required for transport (ESCRTs) and other associated proteins such as ALIX and CD63 and lipids according to ESCRT-dependent machinery. Finally, MVBs are transported to plasma membrane through the cytoskeletal and microtubule networks and either fuse with lysosomes or autophagosomes to be degraded or fuse with the cell surface, whereby exosomes are secreted (39, 40). Some other studies reported that sEV formation can occur without ESCRTs since multivesicular endosomes containing ILVs existed despite the absence of all four ESCRT complexes (ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III) (41–43). The mechanism of sEV biogenesis in an ESCRT-dependent or ESCRT-independent manner may not be completely separated. Furthermore, the cell type and/or cellular homeostasis may have an important influence on the secretion of sEVs.

Bioactive cargoes of small extracellular vesicles

sEVs accommodate proteins (surface and intra-vesicular molecules), lipids, and nucleic acids (DNA, mRNA, and non-coding RNA), as well as signaling molecules with the lipid bilayer membrane outside (44), as shown in Figure 1. It was identified that some common proteins specifically enriched in sEVs, such as CD63, CD9, and CD81, could serve as sEV markers (45). Some other frequent proteins present in sEVs include ESCRT-I-related protein (Tsg101), lysosome-related membrane glycoproteins (LAMP-1 and 2B), MVB-related protein (ALIX-1), heat shock proteins (Hsp60, 70, and 90), adhesion molecules, major histocompatibility molecules (MHC-II), and membrane-binding proteins (annexins) (46–49). These common proteins possess the potential of packaging specific protein molecules into sEVs or carrying targeting molecules on the surface of sEVs, and most of them are transmembrane proteins. It was reported that ALIX recruited ESCRT-III proteins onto late endosomes containing lysobisphosphatidic acid (LBPA) and triggered the formation of ILVs containing CD9, CD81, and CD63 in an ESCRT-independent way (50). Although sEVs contain a common series of components, different results were found in different studies. This may be due to those isolated vesicles being a heterogeneous subpopulation. The heterogeneity is reflective of their cell source,

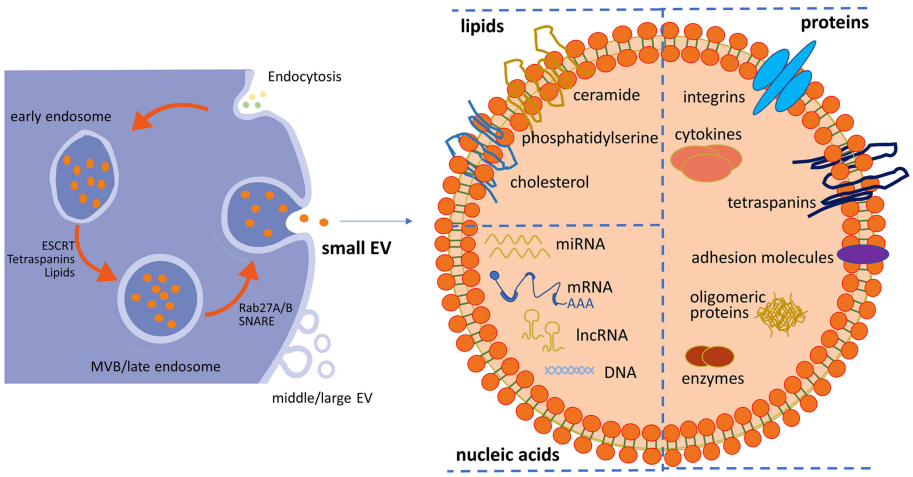


FIGURE 1
Schematic representation of small EV (sEV) biogenesis and typical structure of sEVs. Within the endosomal system, internalized contents through endocytosis are sorted into early endosomes, which subsequently mature into late endosomes/multivesicular bodies (MVBs). sEVs are released from the fusion of MVBs and the plasma membrane. sEVs accommodate lipids, nucleic acids (DNA, mRNA, and non-coding RNA), and proteins (surface and intra-vesicular molecules). Middle/large EVs bud directly from the plasma membrane. EV, extracellular vesicle.

contents, and functional effects on recipient cells. For instance, proteomic analysis of breast cancer cell lines and their sEVs showed that the cell of origin was epithelial-like or mesenchymal-like (51). Proteomic analysis of sEVs isolated from cells with different metastatic propensity demonstrated that the amount and the extent of cancer-related protein cargo vary significantly between non-metastatic and metastatic cell-derived sEVs (52). It was identified that the expression levels of several members of the tetraspanins family (Tetraspanin-14, CD9, CD63, and CD81) were increased in tumor-derived sEVs compared to non-invasive cell line-secreted sEVs. Moreover, sEVs from highly metastatic breast cancer cells induced greater motility (53).

Apart from proteins and peptides, RNA contents, especially miRNAs, have attracted much attention due to their regulatory roles in gene expression. Through a deep sequence of global expression data of a series of cell lines, a subset of miRNAs such as miR-150, miR-142-3p, and miR-451 were generally selected and enriched in sEVs (54). However, some reports have shown that expression levels of sEV-miRNAs differed among various cell lines, as well as the same cell lines under different physiological conditions. The expression level of sEV-miR-21 was lower in the serum of healthy donors than that from glioblastoma patients' serum (55). Moreover, it was found that miR-451 was highly expressed in sEVs derived from normal cells (e.g., primary T lymphocytes and HMC1 cell) (56, 57). The sorting of miRNAs into exosomes did not randomly occur. It was described that heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) was sumoylated and controlled the loading of miRNA into sEVs by binding to them (58). In addition, SYNCRIP, HuR, and major vault protein (MVP) were identified to be involved in the selective incorporation of bioactive cargos into sEVs (59–61). The sEV-lipid composition should be normally consistent with the composition of a lipid bilayer. It was well established that there is an asymmetric distribution of lipid classes in the two leaflets of the plasma membrane, with sphingolipids and phosphatidylcholine (PC) present in the outer leaflet, and other lipid classes located in the inner leaflet (62). The microenvironment and the inherent property may influence the number, contents, and biomarkers of sEVs, but the precise mechanisms of whether and how these bioactive cargoes are sorted and uploaded into sEVs remain unknown.

Components of small extracellular vesicles involved in triple-negative breast cancer progression

Since sEVs are involved in intercellular communication through transferring content cargoes, they can contribute to tumor microenvironment interactions, including angiogenesis, immune escape, tumor proliferation, invasion, distant metastasis, and drug resistance (63–65). It was reported that the secretion level of sEVs in plasma from patients with breast cancer was higher than that in plasma from healthy controls. The sEV-miRNA expression patterns were different between TNBC and HER2-positive patients, such as miR-335, miR-422a, and miR-628 (66). Furthermore, sEV-miR-374 was associated with higher tumor size in TNBC patients, whereas several miRNAs (miR-185, miR-376a, miR-382, miR-410, miR-433, and miR-628) showed association in HER2-positive patients (66). The excessive release of sEVs can be partly ascribed to the upregulation of TSAP6 transcription by activated p53 in response to DNA damage (67). sEVs derived from more invasive TNBC cell lines significantly increased the proliferation, migration, and invasion capacity of all three recipient cell lines (SKBR3, MDA-MB-231, and HCC1954). These vesicles promoted vasculogenesis and subsequent angiogenesis *via* by stimulating the formation of endothelial tubules (68). sEVs isolated from MDA-MB-231 cells, which are resistant to cisplatin, contained higher expression levels of more than 60 miRNAs compared to those collected from MDA-MB-231 cells. Among these miRNAs, miR-370-3p, miR-423-5p, and miR-373 were the most differentially expressed miRNAs (69). These functional miRNAs may have differential expression levels and possess the potential as diagnostic tools and therapeutic interventions.

In addition to the delivery of miRNAs in sEVs, some sEV proteins were found to participate in cancer progression and metastasis. It was revealed that Rab27A promoted the invasive and pulmonary metastatic potentials of TNBC MDA-MB-231 and HER2+ MDA-MB-435 breast cancer cells (70). Consistently, Rab27a was found to promote tumor progression in part by inducing the secretion of sEVs (71). Treatment with sEVs derived from MDA-MB-231 cells could also promote breast cancer cells migrating to the zebrafish tail, which was mediated by overexpression of thrombospondin-1 (TSP1) suppressing intercellular junction molecules (72). For bone metastasis, sEV release of L-plastin and peroxiredoxin-4 (PRDX4) from MDA-MB-231 cells mediated breast cancer-induced osteolysis. The specific mechanism was that L-plastin stimulated osteoclast formation from late osteoclast precursors in the absence of RANKL through stimulation of calcium oscillations and nuclear translocation of NFATc1 transcription factor (73). It was also proved that CD151 transferred by sEVs derived from MDA-MB-231 helped enhance TNBC cell line (MDA-MB-231 and MDA-MB-468) migration and invasion abilities, and sEV-CD151 was significantly enriched in the serum from TNBC patients (74). These results offer evidence that exosomes have a pathophysiological role in TNBC.

Other components of sEVs were reported to participate in the tumor microenvironment as well. For instance, long non-coding RNAs (LncRNAs) are non-coding RNAs with more than 200 nucleotides that lack protein-coding capability due to the absence of open reading frames and start and stop codons (75).

Enhanced expression levels of LncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) were found in breast cancer cells and secreted sEVs. sEV-MALAT1 from cancer cells could significantly induce TNBC cell proliferation (76). Circular RNAs (CircRNAs) are formed by exon back-splicing by connecting the downstream 5′ splicing site to the upstream 3′ splicing site, and they are characterized by evolutional conservation, high stability, and insensitivity to exoribonucleases (77, 78). It was reported that circular RNA arose from HIF1A gene that was overexpressed in breast cancer tissues and sEVs from the plasma of breast cancer patients. CircHIF1A was demonstrated to enhance TNBC cell growth and migration through modulation of miR-149-5p and NFIB and further promote TNBC progression and metastasis (79). Although a variety of progress has been made in the study of sEVs in recent years, its function in TNBC tumorigenesis is still beginning to be understood. Further, the specific role of sEVs in the TNBC microenvironment should be identified, thereby better applying sEVs in clinical treatment.

The potential for clinical application of small extracellular vesicles in triple-negative breast cancer

Isolation and characterization of small extracellular vesicles

sEVs contain various cargoes (DNA, RNA, protein, lipid, and metabolites) and are enriched with specific cancer-associated contents. They are detected to be relatively stable in biological fluids, such as plasma, urine, semen, saliva, amniotic fluid, and tears. The concentration of sEVs was reported to be higher in the systemic circulation of patients with ovarian, breast, and pancreatic cancers (80, 81). sEVs inherit distinct molecules from their cell source and mimic the behavior of the parental cells. Therefore, sEVs have attracted tremendous interest in the biomarker research field.

To be utilized as diagnostic biomarkers, the first key point is standard isolation and characterization of sEVs. A variety of methods have been proposed to isolate and purify sEVs, and they are generally developed based on isolation by size, immunoaffinity capture, and precipitation (Table 1). However, these methods fail to exclusively isolate sEVs and typically result in complex mixtures of sEVs and other components of extracellular space. Among these methods, differential ultracentrifugation was the first method to be used for sEV isolation and remains the gold standard for sEV isolation (82) (83). The representative protocol for sEV isolation is differential ultracentrifugation. The yield can be increased *via* ultracentrifugation at the spin of 100,000 × *g* for a longer time, but ultracentrifugation for a too long time (>4 h) may induce mechanical damage to sEVs and contamination of soluble proteins in the final pellets (84). Differential ultracentrifugation does not require too much technical expertise and sample pretreatment, although it costs time and a large volume of samples or cell culture medium. In order to collect sEVs from a relatively small volume of clinical samples such as plasma, size exclusion chromatography (SEC) is a more clinical setting-friendly option since it allows for sEV isolation from 150 µl to 10 ml of biofluid with resins of selected size (85) (86). Moreover, SEC can protect sEVs from aggregation and improve the removal of protein contaminants (87). In addition, size exclusion chromatography is applied as the purification step after ultracentrifugation methods. An optimized isolation and purification protocol for collected high yields of sEVs from blood was determined as below: firstly, the plasma or serum was centrifuged at 18,000 × *g* for 30 min at 4°C. Then, proteinase K was added to the supernatant (25 µg per 10 mg total proteins of sEV sample) to decrease the amount of albumin and apolipoproteins A-1 and B. Finally, a SEC resin with a molecular weight cutoff (MWCO) of 700 kDa was used to further clear small peptides or proteins (88). Microfluidic isolation can isolate sEVs based on their physical and biochemical properties at the same time. It requires a smaller volume of samples and can be developed into innovative separation, which makes clinical use of sEVs more feasible (89). Immuno-based microfluidic isolation is dependent on the

TABLE 1 Comparison of separation technologies of sEVs.

Isolation method	Advantages	Limitation
Ultracentrifugation	Large sample volume, high yields	Long operation time, equipment requirement, mechanical damage, and protein contamination
Filtration	Fast process, low equipment requirement	sEV damage due to shear stress and loss due to membrane trapping
Size exclusion chromatography	High purity, fast preparation, good reproducibility	Combination with sEV enrichment
Microfluidics	High efficiency, low cost, high sample capacity	Low specificity, contamination of protein and polymeric materials
Immunoaffinity capture	High specificity, high purity	High cost, low sample capacity, and low yields

sEVs, small extracellular vesicles.

interaction between a membrane-binding protein on sEVs and an antibody against the protein, which is immobilized on a microfluidic chip. The predominant advantage of this method is that it requires the smallest volume of the plasma/serum, the least amount of time, minimal expertise, and the least cost to date.

The identification and characterization of sEVs are divided into two types: physical analysis and chemical or compositional analysis. Physical analysis determines particle size and concentration through nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), and dynamic light scattering (DLS). Chemical or compositional analysis evaluates specific contents such as miRNA and protein *via* sequencing, immunoblotting, and staining. The obstacle is still how to differentiate subpopulations of extracellular vesicles with distinct markers and sizes. What makes it more challenging is the fact that the isolation method affects the profiles of sEVs.

Small extracellular vesicles as diagnostic and prognostic biomarkers for triple-negative breast cancer

Following further knowledge of the molecular heterogeneity of TNBC, liquid biopsy has attracted much attention since traditional cancer detection approaches showed weakness in the analysis of the genomic landscape of TNBC. Additionally, liquid biopsy can monitor cancer progress or clinical outcome after treatment in a non-invasive manner. A series of components are released in the tumor microenvironment, for instance, circulating tumor cells (CTCs), cell-free DNA, and EVs in blood circulation (90). sEVs display superiority over other components, as they generally exist in biological liquids and can be easily isolated, stored, and transported. Furthermore, abundant sEV inclusions allow for diverse expression profile analysis.

sEVs collected from breast cancer patients have distinct protein and RNA contents as compared to sEVs derived from healthy donors. As listed in Table 2, it was reported that the serum level of sEV-miR-373 was significantly upregulated in patients with TNBC compared to other breast cancer subtypes, and sEV-miR-372 was increased in breast cancer patients than that in healthy controls (91). Subsequently, functional analyses revealed that miR-373 might downregulate the protein expression level of ER and inhibit apoptosis *via* camptothecin. Interestingly, it was found that the majority of miRNAs detectable in plasma were concentrated in sEVs. The high miRNA concentration observed in sEVs may be due to sEV protection from digestion by RNase. The number of sEVs from plasma was obviously larger in TNBC and HER2+ patients than that in healthy donors (66). A panel of sEV-miR-335, miR-628, and miR-422a could discriminate between TNBC and HER2+ patients. Moreover, in TNBC patients, sEV-miR-374 showed an association with tumor size. These findings suggest a combination of sEV-serum miRNA levels as TNBC-specific markers.

sEV-LncRNA has been revealed to be associated with tumor development and cancer progression. The well-studied LncRNA, HOX transcript antisense intergenic RNA (HOTAIR), was detected in sEVs derived from breast cancer patients, and the expression level of HOTAIR was positively correlated with HER2 in tumor tissues (97). These results suggested HOTAIR as a novel liquid biopsy biomarker for breast cancer. The expression level of serum sEV-LncRNA small ubiquitin-like pseudogene 3 (SUMO1P3) was significantly higher in patients with TNBC compared to that in patients with non-TNBC, patients with benign breast disease, and healthy controls (92). Serum sEV-SUMO1P3 was closely correlated with lymph vascular invasion, lymph node metastasis, and histological grade and positively corresponded to overall survival. Furthermore, serum sEV-SUMO1P3 levels were markedly decreased in chemosensitive cases. These findings showed the potential of serum sEV-LncRNA SUMO1P3 as an independent prognostic factor for TNBC. It was identified that serum sEV-LncRNA XIST obviously increased in TNBC recurrence and could distinguish TNBC patients from healthy controls through receiver operating characteristic (ROC) curve analysis, implying the function of sEV-XIST as a diagnostic and prognostic biomarker for TNBC (93). However, the underlying molecular

TABLE 2 sEV contents as biomarkers for BC and TNBC.

Source	Species	Cargo	Reported effects	References
TNBC serum	RNA	miR-373, miR272 ↑	Decrease ER, inhibit apoptosis	(91)
TNBC plasma		miR-335, miR-628, miR-422a ↑	Promote proliferation	(66)
TNBC serum		lnc-SUMO1P3 ↑	Correlate with lymph vascular invasion, lymph node metastasis	(92)
TNBC serum		lnc-XIST ↑	Correlate with TNBC recurrence	(93)
BC plasma	Protein	Phosphoproteins ↑	Participate in phosphorylation	(94)
Biological fluids		Lipid raft proteins	Function in membrane signaling and trafficking	(95, 96)

sEVs, small extracellular vesicles; BC, breast cancer; TNBC, triple-negative breast cancer; ER, estrogen receptor.
↑ means increase.

mechanisms remain largely unknown, and further supporting evidence is required from larger independent studies.

In addition to RNAs, sEV proteins possess unique features as biomarkers. Specifically, phosphoproteins have the potential as cancer markers because protein phosphorylation is involved in almost all cellular processes (98, 99). It was identified that the expression levels of 144 phosphoproteins were significantly higher in plasma sEVs from patients diagnosed with breast cancer than those in healthy controls through label-free quantitative phosphoproteomics (94). Moreover, lipid rafts proteins are also enriched in the sEV membrane since they organize and stabilize the liquid-ordered regions of the membrane and compartmentalize the processes of intracellular signaling, creating the signaling platforms where interacting components (receptors, effector proteins, and coupling factors) are colocalized in spatial proximity (95, 96). A high abundance of stomatin was shown in sEVs derived from biological fluids, including blood plasma, ascitic fluids, and uterine flushings (100). The expression level of stomatin protein in sEVs from different sources corresponds well to that of CD9, whereas the level of caveolin-1 varies drastically depending on cell type.

The first commercial sEV-based ExoDx™ Prostate (IntelliScore) (EPI) test has been applied for prostate cancer in 2016 (101, 102). This novel non-invasive urine test assessed the expression level of three sEV-RNA transcripts (ERG, PCA3, and SPDEF) for the risk management of men over 50 years of age with PSA level in the "gray zone" of 2–10 ng/ml. The test was validated at a cut point of 15.6 to rule out high-grade prostate cancer and would avoid 27% of invasive biopsies. This sEV-based test has been included in the National Comprehensive Cancer Network guidelines for early prostate cancer detection. We believe this milestone product will promote the development of sEV-based early cancer diagnosis.

Small extracellular vesicles as drug delivery system for treatment approach

sEVs are enriched in biological fluids (such as blood, saliva, and urine), encapsulated with various bioactive cargoes, and mediate intercellular communication by delivering cargoes from parental cells to recipient cells. There is compelling evidence that sEVs can penetrate through the hematoencephalic barrier, keep stability in long circulation, and maintain specific targeting effects (103–105). sEVs derived from different sources carry diverse surface molecules and contents and exert various effects. sEVs serving as drug delivery vehicles should have specific quality standards including size, yield, surface protein, and intracavitary composition.

Mesenchymal stem cells (MSCs) have advantages in terms of ease of expansion, harvesting, and low immunogenic ability. As shown in Table 3, sEVs from MSCs derived from human induced pluripotent stem cells (iPSCs) were loaded with the chemotherapeutic drug doxorubicin (DOX) and showed superior cytotoxic effects on doxorubicin-resistant TNBC cells compared with free or liposomal DOX (106). These vesicles significantly inhibited metastases in TNBC mouse models without detectable immunogenicity. sEVs inherit the essential immunostimulatory faculties from parental dendritic cells (DCs) and lack the risk of *in vivo* replication. It was initially reported that sEVs derived from DCs modified with RVG-targeted Lamp2b peptide delivered siRNA to neurons, microglia, and oligodendrocytes in the mouse brain and strongly downregulated the expression of BACE1 mRNA and protein (107). These results suggested the therapeutic benefit of DC exosomes in Alzheimer's disease since BACE1 is responsible for the N-terminal cleavage of amyloid precursor protein that produces the aggregate-forming β-amyloid peptide in Alzheimer's disease pathogenesis (112). Macrophages are a group of heterogeneous cells that can be phenotypically polarized in the tumor microenvironment to initiate the adaptive immune response (113, 114). Feng et al. modified macrophage-derived sEV-coated nanoparticles carrying DOX for targeted chemotherapy of TNBC (108). It was firstly reported that sEVs from macrophages could penetrate the blood–brain barrier without targeting modification (115). The expression of the integrin lymphocyte function-associated antigen 1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) in naïve macrophage sEVs mediated the uptake of exosomes in brain endothelial cells, thereby helping sEVs deliver brain-derived

TABLE 3 sEVs derived from different types of origins served as drug delivery system.

Source		Cargo	Disease	Reference
Cell source	MSCs	Doxorubicin	TNBC	(106)
	DCs	siRNA	Alzheimer's disease	(107)
	Macrophages	Doxorubicin	TNBC	(108)
	Tumor cells	Doxorubicin	Breast cancer	(109)
Acellular origin	Saliva	mRNA	Wound healing	(110)
	Plasma	Quercetin	Alzheimer's disease	(109)
	Milk	Withaferin A, paclitaxel, docetaxel	Lung cancer	(111)

sEVs, small extracellular vesicles; MSCs, mesenchymal stem cells; DCs, dendritic cells; TNBC, triple-negative breast cancer.

neurotrophic factor (BNDF) to the brain, especially in the presence of brain inflammation. Since patients with TNBC are at a high risk of incidence of brain metastases, the natural crossing blood–brain barrier feature of sEVs holds the promise of improving the survival and life quality of TNBC patients with brain metastasis (110, 116). Compared with sEVs derived from non-cancerous cells, sEVs that originated from tumor cells specifically carry tumor antigens and costimulatory molecules and can lead to an anti-tumor immune response (109). It was reported that sEV-like nanovesicles developed from metastatic breast cancer 4T1 cells could effectively deliver doxorubicin to the lung of the mouse model and inhibited breast cancer lung metastasis (111).

Apart from cell-derived sEVs, these vesicles from biological liquid also possessed advantages as a drug delivery system. For instance, saliva sEVs accelerated wound healing by transferring UBE20, which enhanced the proliferation, migration, and angiogenesis of human umbilical vein endothelial cells (HUVECs) (117). Meanwhile, it was found that saliva sEVs have unique features including distinct elastic properties and substructures carrying specific transmembrane receptors (118). It was firstly proved by Valadi's group that plasma sEVs were uploaded siRNA through chemical transfection and electroporation and delivered the siRNA to monocytes and lymphocytes, leading to gene silencing of mitogen-activated protein kinase 1 (11). Plasma sEVs were lately packed with quercetin, inhibited the activity of CDK5 and decreased tau protein hyperphosphorylation, and attenuated neurodegeneration by reducing the apoptosis of neuron cells and improving memory and spatial learning (119). These findings suggest that sEVs isolated from plasma can be applied as a delivery vehicle of exogenous nucleic acids and chemical drugs for better treatment of central neurological diseases *via* crossing the blood–brain barrier. Bovine milk is generally considered to be a potentially scalable source of sEVs serving as drug delivery vehicles. It was investigated that milk sEVs could encapsulate with both hydrophilic and lipophilic small molecule drugs and exhibit tumor targetability without adverse immune and inflammatory responses (120). In addition to bovine milk exosomes, human breast milk-derived sEVs (HBM-sEVs) also have the potential to be utilized for drug delivery. HBM-sEVs were reported to protect the intestine from damage through intervening intestinal immune response (121) (122). It is worth noting that HBM-sEVs promoted cell proliferation of normal colon epithelial cells, whereas they exerted no beneficial effects on tumor cells (123). These results revealed that HBM-sEVs possess superiorities over other types of sEVs due to their intestinal protection and transferring anti-tumor drug without inducing tumor cell proliferation.

sEVs can be uploaded with drugs (chemical molecules and/or RNAs) through different techniques, which are mainly discussed in two manners (Figure 2). One approach is to load drugs into the donor cells of sEVs, and then the drugs are sorted into sEVs. There are two representative methods, including transfection and electroporation for RNAs and co-incubation for chemical drugs (107–125). Transfection ensures that target miRNA or siRNA is encapsulated into sEVs and released after sEV internalization by recipient cells. It was previously reported that donor cells, HEK293, and COS-7 cells were transfected with miRNA, which targeted EGFR, and secreted sEVs

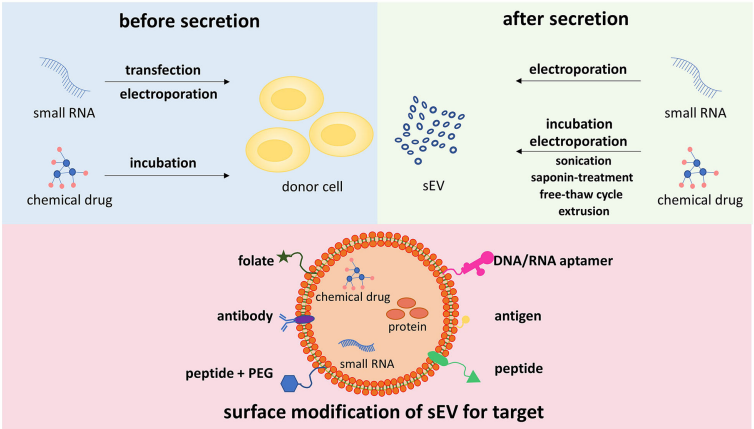


FIGURE 2
Different methods for drug loading into sEVs, and surface engineering for targeting specificity sEVs can be uploaded with drugs *via* two types of methods, introducing drugs into cell origin before sEV secretion and loading drugs into sEVs directly. The former approach includes transfection, electroporation, and incubation. The latter approach consists of electroporation, incubation, sonication, saponin treatment, free–thaw cycle, extrusion, and so on. To enhance targeting activity, the surface of sEVs is modified to express affinity molecules, such as peptides, DNA/RNA aptamers, folate, antibodies, and antigens. sEVs, small extracellular vesicles.

overexpressing the miRNA (126). However, the disadvantage of transfection is the unstable encapsulation efficiency of RNA, which may have an influence on downstream targeting effects. The basic principle of electroporation is that the application of short, high-voltage pulses penetrates the lipid membrane of cells or sEVs, and then the drugs are loaded into sEVs inside (127). Co-incubation is another method to load drugs especially small chemical molecules into sEVs. By exposure of MSCs to high concentrations of paclitaxel (PTX), PTX was incorporated into MSCs and subsequently released sEVs (125). However, after incubation of parental cells with drugs, the synthesis and secretion of drug-carrying sEVs are difficult to be managed. Another way is to introduce drugs directly into sEVs after they are released and isolated, consisting of co-incubation, electroporation, sonication, saponin treatment, extrusion, and freeze–thaw cycles. Compared to the incubation mentioned above, sEVs can be mixed with drugs directly, which is simpler and more effective. Based on the lipophilicity of PTX and passive diffusion, PTX was loaded into sEVs directly by co-incubation with relatively high loading efficiency (128). It was demonstrated that PAK4-specific siRNA was encapsulated into sEVs derived from PANC-1 cells through electroporation, and the encapsulation efficiency and the loading efficiency were 10%–20% and 5%, respectively (129). It is inferred that the aggregation of sEVs during electroporation and the intraluminal space within sEVs, which is occupied by siRNA, is fully saturated, leading to the lower encapsulation efficiency of electroporation (130, 131). In addition to co-incubation and electroporation, there are several other approaches for drug loading in sEVs after their releases, such as sonication, saponin treatment, free–thaw cycle, and extrusion (132). Despite the natural origin of sEVs endowed with homing features, sEVs can be surface-engineered to enhance targeting specificity. As shown in Figure 2, genetic modification links antibodies, peptides, DNA/RNA aptamers, and tumor antigens with the transmembrane domain. Tian et al. engineered immature DC-derived sEVs with αv integrin-specific iRGD peptides and uploaded DOX into these vesicles through electroporation (133). These modified sEVs showed highly efficient targeting and DOX delivery to αv integrin-positive breast cancer cells, leading to the inhibition of tumor growth without overt toxicity. In another study, sEVs were labeled with folate to target TNBC cells with overexpression of folate receptors, and these sEVs exerted a better inhibitory effect on the proliferation and migration of TNBC cells (134). Targeting sEV-based drug delivery system helps generate sEVs with a high yield and low toxicity.

A variety of administration approaches have been exploited to deliver sEVs to target tissues in different disease models, such as direct injection, intravenous injection, intraperitoneal injection, oral administration, and, recently, inhalation. Direct injection showed high efficiency in inhibiting the proliferation of cancer cells and decreasing tumor mass (135). However, direct injection was more invasive than a systemic approach (intravenous injection) (136). Intravenous injection is generally selected for sEV delivery; however, the clearance of this route is rapid (137). It was fluorescently detected that exosomes were predominantly accumulated in liver, lung, kidney, and splenic tissues after intravenous injection (138). With the use of chemiluminescence, sEVs were detected primarily in the liver and the lung, and the signal was retained in the lung longer than that in other organs (137). Moreover, sEVs were found to distribute to the brain and intestines after intranasal administration (138). When sEVs were modified with neuron-specific targeting peptides, they were detected in the central nervous system after intravenous injection (107). Inhaled sEV treatment provides beneficial effects for inflammatory lung diseases including asthma, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), and COVID-19 since it can prevent first-pass hepatic metabolism, improve drug solubility and distribution, and reduce drug side effects (139). The outstanding advantages of sEVs as drug delivery systems lie in their biological origin, which is strongly associated with good biocompatibility. However, some critical questions remain to be answered before clinical application. One of the major challenges is the large-scale standardized production of therapeutic sEVs, which include the origin choice, isolation and purification method, external modification and drug encapsulation, storage, and transportation.

New advances in small extracellular vesicle-based therapy for triple-negative breast cancer

sEVs as nano-sized drug delivery vehicles have attracted attention in TNBC. Some TNBC cells have been demonstrated to be sensitive to erastin-induced reactive oxygen species (ROS)-dependent ferroptosis, followed by significant suppression of cell proliferation and migration (140, 141). However, the poor water solubility of erastin results in low absorption, and renal toxicity limited its clinical application (142). It was reported that erastin was loaded into folate-modified sEVs and can be successfully transported to TNBC tumor sites, thereby increasing the inhibition rate of erastin on the cytotoxicity, proliferation, and migration of TNBC MDA-MB-231 cells (134). Erastin carried by folate-vectorized sEVs caused more ferroptosis with intracellular depletion of glutathione and reactive oxygen species overgeneration than erastin carried by natural sEVs and free erastin (134). The results revealed that erastin loaded in a sEV-targeted delivery system increased the uptake efficiency of erastin into TNBC cells with a longer duration of action and higher activity. Genetically engineered chimeric antigen receptor T cell (CAR-T) therapy has rapidly developed into a powerful and innovative treatment for cancer patients (143, 144). Despite the unprecedented success of CAR-T therapy in B-cell leukemia

or lymphoma, many challenges limited its therapeutic effects in solid tumors such as dose-dependent systemic toxicity. sEVs derived from mesothelin-targeted CAR-T cells inherited surface expression of the CARs and CD3 from parental CAR-T cells and strongly inhibited the growth of both endogenous and exogenous mesothelin-positive TNBC cells (145). The cytotoxicity against TNBC cells of CAR-T-derived-sEVs is exerted through the release of effector molecules perforin and granzyme B with low toxicity *in vivo*. Hence, it was suggested that CAR-T-derived sEVs as a cell-free alternative therapy with efficient cytotoxicity and favorable safety. Immune checkpoints play critical roles in tumor immune surveillance. After analysis of the expression pattern of immune checkpoints using ICP array, sEVs derived from activated tumor-associated effector T cells carry membrane-bound PD-1 (146). Furthermore, they enhanced the cytotoxicity of T cells against TNBC cells by occupying PD-L1 and attenuating subsequent T-cell dysfunction. Altogether, activated T cells in TNBC tumor microenvironment inhibited tumor growth and enhanced anti-tumor immunity. Not only T cell-derived sEVs but also other types of immune cell-derived sEVs can target tumor cells. A macrophage-secreted sEV-based nanosystem was developed, which was modified with peptide targeting the mesenchymal–epithelial transition factor (overexpressed by TNBC cells) and loaded DOX (108). These engineered sEVs obviously prolonged the circulation time of DOX, specifically targeted tumors, and promoted apoptosis of tumor cells with low hepatotoxicity.

In addition to target-modified sEVs, sEVs secreted from breast cancer cells were demonstrated to exhibit excellent lung targeting properties owing to their functional surface integrins, which co-located in the laminin-rich lung microenvironment (147). In order to utilize the natural targeting characteristic, the membrane of sEVs derived from breast cancer cells was extracted and wrapped around cationic bovine serum albumin-conjugated S100A4 siRNA (148). These biomimetic nanoparticles displayed gene-silencing effects on S100A4, which was an important metastasis-related protein that promotes tumor progression and metastasis and suppressed postoperative breast cancer metastasis (149, 150). TNBC cell-derived sEVs were reported to be utilized as a DC-primed vaccine to induce antitumor immunity (151). In specific, sEVs originating from MDA-MB-231 cells were genetically engineered to overexpress α-lactalbumin, which was expressed in the majority of human breast cancers, hence showing enhanced tumor-targeting capability and immunogenicity. The sEVs were subsequently loaded with the immunogenic cell death (ICD) inducers human neutrophil elastase (ELANE) and Hiltonol. This combined delivery system activated DCs *in situ* and cross-primed tumor-reactive $CD8^+$ T-cell responses, leading to tumor inhibition in a poorly immunogenic TNBC mouse xenograft model and patient-derived tumor organoids. These results are promising for clinical application, but till now, there are no clinically approved exosome-based therapies. Further cohort studies are required to demonstrate the indicative role of exosomes in TNBC.

Conclusion

sEVs are natural nano-sized extracellular vesicles with lipid membranes outside and bioactive contents inside. They generally can be secreted by almost all types of cells and play a critical role in intercellular signaling networks. They exhibit several properties such as targeted homing, stability, biocompatibility, low toxicity, and low immunogenicity. The distribution of various biological molecules including DNA, RNA, proteins, and cytokines within exosomes during physiological and pathological processes, including cancers, suggest that sEVs are involved in cancer occurrence and progression. sEVs derived from both tumoral and normal cells have emerged as important components of the tumor microenvironment. TNBC is a particularly aggressive subtype of breast cancer with earlier onset of metastatic disease, visceral metastases, rapid progression, short response duration to available treatment, and worse clinical outcomes. There is an urgent need to develop novel early diagnosis tools and therapies with good efficacy. sEVs have been shown to contribute to angiogenesis, immune escape, tumor proliferation, invasion and distant metastasis, and drug resistance in TNBC. In addition, sEVs can be easily isolated and detected in body fluids. Hence, they hold great promise as biomarkers for early diagnosis, prognosis, and treatment approach of TNBC.

The studies mentioned above provide the basis for the development of sEV-based biomarkers and therapeutics. It is also necessary to further explore the characteristics of sEVs, for instance, content sorting, transportation and internalization, circulation, and tissue clearance, to validate their role in the onset and development of TNBC. Moreover, answering the following questions may promote the clinical application of sEVs. Firstly, there have been no established standardized isolation and purification methods. Then methods such as miRNA quantification are not determined. Next, the precise mechanisms involved in the uploading of drugs into sEVs are unknown. Finally, the complexity of inclusions in sEVs may result in side effects and toxicity *in vivo*. There is still a need to conduct research and clinical studies on how sEVs participate in TNBC, as well as how to utilize sEVs in cancer diagnosis and treatment.

Author contributions

The corresponding author is responsible for ensuring that the descriptions are accurate and agreed upon by all authors. The authors have contributed in multiple roles. YZ is responsible for writing the original drafts and literature search. ZX and WZ are responsible for literature search and editing for the original draft.

All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* (2000) 406(6797):747–52. doi: 10.1038/35021093

2. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. *Mol Oncol* (2011) 5(1):5–23. doi: 10.1016/j.molonc.2010.11.003

3. Kohler BA, Sherman RL, Howlader N, Jemal A, Ryerson AB, Henry KA, et al. Annual report to the nation on the status of cancer, 1975-2011, featuring incidence of breast cancer subtypes by Race/Ethnicity, poverty, and state. *JNCI J Natl Cancer Inst [Internet]* (2015) 107(6). doi: 10.1093/jnci/djv048

4. Dunnwald LK, Rossing MA, Li CI. Hormone receptor status, tumor characteristics, and prognosis: A prospective cohort of breast cancer patients. *Breast Cancer Res* (2007) 9(1):R6. doi: 10.1186/bcr1639

5. Dean-Colomb W, Esteva FJ. Her2-positive breast cancer: Herceptin and beyond. *Adv Transl Res Breast Cancer Bridge Future Ther* (2008) 44(18):2806–12. doi: 10.1016/j.ejca.2008.09.013

6. Januškevičienė I, Petrikaitė V. Heterogeneity of breast cancer: The importance of interaction between different tumor cell populations. *Life Sci* (2019) 239:117009. doi: 10.1016/j.lfs.2019.117009

7. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype. *Cancer* (2007) 109(9):1721–8. doi: 10.1002/cncr.22618

8. Keller S, Sanderson MP, Stoeck A, Altevogt P. Exosomes: From biogenesis and secretion to biological function. *Immunol Lett* (2006) 107(2):102–8. doi: 10.1016/j.imlet.2006.09.005

9. Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D. Extracellular vesicles in cancer: Cell-to-Cell mediators of metastasis. *Cancer Cell* (2016) 30 (6):836–48. doi: 10.1016/j.ccell.2016.10.009

10. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* (2007) 9(6):654–9. doi: 10.1038/ncb1596

11. Wahlgren J, Karlson TDL, Brisslert M, Vaziri Sani F, Telemo E, Sunnerhagen P, et al. Plasma exosomes can deliver exogenous short interfering RNA to monocytes and lymphocytes. *Nucleic Acids Res* (2012) 40(17):e130–0. doi: 10.1093/nar/gks463

12. György B, Szabó TG, Pásztói M, Pál Z, Misják P, Aradi B, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci* (2011) 68(16):2667–88. doi: 10.1007/s00018-011-0689-3

13. Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol* (2009) 9(8):581–93. doi: 10.1038/nri2567

14. Mathivanan S, Ji H, Simpson RJ. Exosomes: Extracellular organelles important in intercellular communication. *Curr Status Cancer Proteomics Far Are We Clin Appl* (2010) 73(10):1907–20. doi: 10.1016/j.jprot.2010.06.006

15. van Dommelen SM, Vader P, Lakhal S, Kooijmans SAA, van Solinge WW, Wood MJA, et al. Microvesicles and exosomes: Opportunities for cell-derived membrane vesicles in drug delivery. *Drug Delivery Res Eur* (2012) 161(2):635–44. doi: 10.1016/j.jconrel.2011.11.021

16. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, et al. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nat Cell Biol* (2008) 10(5):619–24. doi: 10.1038/ncb1725

17. Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: Artefacts no more. *Trends Cell Biol* (2009) 19(2):43–51. doi: 10.1016/j.tcb.2008.11.003

18. Hristov M, Erl W, Linder S, Weber PC. Apoptotic bodies from endothelial cells enhance the number and initiate the differentiation of human endothelial progenitor cells *in vitro*. *Blood* (2004) 104(9):2761–6. doi: 10.1182/blood-2003-10-3614

19. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* (2018) 7(1):1535750. doi: 10.1080/20013078.2018.1535750

20. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U.S.A.* (2001) 98(19):10869–74. doi: 10.1073/pnas.191367098

21. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U.S.A.* (2003) 100(14):8418–23. doi: 10.1073/pnas.0932692100

22. Prat A, Adamo B, Cheang MCU, Anders CK, Carey LA, Perou CM. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. *Oncol* (2013) 18(2):123–33. doi: 10.1634/theoncologist.2012-0397

23. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* (2011) 121(7):2750–67. doi: 10.1172/JCI45014

24. Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreras A, Fuqua SAW, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. *Clin Cancer Res Off J Am Assoc Cancer Res* (2015) 21(7):1688–98. doi: 10.1158/1078-0432.CCR-14-0432

25. Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes *in vitro*: Selective externalization of the receptor. *Cell* (1983) 33(3):967–78. doi: 10.1016/0092-8674(83)90040-5

26. Pan BT, Johnstone R. Selective externalization of the transferrin receptor by sheep reticulocytes *in vitro*. Response to ligands and inhibitors of endocytosis. *J Biol Chem* (1984) 259(15):9776–82. doi: 10.1016/S0021-9258(17)42767-0

27. Johnstone RM, Bianchini A, Teng K. Reticulocyte maturation and exosome release: Transferrin receptor containing exosomes shows multiple plasma membrane functions. *Blood* (1989) 74(5):1844–51. doi: 10.1182/blood.V74.5.1844.1844

28. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J Biol Chem* (1987) 262(19):9412–20. doi: 10.1016/S0021-9258(18)48095-7

29. Baixauli F, López-Otín C, Mittelbrunn M. Exosomes and autophagy: Coordinated mechanisms for the maintenance of cellular fitness. *Front Immunol* (2014) 5:403–3. doi: 10.3389/fimmu.2014.00403

30. Minakaki G, Menges S, Kittel A, Emmanouilidou E, Schaeffner I, Barkovits K, et al. Autophagy inhibition promotes SNCA/alpha-synuclein release and transfer *via* extracellular vesicles with a hybrid autophagosome-exosome-like phenotype. *Autophagy* (2018) 14(1):98–119. doi: 10.1080/15548627.2017.1395992

31. Ludwig AK, Giebel B. Exosomes: Small vesicles participating in intercellular communication. *Int J Biochem Cell Biol* (2012) 44(1):11–5. doi: 10.1016/j.biocel.2011.10.005

32. Pérez-Cabezas B, Santarém N, Cecílio P, Silva C, Silvestre R, Catita J AM, et al. More than just exosomes: Distinct leishmania infantum extracellular products potentiate the establishment of infection. *J Extracell Vesicles* (2018) 8(1):1541708–1541708. doi: 10.1080/20013078.2018.1541708

33. Liu K, Gao X, Kang B, Liu Y, Wang D, Wang Y. The role of tumor stem cell exosomes in cancer invasion and metastasis. *Front Oncol* (2022) 12:836548–8. doi: 10.3389/fonc.2022.836548

34. Eisenblaetter M, Flores-Borja F, Lee JJ, Wefers C, Smith H, Hueting R, et al. Visualization of tumor-immune interaction - target-specific imaging of S100A8/A9

reveals pre-metastatic niche establishment. *Theranostics* (2017) 7(9):2392–401. doi: 10.7150/thno.17138

35. Rodrigues G, Hoshino A, Kenific CM, Matei IR, Steiner L, Freitas D, et al. Tumour exosomal CEMIP protein promotes cancer cell colonization in brain metastasis. *Nat Cell Biol* (2019) 21(11):1403–12. doi: 10.1038/s41556-019-0404-4

36. Morvan J, Rinaldi B, Friant S. Pkh1/2-dependent phosphorylation of Vps27 regulates ESCRT-I recruitment to endosomes. *Mol Biol Cell* (2012) 23(20):4054–64. doi: 10.1091/mbc.E12-01-0001

37. Adell MAY, Vogel GF, Pakdel M, Müller M, Lindner H, Hess MW, et al. Coordinated binding of Vps4 to ESCRT-III drives membrane neck constriction during MVB vesicle formation. *J Cell Biol* (2014) 205(1):33–49. doi: 10.1083/jcb.201310114

38. Buschow SI, Nolte-'t Hoen ENM, Van Niel G, Pols MS, Ten Broeke T, Lauwen M, et al. MHC II in dendritic cells is targeted to lysosomes or T cell-induced exosomes *Via* distinct multivesicular body pathways. *Traffic* (2009) 10 (10):1528–42. doi: 10.1111/j.1600-0854.2009.00963.x

39. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science* (2020) 367(6478):eaau6977. doi: 10.1126/science.aau6977

40. Pocsfalvi G, Stanly C, Vilasi A, Fiume I, Capasso G, Turiák L, et al. Mass spectrometry of extracellular vesicles. *Mass Spectrom Rev* (2016) 35(1):3–21. doi: 10.1002/mas.21457

41. Bache KG, Stuffers S, Malerød L, Slagsvold T, Raiborg C, Lechardeur D, et al. The ESCRT-III subunit hVps24 is required for degradation but not silencing of the epidermal growth factor receptor. *Mol Biol Cell* (2006) 17(6):2513–23. doi: 10.1091/mbc.e05-10-0915

42. Malerød L, Stuffers S, Brech A, Stenmark H. Vps22/EAP30 in ESCRT-II mediates endosomal sorting of growth factor and chemokine receptors destined for lysosomal degradation. *Traffic* (2007) 8(11):1617–29. doi: 10.1111/j.1600-0854.2007.00630.x

43. Stuffers S, Sem Wegner C, Stenmark H, Brech A. Multivesicular endosome biogenesis in the absence of ESCRTs. *Traffic* (2009) 10(7):925–37. doi: 10.1111/j.1600-0854.2009.00920.x

44. Haraszti RA, Didiot MC, Sapp E, Leszyk J, Shaffer SA, Rockwell HE, et al. High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources. *J Extracell Vesicles* (2016) 5:32570–0. doi: 10.3402/jev.v5.32570

45. Kowal J, Arras G, Colombo M, Jouve M, Morath JP, Primdal-Bengtson B, et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proc Natl Acad Sci U.S.A.* (2016) 113 (8):E968–77. doi: 10.1073/pnas.1521230113

46. Baietti MF, Zhang Z, Mortier E, Melchior A, Degeest G, Geeraerts A, et al. Syndecan–syntenin–ALIX regulates the biogenesis of exosomes. *Nat Cell Biol* (2012) 14(7):677–85. doi: 10.1038/ncb2502

47. Simpson RJ, Jensen SS, Lim JWE. Proteomic profiling of exosomes: Current perspectives. *PROTEOMICS* (2008) 8(19):4083–99. doi: 10.1002/pmic.200800109

48. Nabhan JF, Hu R, Oh RS, Cohen SN, Lu Q. Formation and release of arrestin domain-containing protein 1-mediated microvesicles (ARMMs) at plasma membrane by recruitment of TSG101 protein. *Proc Natl Acad Sci U.S.A.* (2012) 109 (11):4146–51. doi: 10.1073/pnas.1200448109

49. Liu C, Su C. Design strategies and application progress of therapeutic exosomes. *Theranostics* (2019) 9(4):1015–28. doi: 10.7150/thno.30853

50. Larios J, Mercier V, Roux A, Gruenberg J. ALIX- and ESCRT-III-dependent sorting of tetraspanins to exosomes. *J Cell Biol* (2020) 219(3):e201904113. doi: 10.1083/jcb.201904113

51. Wen SW, Lima LG, Lobb RJ, Norris EL, Hastie ML, Krumeich S, et al. Breast cancer-derived exosomes reflect the cell-of-Origin phenotype. *PROTEOMICS* (2019) 19(8):1800180. doi: 10.1002/pmic.201800180

52. Gangoda L, Liem M, Ang CS, Keerthikumar S, Adda CG, Parker BS, et al. Proteomic profiling of exosomes secreted by breast cancer cells with varying metastatic potential. *PROTEOMICS* (2017) 17(23–24):1600370. doi: 10.1002/pmic.201600370

53. Harris DA, Patel SH, Gucek M, Hendrix A, Westbroek W, Taraska JW. Exosomes released from breast cancer carcinomas stimulate cell movement. *PloS One* (2015) 10(3):e0117495–e0117495. doi: 10.1371/journal.pone.0117495

54. Guduric-Fuchs J, O'Connor A, Camp B, O'Neill CL, Medina RJ, Simpson DA. Selective extracellular vesicle-mediated export of an overlapping set of microRNAs from multiple cell types. *BMC Genomics* (2012) 13:357–7. doi: 10.1186/1471-2164-13-357

55. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* (2008) 10(12):1470–6. doi: 10.1038/ncb1800

56. Ekström K, Valadi H, Sjöstrand M, Malmhäll C, Bossios A, Eldh M, et al. Characterization of mRNA and microRNA in human mast cell-derived exosomes and their transfer to other mast cells and blood CD34 progenitor cells. *J Extracell Vesicles* (2012) 1:1–12. doi: 10.3402/jev.v1i0.18389

57. Koppers-Lalic D, Hackenberg M, Bijnsdorp IV, van Eijndhoven MAJ, Sadek P, Sie D, et al. Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. *Cell Rep* (2014) 8(6):1649–58. doi: 10.1016/j.celrep.2014.08.027

58. Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F, Pérez-Hernández D, Vázquez J, Martin-Cofreces N, et al. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. *Nat Commun* (2013) 4:2980–0. doi: 10.1038/ncomms3980

59. Santangelo L, Giurato G, Cicchini C, Montaldo C, Mancone C, Tarallo R, et al. The RNA-binding protein SYNCRIP is a component of the hepatocyte exosomal machinery controlling MicroRNA sorting. *Cell Rep* (2016) 17(3):799–808. doi: 10.1016/j.celrep.2016.09.031

60. Mukherjee K, Ghoshal B, Ghosh S, Chakrabarty Y, Shwetha S, Das S, et al. Reversible HuR-microRNA binding controls extracellular export of miR-122 and augments stress response. *EMBO Rep* (2016) 17(8):1184–203. doi: 10.15252/embr.201541930

61. Hobor F, Dallmann A, Ball NJ, Cicchini C, Battistelli C, Ogrodowicz RW, et al. A cryptic RNA-binding domain mediates syncrip recognition and exosomal partitioning of miRNA targets. *Nat Commun* (2018) 9(1):831–1. doi: 10.1038/s41467-018-03182-3

62. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol* (2008) 9(2):112–24. doi: 10.1038/nrm2330

63. Dioufa N, Clark AM, Ma B, Beckwitt CH, Wells A. Bi-directional exosome-driven intercommunication between the hepatic niche and cancer cells. *Mol Cancer* (2017) 16(1):172–2. doi: 10.1186/s12943-017-0740-6

64. Yousafzai NA, Wang H, Wang Z, Zhu Y, Zhu L, Jin H, et al. Exosome mediated multidrug resistance in cancer. *Am J Cancer Res* (2018) 8(11):2210–26.

65. Choi JU, Park IK, Lee YK, Hwang SR. The biological function and therapeutic potential of exosomes in cancer: Exosomes as efficient nanocommunicators for cancer therapy. *Int J Mol Sci* (2020) 21(19):7363. doi: 10.3390/ijms21197363

66. Stevic I, Müller V, Weber K, Fasching PA, Karn T, Marmé F, et al. Specific microRNA signatures in exosomes of triple-negative and HER2-positive breast cancer patients undergoing neoadjuvant therapy within the GeparSixto trial. *BMC Med* (2018) 16(1):179–9. doi: 10.1186/s12916-018-1163-y

67. Yu X, Harris SL, Levine AJ. The regulation of exosome secretion: a novel function of the p53 protein. *Cancer Res* (2006) 66(9):4795–801. doi: 10.1158/0008-5472.CAN-05-4579

68. O'Brien K, Rani S, Corcoran C, Wallace R, Hughes L, Friel AM, et al. Exosomes from triple-negative breast cancer cells can transfer phenotypic traits representing their cells of origin to secondary cells. *Eur J Cancer* (2013) 49(8):1845–59. doi: 10.1016/j.ejca.2013.01.017

69. Wang B, Zhang Y, Ye M, Wu J, Ma L, Chen H. Cisplatin-resistant MDA-MB-231 cell-derived exosomes increase the resistance of recipient cells in an exosomal miR-423-5p-dependent manner. *Curr Drug Metab* (2019) 20(10):804–14. doi: 10.2174/1389200220666190819151946

70. Wang JS, Wang FB, Zhang QG, Shen ZZ, Shao ZM. Enhanced expression of Rab27A gene by breast cancer cells promoting invasiveness and the metastasis potential by secretion of insulin-like growth factor-II. *Mol Cancer Res* (2008) 6 (3):372–82. doi: 10.1158/1541-7786.MCR-07-0162

71. Bobrie A, Krumeich S, Reyal F, Recchi C, Moita LF, Seabra MC, et al. Rab27a supports exosome-dependent and -independent mechanisms that modify the tumor microenvironment and can promote tumor progression. *Cancer Res* (2012) 72(19):4920–30. doi: 10.1158/0008-5472.CAN-12-0925

72. Cen J, Feng L, Ke H, Bao L, Li LZ, Tanaka Y, et al. Exosomal thrombospondin-1 disrupts the integrity of endothelial intercellular junctions to facilitate breast cancer cell metastasis. *Cancers* (2019) 11(12):1946. doi: 10.3390/cancers11121946

73. Tiedemann K, Sadvakassova G, Mikolajewicz N, Juhas M, Sabirova Z, Tabariès S, et al. Exosomal release of l-plastin by breast cancer cells facilitates metastatic bone osteolysis. *Transl Oncol* (2019) 12(3):462–74. doi: 10.1016/j.tranon.2018.11.014

74. Li S, Li X, Yang S, Pi H, Li Z, Yao P, et al. Proteomic landscape of exosomes reveals the functional contributions of CD151 in triple-negative breast cancer. *Mol Cell Proteomics* (2021) 20:100121. doi: 10.1016/j.mcpro.2021.100121

75. Ransohoff JD, Wei Y, Khavari PA. The functions and unique features of long intergenic non-coding RNA. *Nat Rev Mol Cell Biol* (2018) 19(3):143–57. doi: 10.1038/nrm.2017.104

76. Zhang P, Zhou H, Lu K, Lu Y, Wang Y, Feng T. Exosome-mediated delivery of MALAT1 induces cell proliferation in breast cancer. *OncoTargets Ther* (2018) 11:291–9. doi: 10.2147/OTT.S155134

77. Zhang XO, Dong R, Zhang Y, Zhang JL, Luo Z, Zhang J, et al. Diverse alternative back-splicing and alternative splicing landscape of circular RNAs. *Genome Res* (2016) 26(9):1277–87. doi: 10.1101/gr.202895.115

78. Hentze MW, Preiss T. Circular RNAs: Splicing's enigma variations. *EMBO J* (2013) 32(7):923–5. doi: 10.1038/emboj.2013.53

79. Chen T, Wang X, Li C, Zhang H, Liu Y, Han D, et al. CircHIF1A regulated by FUS accelerates triple-negative breast cancer progression by modulating NFIB expression and translocation. *Oncogene* (2021) 40(15):2756–71. doi: 10.1038/s41388-021-01739-z

80. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol Oncol* (2008) 110 (1):13–21. doi: 10.1016/j.ygyno.2008.04.033

81. Melo SA, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* (2015) 523(7559):177–82. doi: 10.1038/nature14581

82. Muller L, Hong CS, Stolz DB, Watkins SC, Whiteside TL. Isolation of biologically-active exosomes from human plasma. *J Immunol Methods* (2014) 411:55–65. doi: 10.1016/j.jim.2014.06.007

83. Zarovni N, Corrado A, Guazzi P, Zocco D, Lari E, Radano G, et al. Integrated isolation and quantitative analysis of exosome shuttled proteins and nucleic acids using immunocapture approaches. *Isol Mol Charact Exosomes* (2015) 87:46–58. doi: 10.1016/j.ymeth.2015.05.028

84. Cvjetkovic A, Lötvall J, Lässer C. The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. *J Extracell Vesicles* (2014) 3. doi: 10.3402/jev.v3.23111

85. Gheinani AH, Vögeli M, Baumgartner U, Vassella E, Draeger A, Burkhard FC, et al. Improved isolation strategies to increase the yield and purity of human urinary exosomes for biomarker discovery. *Sci Rep* (2018) 8(1):3945–5. doi: 10.1038/s41598-018-22142-x

86. Monguió-Tortajada M, Gálvez-Montón C, Bayes-Genis A, Roura S, Borràs FE. Extracellular vesicle isolation methods: Rising impact of size-exclusion chromatography. *Cell Mol Life Sci* (2019) 76(12):2369–82. doi: 10.1007/s00018-019-03071-y

87. Welton JL, Webber JP, Botos LA, Jones M, Clayton A. Ready-made chromatography columns for extracellular vesicle isolation from plasma. *J Extracell Vesicles* (2015) 4:27269–9. doi: 10.3402/jev.v4.27269

88. Diaz G, Bridges C, Lucas M, Cheng Y, Schorey JS, Dobos KM, et al. Protein digestion, ultrafiltration, and size exclusion chromatography to optimize the isolation of exosomes from human blood plasma and serum. *J Vis Exp JoVE* (2018) 134):57467. doi: 10.3791/57467

89. Lee K, Shao H, Weissleder R, Lee H. Acoustic purification of extracellular microvesicles. *ACS Nano* (2015) 9(3):2321–7. doi: 10.1021/nn506538f

90. Ulz P, Thallinger GG, Auer M, Graf R, Kashofer K, Jahn SW, et al. Inferring expressed genes by whole-genome sequencing of plasma DNA. *Nat Genet* (2016) 48(10):1273–8. doi: 10.1038/ng.3648

91. Eichelser C, Stückrath I, Müller V, Milde-Langosch K, Wikman H, Pantel K, et al. Increased serum levels of circulating exosomal microRNA-373 in receptor-negative breast cancer patients. *Oncotarget* (2014) 5(20):9650–63. doi: 10.18632/oncotarget.2520

92. Na-Er A, Xu Y-Y, Liu Y-H, Gan Y-J. Upregulation of serum exosomal SUMO1P3 predicts unfavorable prognosis in triple negative breast cancer. *Eur Rev Med Pharmacol Sci* (2021) 25(1):154–60. doi: 10.26355/eurrev_202101_24379

93. Lan F, Zhang X, Li H, Yue X, Sun Q. Serum exosomal lncRNA XIST is a potential non-invasive biomarker to diagnose recurrence of triple-negative breast cancer. *J Cell Mol Med* (2021) 25(16):7602–7. doi: 10.1111/jcmm.16009

94. Chen IH, Xue L, Hsu CC, Paez JSP, Pan L, Andaluz H, et al. Phosphoproteins in extracellular vesicles as candidate markers for breast cancer. *Proc Natl Acad Sci U.S.A.* (2017) 114(12):3175–80. doi: 10.1073/pnas.1618088114

95. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science* (2010) 327(5961):46–50. doi: 10.1126/science.1174621

96. Simons K, Gerl MJ. Revitalizing membrane rafts: New tools and insights. *Nat Rev Mol Cell Biol* (2010) 11(10):688–99. doi: 10.1038/nrm2977

97. Wang YL, Liu LC, Hung Y, Chen CJ, Lin YZ, Wu WR, et al. Long non-coding RNA HOTAIR in circulatory exosomes is correlated with ErbB2/HER2 positivity in breast cancer. *Breast* (2019) 46:64–9. doi: 10.1016/j.breast.2019.05.003

98. Cohen P. The origins of protein phosphorylation. *Nat Cell Biol* (2002) 4(5): E127–30. doi: 10.1038/ncb0502-e127

99. Singh V, Ram M, Kumar R, Prasad R, Roy BK, Singh KK. Phosphorylation: Implications in cancer. *Protein J* (2017) 36(1):1–6. doi: 10.1007/s10930-017-9696-z

100. Skryabin GO, Komelkov AV, Galetsky SA, Bagrov DV, Evtushenko EG, Nikishin II, et al. Stomatin is highly expressed in exosomes of different origin and is a promising candidate as an exosomal marker. *J Cell Biochem* (2021) 122(1):100–15. doi: 10.1002/jcb.29834

101. McKiernan J, Donovan MJ, O'Neill V, Bentink S, Noerholm M, Belzer S, et al. A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy. *JAMA Oncol* (2016) 2(7):882–9. doi: 10.1001/jamaoncol.2016.0097

102. McKiernan J, Donovan MJ, Margolis E, Partin A, Carter B, Brown G, et al. A prospective adaptive utility trial to validate performance of a novel urine exosome gene expression assay to predict high-grade prostate cancer in patients with prostate-specific antigen 2–10ng/ml at initial biopsy. *Eur Urol* (2018) 74 (6):731–8. doi: 10.1016/j.eururo.2018.08.019

103. András IE, Leda A, Contreras MG, Bertrand L, Park M, Skowronska M, et al. Extracellular vesicles of the blood-brain barrier: Role in the HIV-1 associated amyloid beta pathology. *Mol Cell Neurosci* (2017) 79:12–22. doi: 10.1016/j.mcn.2016.12.006

104. Zhang K, Zhao X, Chen X, Wei Y, Du W, Wang Y, et al. Enhanced therapeutic effects of mesenchymal stem cell-derived exosomes with an injectable hydrogel for hindlimb ischemia treatment. *ACS Appl Mater Interfaces* (2018) 10 (36):30081–91. doi: 10.1021/acsami.8b08449

105. Tian T, Zhang HX, He CP, Fan S, Zhu YL, Qi C, et al. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. *Biomaterials* (2018) 150:137–49. doi: 10.1016/j.biomaterials.2017.10.012

106. Zhao Q, Hai B, Zhang X, Xu J, Koehler B, Liu F. Biomimetic nanovesicles made from iPS cell-derived mesenchymal stem cells for targeted therapy of triple-negative breast cancer. *Nanomed Nanotechnol Biol Med* (2020) 24:102146. doi: 10.1016/j.nano.2019.102146

107. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJA. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* (2011) 29(4):341–5. doi: 10.1038/nbt.1807

108. Li S, Wu Y, Ding F, Yang J, Li J, Gao X, et al. Engineering macrophage-derived exosomes for targeted chemotherapy of triple-negative breast cancer. *Nanoscale* (2020) 12(19):10854–62. doi: 10.1039/D0NR00523A

109. Li Q, Cai S, Li M, Salma KI, Zhou X, Han F, et al. Tumor-derived extracellular vesicles: Their role in immune cells and immunotherapy. *Int J Nanomed* (2021) 16:5395–409. doi: 10.2147/IJN.S313912

110. Kennecke H, Yerushalmi R, Woods R, Cheang MCU, Voduc D, Speers CH, et al. Metastatic behavior of breast cancer subtypes. *J Clin Oncol* (2010) 28 (20):3271–7. doi: 10.1200/JCO.2009.25.9820

111. Qiu X, Li Z, Han X, Zhen L, Luo C, Liu M, et al. Tumor-derived nanovesicles promote lung distribution of the therapeutic nanovector through repression of kupffer cell-mediated phagocytosis. *Theranostics* (2019) 9(9):2618–36. doi: 10.7150/thno.32363

112. Sathya M, Premkumar P, Karthick C, Moorthi P, Jayachandran KS, Anusuyadevi M. BACE1 in alzheimer's disease. *Clin Chim Acta* (2012) 414:171–8. doi: 10.1016/j.cca.2012.08.013

113. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* (2010) 11(10):889–96. doi: 10.1038/ni.1937

114. Gordon S, Martinez FO. Alternative activation of macrophages: Mechanism and functions. *Immunity* (2010) 32(5):593–604. doi: 10.1016/j.immuni.2010.05.007

115. Yuan D, Zhao Y, Banks WA, Bullock KM, Haney M, Batrakova E, et al. Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain. *Biomaterials* (2017) 142:1–12. doi: 10.1016/j.biomaterials.2017.07.011

116. Hosonaga M, Saya H, Arima Y. Molecular and cellular mechanisms underlying brain metastasis of breast cancer. *Cancer Metastasis Rev* (2020) 39 (3):711–20. doi: 10.1007/s10555-020-09881-y

117. Mi B, Chen L, Xiong Y, Yan C, Xue H, Panayi AC, et al. Saliva exosomes-derived UBE2O mRNA promotes angiogenesis in cutaneous wounds by targeting SMAD6. *J Nanobiotechnol* (2020) 18(1):68–8. doi: 10.1186/s12951-020-00624-3

118. Sharma S, Rasool HI, Palanisamy V, Mathisen C, Schmidt M, Wong DT, et al. Structural-mechanical characterization of nanoparticle exosomes in human saliva, using correlative AFM, FESEM, and force spectroscopy. *ACS Nano* (2010) 4 (4):1921–6. doi: 10.1021/nn901824n

119. Qi Y, Guo L, Jiang Y, Shi Y, Sui H, Zhao L. Brain delivery of quercetin-loaded exosomes improved cognitive function in AD mice by inhibiting phosphorylated tau-mediated neurofibrillary tangles. *Drug Delivery* (2020) 27 (1):745–55. doi: 10.1080/10717544.2020.1762262

120. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. *Cancer Lett* (2016) 371(1):48–61. doi: 10.1016/j.canlet.2015.10.020

121. Melnik BC, Stremmel W, Weiskirchen R, John SM, Schmitz G. Exosome-derived MicroRNAs of human milk and their effects on infant health and development. *Biomolecules* (2021) 11(6):851. doi: 10.3390/biom11060851

122. He S, Liu G, Zhu X. Human breast milk-derived exosomes may help maintain intestinal epithelial barrier integrity. *Pediatr Res* (2021) 90(2):366–72. doi: 10.1038/s41390-021-01449-y

123. Reif S, Elbaum Shiff Y, Golan-Gerstl R. Milk-derived exosomes (MDEs) have a different biological effect on normal fetal colon epithelial cells compared to colon tumor cells in a miRNA-dependent manner. *J Transl Med* (2019) 17(1):325–5. doi: 10.1186/s12967-019-2072-3

124. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of MicroRNAs in living cells*◆. *J Biol Chem* (2010) 285(23):17442–52. doi: 10.1074/jbc.M110.107821

125. Pascucci L, Coccè V, Bonomi A, Ami D, Ceccarelli P, Ciusani E, et al. Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit *in vitro* tumor growth: A new approach for drug delivery. *J Controlled Release* (2014) 192:262–70. doi: 10.1016/j.jconrel.2014.07.042

126. S.-ichiro O, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, et al. Systemically injected exosomes targeted to EGFR deliver antitumor MicroRNA to breast cancer cells. *Mol Ther* (2013) 21(1):185–91. doi: 10.1038/mt.2012.180

127. Gehl J. Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. *Acta Physiol Scand* (2003) 177(4):437–47. doi: 10.1046/j.1365-201X.2003.01093.x

128. Saari H, Lázaro-Ibáñez E, Viitala T, Vuorimaa-Laukkanen E, Siljander P, Yliperttula M. Microvesicle- and exosome-mediated drug delivery enhances the cytotoxicity of paclitaxel in autologous prostate cancer cells. *Sel Contrib 17th Int Symp Recent Adv Drug Delivery Syst Salt Lake City USA* (2015) 220:727–37. doi: 10.1016/j.jconrel.2015.09.031

129. Xu L, Faruqu FN, Lim YM, Lim KY, Liam-Or R, Walters AA, et al. Exosome-mediated RNAi of PAK4 prolongs survival of pancreatic cancer mouse model after loco-regional treatment. *Biomaterials* (2021) 264:120369. doi: 10.1016/j.biomaterials.2020.120369

130. Kooijmans SAA, Stremersch S, Braeckmans K, de Smedt SC, Hendrix A, Wood MJA, et al. Electroporation-induced siRNA precipitation obscures the efficiency of siRNA loading into extracellular vesicles. *J Controlled Release* (2013) 172(1):229–38. doi: 10.1016/j.jconrel.2013.08.014

131. Hood JL, Scott MJ, Wickline SA. Maximizing exosome colloidal stability following electroporation. *Anal Biochem* (2014) 448:41–9. doi: 10.1016/j.ab.2013.12.001

132. Liao W, Du Y, Zhang C, Pan F, Yao Y, Zhang T, et al. Exosomes: The next generation of endogenous nanomaterials for advanced drug delivery and therapy. *Acta Biomater* (2019) 86:1–14. doi: 10.1016/j.actbio.2018.12.045

133. Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, et al. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. *Biomaterials* (2014) 35(7):2383–90. doi: 10.1016/j.biomaterials.2013.11.083

134. Yu M, Gai C, Li Z, Ding D, Zheng J, Zhang W, et al. Targeted exosome-encapsulated erastin induced ferroptosis in triple negative breast cancer cells. *Cancer Sci* (2019) 110(10):3173–82. doi: 10.1111/cas.14181

135. Zhou Y, Yamamoto Y, Takeshita F, Yamamoto T, Xiao Z, Ochiya T. Delivery of miR-424-5p *via* extracellular vesicles promotes the apoptosis of MDA-MB-231 TNBC cells in the tumor microenvironment. *Int J Mol Sci* (2021) 22(2). doi: 10.3390/ijms22020844

136. Kosaka N, Iguchi H, Yoshioka Y, Hagiwara K, Takeshita F, Ochiya T. Competitive interactions of cancer cells and normal cells *via* secretory MicroRNAs*. *J Biol Chem* (2012) 287(2):1397–405. doi: 10.1074/jbc.M111.288662

137. Takahashi Y, Nishikawa M, Shinotsuka H, Matsui Y, Ohara S, Imai T, et al. Visualization and *in vivo* tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection. *J Biotechnol* (2013) 165(2):77–84. doi: 10.1016/j.jbiotec.2013.03.013

138. Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, et al. A novel nanoparticle drug delivery system: The anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Mol Ther* (2010) 18(9):1606–14. doi: 10.1038/mt.2010.105

139. Gulati N, Chellappan DK, MacLoughlin R, Dua K, Dureja H. Inhaled nano-based therapeutics for inflammatory lung diseases: Recent advances and future prospects. *Life Sci* (2021) 285:119969. doi: 10.1016/j.lfs.2021.119969

140. Lin X, Ping J, Wen Y, Wu Y. The mechanism of ferroptosis and applications in tumor treatment. *Front Pharmacol [Internet]* (2020) 11:1061. doi: 10.3389/fphar.2020.01061

141. Verma N, Vinik Y, Saroha A, Nair NU, Ruppin E, Mills G, et al. Synthetic lethal combination targeting BET uncovered intrinsic susceptibility of TNBC to ferroptosis. *Sci Adv* (2020). 6(34):eaba8968. doi: 10.1126/sciadv.aba8968

142. Yu Y, Xie Y, Cao L, Yang L, Yang M, Lotze MT, et al. The ferroptosis inducer erastin enhances sensitivity of acute myeloid leukemia cells to chemotherapeutic agents. *Mol Cell Oncol* (2015) 2(4):e1054549. doi: 10.1080/23723556.2015.1054549

143. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory Large b-cell lymphoma. *N Engl J Med* (2017) 377(26):2531–44. doi: 10.1056/NEJMoa1707447

144. June Carl H, O'Connor Roddy S, Kawalekar Omkar U, Saba G, Milone Michael C. CAR T cell immunotherapy for human cancer. *Science* (2018) 359 (6382):1361–5. doi: 10.1126/science.aar6711

145. Yang P, Cao X, Cai H, Feng P, Chen X, Zhu Y, et al. The exosomes derived from CAR-T cell efficiently target mesothelin and reduce triple-negative breast cancer growth. *Cell Immunol* (2021) 360:104262. doi: 10.1016/j.cellimm.2020.104262

146. Qiu Y, Yang Y, Yang R, Liu C, Hsu JM, Jiang Z, et al. Activated T cell-derived exosomal PD-1 attenuates PD-L1-induced immune dysfunction in triple-negative breast cancer. *Oncogene* (2021) 40(31):4992–5001. doi: 10.1038/s41388-021-01896-1

147. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, et al. Tumour exosome integrins determine organotropic metastasis. *Nature* (2015) 527(7578):329–35. doi: 10.1038/nature15756

148. Zhao L, Gu C, Gan Y, Shao L, Chen H, Zhu H. Exosome-mediated siRNA delivery to suppress postoperative breast cancer metastasis. *J Controlled Release* (2020) 318:1–15. doi: 10.1016/j.jconrel.2019.12.005

149. Mishra SK, Siddique HR, Saleem M. S100A4 calcium-binding protein is key player in tumor progression and metastasis: Preclinical and clinical evidence. *Cancer Metastasis Rev* (2012) 31(1):163–72. doi: 10.1007/s10555-011-9338-4

150. Liu L, Qi L, Knifley T, Piecoro DW, Rychahou P, Liu J, et al. S100A4 alters metabolism and promotes invasion of lung cancer cells by up-regulating mitochondrial complex I protein NDUFS2. *J Biol Chem* (2019) 294(18):7516–27. doi: 10.1074/jbc.RA118.004365

151. Huang L, Rong Y, Tang X, Yi K, Qi P, Hou J, et al. Engineered exosomes as an *in situ* DC-primed vaccine to boost antitumor immunity in breast cancer. *Mol Cancer* (2022) 21(1):45. doi: 10.1186/s12943-022-01515-x

frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 28 September 2022
DOI 10.3389/fonc.2022.954179

OPEN ACCESS

EDITED BY
Claudia Mello-Thoms,
The University of Iowa, United States

REVIEWED BY
Phuong D (Yun) Trieu,
The University of Sydney, Australia
Valeria Sebri,
European Institute of Oncology (IEO), Italy

*CORRESPONDENCE
Jing Gao
19942021@cdutcm.edu.cn
Chaoming Hou
983729484@qq.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 27 May 2022
ACCEPTED 29 August 2022
PUBLISHED 28 September 2022

CITATION
Chen X, Wu C, Bai D, Gao J, Hou C, Chen T, Zhang L and Luo H (2022) Health-related quality of life in breast cancer patients in Asia: A meta-analysis and systematic review. *Front. Oncol.* 12:954179. doi: 10.3389/fonc.2022.954179

Health-related quality of life in breast cancer patients in Asia: A meta-analysis and systematic review

Xinyu Chen†, Chenxi Wu†, Dingxi Bai†, Jing Gao*, Chaoming Hou*, Tingting Chen, Lulu Zhang and Huan Luo

School of Nursing, Chengdu University of Traditional Chinese Medicine (TCM), Chengdu, China

Objectives: The primary purposes of this meta-analysis and systematic review were to evaluate the health-related quality of life (HRQoL) of Asian breast cancer (BC) patients to understand their holistic HRQoL level and provide medical and nursing recommendations to improve and preserve their quality of life.

Methods: A comprehensive literature search was conducted to find cross-sectional studies published in Chinese and English concerning HRQoL in BC patients from the inceptions of databases to 14 March 2022. The databases consulted were PubMed, Web of Science, Embase, Cochrane, PsycINFO, CINAHL, and CNKI. Literature screening, data extraction, risk bias assessment, and data synthesis were independently carried out by two researchers. The Endnote X9 and Stata 15.0 software programs were used during the meta-analysis process.

Results: Out of the 8,563 studies identified, 23 cross-sectional studies involving 3,839 Asian BC patients were included in this meta-analysis. Two tools, namely, European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC QLQ-C30) and Quality of Life Questionnaire Breast Cancer module 23 (EORTC QLQ-BR23)—were used to evaluate the HRQoL of BC patients in Asia. The pooled mean of the global health status of Asian BC patients was 58.34 (95% confidence interval [CI]: 53.66–63.02). According to functional subscales of EORTC QLQ-C30 and EORTC QLQ-BR23, Asian BC patients suffered from the worst emotional functioning (pooled mean=66.38; 95% CI: 59.66–73.11) and sexual enjoyment (pooled mean=49.31; 95% CI: 31.97–63.36). In addition, fatigue (pooled mean=42.17; 95% CI: 34.46–49.88) and being upset by hair loss (pooled mean=48.38; 95% CI: 36.64–60.12) were the most obvious symptoms that Asian BC patients experienced according to the meta-analysis results of the EORTC QLQ-C30 and EORTC QLQ-BR23 symptom subscales.

Conclusion: Asian BC patients experience a relatively low HRQoL due to the prominent decline in their body functions, as well as the unpleasant experiences caused by their symptoms. It is suggested that timely, appropriate, and targeted

intervention should be provided in relation to the physical, psychological, and social aspects of Asian BC patients' lives to enhance their ability to function, relieve them of adverse symptoms, and improve their overall HRQoL.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42022321165.

KEYWORDS

Asia, breast cancer, health-related quality of life, meta-analysis, systematic review

Introduction

Breast cancer (BC), the most common malignant tumor, with the highest mortality among women globally, continues to threaten women's lives, with an increasing number of cases yearly. The latest data pertaining to cancer worldwide in 2020 show that the number of new cases of BC has reached 2.26 million (11.7%), surpassing that of lung cancer, and so BC has become the world's leading cancer (1, 2). Various factors may increase the likelihood of incidence of BC, and may also be influenced by socioeconomic developments, ethnicity, and lifestyle, among others (3–5). There are significant differences between Western countries and Asian countries in terms of clinical presentation, epidemiology, treatment methods, and prognosis of the disease. For instance, developing countries in Asia have a lower incidence of BC than Western countries but have a higher mortality rate (6). In addition, there are differences in the choice of BC treatment strategies between Asian and Western countries. To preserve their self-image and sexuality, young women with early-stage BC are more likely to choose breast-conserving surgery (BCS) as the primary treatment (7, 8). However, despite BC being diagnosed in Asian women at an earlier age approximately 10 years earlier than in Western countries, and more than 50% of Asian BC patients suffering from locally advanced cancers (9–11), findings from many studies have demonstrated that breast-conserving surgery is less common in Asia than in Europe and the United States (12, 13). The unique characteristics of BC in Asia and the different therapies adopted by Asian BC patients have led to a different health-related quality of life (HRQoL) compared to other continents.

HRQoL refers to an individual's health status under the influence of illness and injury, medical intervention, aging, and changes in the social environment, as well as subjective satisfaction related to one's economic and cultural backgrounds and values orientation (14). Owing to the complex treatment and prognosis of BC, patients usually experience chronic or prolonged diagnostic procedures and treatment processes, such as surgery, chemotherapy, radiotherapy, and hormonotherapy, which consequently lead to severe physical disorders, including breast removal, skin discoloration, hair loss, fatigue, sexual dysfunction, and distortions in body image (15, 16). However, as a chronic disease, BC usually affects more than just the body's integrity (17). The treatment experiences and associated symptoms in BC patients also give rise to negative effects on mental health. Anxiety, depression, and distress in BC patients are found at high levels, even years after the acute phase or successful treatment in some cases (18–20). These negative effects on mental health can be caused by various factors, including, but not limited to, treatment-related side effects, interruption of the desire for childbearing (or even loss of fertility), and fear of cancer recurrence (FCR) (21, 22).

Impaired HRQoL is the most accurate indication of the gap between an individual's actual functional level and the ideal standard (23). In Asia, a higher proportion of cancers are diagnosed at advanced stages due to a lack of early screening and insufficient understanding of the disease. Therefore, cancer patients in Asia tend to have more severe symptoms, such as pain, fatigue, insomnia, and anxiety, which are more likely to lower their HRQoL. There exists a consensus that the treatment of cancer patients should not only control their pathological reactions and relieve the discomfort caused by the disease and treatment but also reduce their psychological and emotional distress to improve their holistic HRQoL. However, in the context of the different types of economic and medical statuses, as well as cultural and habitual practices, such as the use of traditional medicine, there is a lack of overall understanding of the level of HRQoL of Asian BC patients (24, 25). Therefore, this meta-analysis aims to investigate the holistic HRQoL of Asian BC patients and provide conclusive evidence for developing more targeted measures to improve their quality of life.

Materials and methods

This research was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analyses

(PRISMA) guidelines (26). The current study's protocol registration number in PROSPERO: International prospective register of systematic reviews is CRD42022321165.

Search strategy

Six English databases (PubMed, Web of Science, Embase, Cochrane, Psyclnfo, and CINAHL) and one Chinese database (CNKI) were searched to retrieve eligible articles from the inceptions of databases to 14 March 2022. The search terms "breast cancer," "breast neoplasm," "breast tumor," "health-related quality of life," "quality of life," "Asia," "Far East," "Southeast Asia," "South eastern Asia," "Asia, Western," "Middle East," "China," "Chine*," "Hong Kong*," "Macau," "Tibet*," "Taiwan*," "Japan*," "Korea*," "Mongoli*," "India*," "Brunei*," "Indonesia*," "Lao*," "Malay*," "Myanmar," "Burmese*," "Philippines*," "Singapore*," "Thai*," "Timor*," "Vietnam*," "Bangladesh," "Bengal*," "Bhutan*," "India*," "Nepal*," "Pakistan*," "Sri Lanka*," "Kazakhstan," "Tajikistan," "Turkmenistan," and "Borneo" were used in various combinations to ensure the capture of related literature. The asterisk (*) symbol is significant as it is the component of the search strategy. Appendix S1 in the Supplementary Materials reveals the search strategies used in the seven databases above. Furthermore, we checked the references listed in the selected articles for additional eligible resources.

Inclusion and exclusion criteria

Inclusion criteria

- Populations: Asian adults (age ≥ 18 years old) diagnosed with breast cancer at any stage of pathology
- Study type: cross-sectional studies that investigated HRQoL of BC patients
- Outcomes: HRQoL scores of BC patients were evaluated by related tools

Exclusion criteria

- Patients with other diseases aside from breast cancer
- Patients who had cognitive impairment or any psychiatric disorder
- Studies not in English or Chinese, gray literature, and studies that are not original
- Incomplete information or studies without the full text available
- Unpublished data and presentations that did not provide data that were accurate and clear with respect to the research variables

Study selection and data extraction

Two researchers (XYC and CXW) screened all of the literature by reading the titles and abstracts, then they excluded the studies that did not clearly meet the inclusion criteria; the researchers then read the full text to determine which should be used in our study. The whole process of screening and reading was carried out by the two researchers independently, and a third reviewer (TTC) stepped in when there was any discrepancy between the first two researchers. Once the literature was chosen, both researchers (XYC and CXW) independently extracted the information regarding the authors, region of the country, year of publication, study design, sampling methods and settings, HRQoL instruments, etc. Finally, all the information was integrated and verified by the two researchers.

Quality appraisal

The quality of the eligible studies was assessed *via* the Joanna Briggs Institute tool for cross-sectional studies (JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies) (27). This tool includes eight aspects of every cross-sectional study, evaluated by "Yes," "No," and "Unclear." The answers of "yes" ≥ 5, 3–4, and 0–2 times are considered to indicate high, moderate, and low methodological quality, respectively. Similarly, two researchers (XYC and CXW) conducted the quality evaluation procedure independently, and the third researcher (LLZ) was asked to address any divergence.

As recommended by the Cochrane Collaboration, we used the Risk of Bias in Non-Randomized Studies - of Intervention (ROBINS-I) tool to assess the risk of bias in the studies included (28) from the following seven domains: (i) bias in the selection of exposed and non-exposed cohorts; (ii) bias in the assessment of exposure; (iii) bias in the presence of outcome of interest at the start of study; (iv) bias in the control of prognostic variables (with matching or adjusting); (v) bias in the assessment of the presence or absence of prognostic factors; (vi) bias in the assessment of outcome; and (vii) bias in adequacy regarding follow-up of cohorts.

Statistical analysis

Stata 15.0 software was used to conduct the meta-analysis, and the pooled mean value was taken as the effect size (ES). The pooled mean of Asian BC patients' HRQoL was estimated using a random effects model with a confidence interval of 95%, and the scores were from the "global health status" for Quality of Life Questionnaire C30 (QLQ-C30), functional scales, and symptom scales for both QLQ-C30 and Quality of Life Questionnaire Breast Cancer module 23 QLQ-BR23. Forest plots were used to present the pooled means (95% CI) of the studies included. Statistical heterogeneity was assessed with the I^2 statistic, and I^2

values above 50% were interpreted as heterogeneous. Meta-regression was then used to analyze the factors related to high heterogeneity. Publication bias was assessed *via* Begg's test, and a *p*-value greater than 0.05 ($p > 0.05$) indicated no publication bias. If publication bias existed, the trim-and-fill method was employed to detect the effects of publication bias on the results. To assess the influence of each study on the pooled ES, sensitivity analysis was conducted by omitting an individual study each time and repeating the analysis.

Results

Study selection and data characteristics

After screening and removing duplicates, 23 cross-sectional studies (29–51) containing 3,839 Asian BC patients were included in this study from 8,563 retrieved records. The flow diagram of literature screening is displayed in Figure 1.

Quality appraisal

The characteristics of the 23 cross-sectional studies are given in Table 1. The details of quality assessment for the studies included are presented in Appendix S2 in the Supplementary Materials section, which shows that the scores of the studies included range from 5 to 8, all being of high methodological quality. Moreover, the assessment results for the risk of bias are given in Appendix S3 in the Supplementary Materials. There are 5 studies with a high risk of bias, 14 with a moderate risk of bias, and 4 with a low risk of bias.

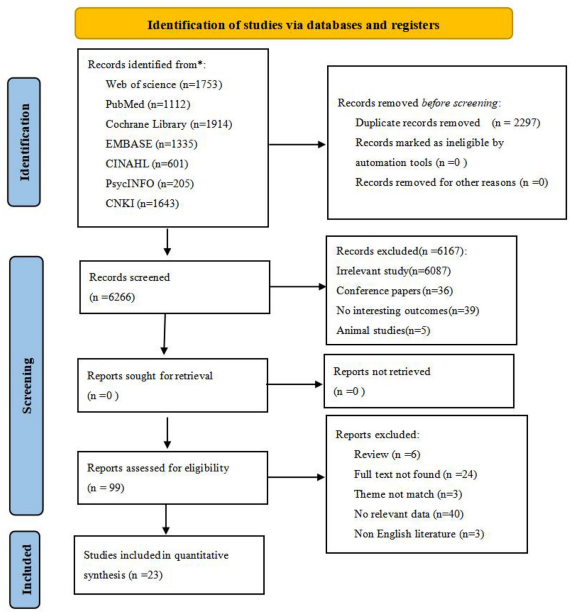


FIGURE 1
Flow diagram of the search strategy and study selection. From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71
For more information, visit: http://www.prisma-statement.org/.
* reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).

Outcomes of meta-analysis

Instruments

Two types of standard tools are used alone or in combination with each other for the studies included: the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC QLQ-C30) (n=23) and the Quality of Life Questionnaire Breast Cancer module 23 (QLQ-BR23) (n=13). QLQ-BR23 is a supplement for the general cancer questionnaire QLQ-C30, and it aims to identify unique concerns of the BC patients (41). The detailed analysis of Asian BC patients' HRQoL, evaluated by the two tools, is as follows.

Meta-analysis results based on the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire C30

EORTC QLQ-C30 was used in all studies included in this systematic review to assess HRQoL of Asian BC patients. The mean value of the global health status of Asian BC patients ranged from 31.2 to 79.43, and the pooled mean value of global health status was 58.34 (95%CI: 53.66–63.02) (I^2 = 98.5%) by using the random effects model (Figure 2). Two subscales of QLQ-C30 also illustrate the HRQoL of BC patients with respect to their functions and symptoms. The functional subscales evaluate the functional status with respect to physical, role, emotional, cognitive, and social functioning. There exist 23 studies (29–51) that have reported the results of physical, role, emotional, and cognitive functioning status, while 22 (29–32, 34–51) have provided the results of social functioning. The pooled means of functional status from low to high are as follows: emotional functioning (pooled mean=66.38; 95% CI: 59.66–73.11), social functioning (pooled mean=71.26; 95% CI: 64.97–77.54), role functioning (pooled mean=72.70; 95% CI: 66.06–79.33), physical functioning (pooled mean=73.15; 95% CI: 66.84–79.46), and cognitive functioning (pooled mean=75.53; 95% CI: 70.58–80.49, Table 2). As for the symptom subscales, a total of nine symptoms are included. All 23 studies (29–51) reported the symptoms of fatigue, pain, insomnia, and appetite loss; 22 (30–51) reported nausea and

TABLE 1 Characteristics of enrolled research.

Study ID, Year	Country	Study design	Sampling method	Setting	Mode of data collection	Instrument	Participants	Age (mean)
Dubashi, 2010 (29)	India	Cross-sectional study	Convenience	Breast clinic	Self-report	QLQ-C30 and QLQ-BR23	51	35
Ghufran, 2013 (30)	Bahrain	Cross-sectional study	Simple random sample	Hospital oncology center	Interview	QLQ-C30 and QLQ-BR23	337	50.2
Min, 2020 (31)	Myanmar	Cross-sectional study	Convenience	Cancer clinic	NR	QLQ-C30	74	–
Chen, 2018 (32)	China	Cross-sectional study	Consecutive	Oncology wards	Interview	QLQ-C30 and QLQ-BR23	608	48
Muna, 2018 (33)	Nepal	Cross-sectional study	Purposive sampling	Outpatient departments	NR	QLQ-C30 and QLQ-BR23	107	47.88
Huang, 2019 (34)	China	Cross-sectional study	Convenience	Breast Cancer Alliance	NR	QLQ-C30 and QLQ-BR23	193	55.52
Najaf, 2016 (35)	Iran	Cross-sectional study	Consecutive	Medical oncology clinic	Self-report	QLQ-C30	155	47.6
Fatemeh, 2021 (36)	Iran	Cross-sectional study	Convenience	Oncology centers	Interview	QLQ-C30 and QLQ-BR23	190	46.9
Safaee, 2008 (37)	Iran	Cross-sectional study	Consecutive	Chemotherapy ward	NR	QLQ-C30	119	48.27
Almutairi, 2016 (38)	Saudi Arabia	Cross-sectional study	Consecutive	Outpatient units	Self-report	QLQ-C30 and QLQ-BR23	145	–
Najmeh, 2013 (39)	Iran	Cross-sectional study	Convenience	Breast Cancer Research Center	NR	QLQ-C30 and QLQ-BR23	68	48
Aishwarya, 2019 (40)	India	Cross-sectional study	Convenience	Department of Oncology	Self-report	QLQ-C30 and QLQ-BR23	50	54.02
Ganesh, 2016 (41)	Malaysia	Cross-sectional study	Systematic random sampling	Oncology clinic	NR	QLQ-C30 and QLQ-BR23	223	52.4
Sajani, 2014 (42)	Nepal	Cross-sectional study	Convenience	National cancer centers	Interview	QLQ-C30 and QLQ-BR23	100	46.79
Azlina, 2013 (43)	Malaysia	Cross-sectional study	Consecutive	Public referral hospitals for breast cancer	NR	QLQ-C30 and QLQ-BR23	58	50.72
Ahmet, 2009 (44)	Turkey	Cross-sectional study	Consecutive	Department of Oncology	Interview	QLQ-C30	55	48.2
Huang, 2017 (45)	China	Cross-sectional study	Convenience	Medical centers	Interview	QLQ-C30	252	54.48
Syarifah, 2022 (46)	Malaysia	Cross-sectional study	Convenience	Oncology clinics	Interview	QLQ-C30	160	51.5

(Continued)

TABLE 1 Continued

Study ID, Year	Country	Study design	Sampling method	Setting	Mode of data collection	Instrument	Participants	Age (mean)
Huda, 2012 (47)	Lebanon	Cross-sectional study	Sequential sampling procedure	Medical Center	Interview	QLQ-C30	89	49.19
Shafika, 2009 (48)	Kuwait	Cross-sectional study	Consecutive	Medical oncology department	Interview	QLQ-C30 and QLQ-BR23	345	48.3
Fatemeh, 2017 (49)	Iran	Cross-sectional study	Convenience	Imam Reza Center	Interview	QLQ-C30	94	45.20
Saleha, 2010 (50)	Pakistan	Cross-sectional study	Consecutive	Department of Clinical Oncology	Interview	QLQ-C30 and QLQ-BR23	200	46.3
Fahimeh, 2018 (51)	Iran	Cross-sectional study	Convenience	Hospitals	Interview	QLQ-C30	166	50

Nothing: NR, no report.

vomiting, dyspnea, constipation, and diarrhea; and 21 (29–36, 38–48, 50, 51) reported financial difficulties. Among the nine symptoms, fatigue was the most common symptom suffered by Asian BC patients, with a pooled mean score of 42.17 (95% CI: 34.46–49.88), followed by financial difficulties (pooled mean=39.07; 95% CI: 39.07–49.07), insomnia (pooled mean=34.96; 95% CI: 26.93–42.99), pain (pooled mean=32.51; 95% CI:25.61–39.42), appetite loss (pooled mean=26.80; 95% CI: 18.50–35.10), dyspnea (pooled mean=24.46; 95% CI: 17.02–31.91), constipation (pooled mean=22.52; 95% CI: 15.24–29.81), nausea and vomiting (pooled mean=18.47; 95% CI: 14.26–22.67), and diarrhea (pooled mean=15.46; 95% CI: 8.60–22.33; Table 3).

Meta-analysis results based on the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Cancer module 23

There are 13 studies (29, 30, 32–34, 36, 38–40, 42, 43, 48, 50) that have used QLQ-BR23. The details of the results are

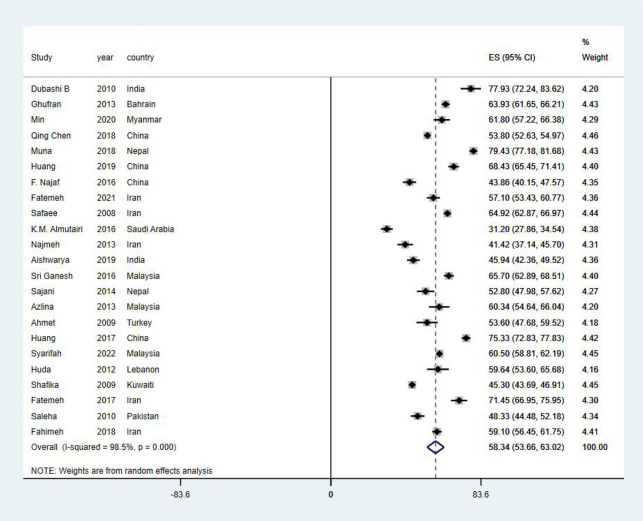


FIGURE 2
Global health status.

10.3389/fonc.2022.954179

TABLE 2 Functional subscales of EORTC QLQ-C30.

Study ID, Year	Country	Functional subscales				
		Physical functioningES (95% CI)	Role functioningES (95% CI)	Emotional functioningES (95% CI)	Cognitive functioningES (95% CI)	Social functioningES (95% CI)
Dubashi, 2010 (29)	India	86.39 (81.57, 91.21)	87.01 (80.79, 93.23)	82.11 (75.74, 88.48)	89.25 (83.81, 94.69)	87.70 (80.95, 94.45)
Ghufran, 2013 (30)	Bahrain	74.92 (72.60, 77.24)	68.84 (65.00, 72.68)	63.41 (59.84, 66.98)	73.38 (70.19, 76.57)	77.52 (74.29, 80.75)
Min, 2020 (31)	Myanmar	80.40 (76.94, 83.86)	66.40 (59.72, 73.08)	73.30 (68.52, 78.09)	83.60 (79.09, 88.11)	80.40 (75.43, 85.37)
Chen, 2018 (32)	China	75.50 (74.13, 76.87)	77.40 (75.37, 79.43)	74.20 (72.63, 75.77)	76.90 (75.35, 78.45)	69.90 (67.95, 71.86)
Muna, 2018 (33)	Nepal	90.21 (89.06, 91.36)	98.28 (97.32, 99.24)	93.06 (91.88, 94.24)	97.19 (96.00, 98.38)	—
Huang, 2019 (34)	China	87.85 (86.24, 89.46)	89.38 (86.93, 91.83)	80.00 (77.36, 82.64)	75.61 (72.89, 78.33)	82.45 (79.41, 85.49)
Najaf, 2016 (35)	China	68.51 (65.42, 71.60)	71.17 (66.91, 75.43)	42.90 (38.88, 46.92)	74.43 (70.77, 78.10)	55.74 (51.63, 59.85)
Fatemeh, 2021 (36)	Iran	71.70 (68.87, 74.53)	72.70 (68.96, 76.44)	54.00 (50.08, 57.92)	77.20 (73.96, 80.44)	67.10 (62.79, 71.41)
Safaee, 2008 (37)	Iran	57.31 (53.04, 61.58)	65.27 (59.00, 71.54)	56.26 (50.72, 61.80)	72.27 (67.33, 77.21)	69.61 (63.69, 75.53)
Almutairi, 2016 (38)	Saudi Arabia	62.90 (58.90, 66.90)	67.60 (62.85, 72.35)	83.30 (79.61, 87.00)	68.30 (63.86, 72.74)	65.00 (59.19, 70.81)
Najmeh, 2013 (39)	Iran	63.14 (58.240, 68.04)	63.93 (57.82, 70.04)	43.38 (38.18, 48.58)	54.41 (48.46, 60.36)	47.55 (41.44, 53.66)
Aishwarya, 2019 (40)	India	55.86 (54.99, 56.73)	35.74 (29.77, 41.71)	33.97 (24.66, 43.28)	65.24 (55.64, 74.84)	58.24 (54.73, 61.75)
Ganesh, 2016 (41)	Malaysia	81.70 (79.39, 84.01)	82.30 (78.99, 85.61)	78.50 (75.89, 81.11)	84.10 (81.74, 86.46)	81.60 (78.74, 84.46)
Sajani, 2014 (42)	Nepal	71.40 (68.05, 74.75)	78.50 (73.64, 83.36)	46.40 (39.66, 53.14)	59.30 (53.11, 65.49)	45.20 (38.99, 51.41)
Azlina, 2013 (43)	Malaysia	76.32 (69.76, 82.88)	67.24 (56.98, 77.50)	65.80 (58.90, 72.70)	84.77 (79.87, 89.68)	75.00 (67.00, 83.00)
Ahmet, 2009 (44)	Turkey	63.10 (56.68, 69.52)	68.20 (59.85, 76.55)	71.50 (65.24, 77.76)	78.30 (71.22, 85.38)	63.40 (55.13, 71.67)
Huang, 2017 (45)	China	91.19 (89.83, 92.55)	94.05 (92.40, 95.70)	84.82 (82.46, 87.18)	77.12 (74.72, 79.52)	86.18 (83.63, 88.73)
Syarifah, 2022 (46)	Malaysia	83.10 (81.42, 84.78)	77.80 (75.70, 79.90)	93.70 (92.03, 95.37)	81.80 (79.62, 83.98)	97.00 (95.56, 98.44)
Huda, 2012 (47)	Lebanon	79.10 (74.72, 83.48)	73.41 (67.09, 79.73)	65.92 (60.01, 71.84)	84.45 (79.85, 89.05)	60.29 (54.37, 66.21)
Shafika, 2009 (48)	Kuwait	52.60 (50.62, 54.58)	55.10 (52.84, 57.36)	60.30 (57.93, 62.68)	59.90 (57.38, 62.42)	61.20 (58.81, 63.60)
Fatemeh, 2017 (49)	Iran	91.35 (89.40, 93.31)	86.70 (82.98, 90.42)	78.55 (74.81, 82.29)	81.56 (78.09, 85.04)	89.18 (85.91, 92.45)
Saleha, 2010 (50)	Pakistan	56.40 (52.60, 60.20)	61.00 (55.20, 66.80)	46.16 (41.03, 51.29)	60.66 (56.76, 64.56)	77.33 (72.99, 81.68)
Fahimeh, 2018 (51)	Iran	60.60 (56.43, 64.77)	61.40 (57.89, 64.91)	51.40 (48.19, 54.61)	74.90 (71.28, 78.52)	68.10 (65.06, 71.14)
Random pool ES (95% CI)		73.15 (66.84, 79.46)	72.70 (66.06, 79.33)	66.38 (59.66, 73.11)	75.53 (70.58, 80.49)	71.26 (64.97, 77.54)

TABLE 3 Symptom subscales of EORTC QLQ-C30.

Study ID, Year	Country	Symptom subscales								
		FatigueES (95% CI)	Nausea and vomitingES (95% CI)	PainES (95% CI)	DyspneaES (95% CI)	InsomniaES (95% CI)	Appetite lossES (95% CI)	ConstipationES (95% CI)	DiarrheaES (95% CI)	Financial difficultiesES (95% CI)
Dubashi, 2010 (29)	India	18.10 (12.04, 24.17)	—	19.60 (12.29, 26.91)	—	8.49 (1.44, 15.54)	5.88 (1.13, 10.63)	—	—	40.50 (29.61, 51.39)
Ghufran, 2013 (30)	Bahrain	35.28 (32.01, 38.55)	10.29 (7.00, 13.58)	29.97 (26.64, 33.30)	20.22 (16.98, 23.46)	30.12 (25.93, 34.32)	13.38 (10.43, 16.33)	17.99 (14.72, 21.26)	6.83 (4.81, 8.85)	34.58 (30.07, 39.09)
Min, 2020 (31)	Myanmar	22.80 (18.70, 26.90)	4.70 (2.15, 7.25)	18.50 (12.71, 24.29)	11.30 (6.38, 16.22)	29.30 (22.31, 36.30)	15.30 (9.79, 20.81)	18.50 (13.01, 23.99)	0.90 (-0.33, 2.13)	57.70 (50.23, 65.17)
Chen, 2018 (32)	China	34.00 (32.56, 35.44)	19.00 (17.29, 20.71)	28.90 (27.32, 30.48)	17.20 (15.44, 18.97)	31.40 (29.46, 33.34)	24.10 (22.09, 26.11)	24.60 (22.50, 26.70)	10.40 (8.90, 11.90)	34.60 (32.32, 36.88)
Muna, 2018 (33)	Nepal	80.36 (77.24, 83.48)	0.46 (-0.06, 0.98)	7.47 (5.84, 9.11)	0.93 (-0.25, 2.11)	4.98 (2.72, 7.24)	6.54 (4.02, 9.06)	0.93 (-0.12, 1.98)	1.24 (0.04, 2.44)	0.93 (-0.12, 1.98)
Huang, 2019 (34)	China	28.39 (25.85, 30.93)	7.14 (5.23, 9.05)	20.02 (17.45, 22.59)	12.09 (9.49, 14.69)	34.75 (30.77, 38.73)	10.99 (8.52, 13.46)	18.34 (15.12, 21.57)	10.41 (7.88, 12.94)	19.50 (15.74, 23.26)
Najaf, 2016 (35)	China	52.77 (48.87, 56.67)	34.68 (30.29, 39.07)	42.34 (38.30, 46.38)	41.21 (36.08, 46.34)	47.29 (42.43, 52.15)	43.91 (39.09, 48.73)	20.49 (16.10, 24.88)	17.11 (12.98, 21.24)	64.18 (59.32, 69.04)
Fatemeh, 2021 (36)	Iran	45.00 (41.28, 48.73)	19.70 (15.98, 23.43)	43.30 (39.49, 47.11)	16.80 (13.13, 20.47)	35.60 (30.89, 40.31)	23.20 (18.52, 27.88)	25.80 (21.21, 30.39)	11.60 (8.42, 14.79)	55.40 (50.08, 60.72)
Safaee, 2008 (37)	Iran	41.74 (36.91, 46.58)	16.39 (11.29, 21.49)	33.19 (28.11, 38.27)	16.25 (11.39, 21.11)	43.70 (36.40, 51.00)	22.69 (16.17, 29.21)	14.85 (9.58, 20.12)	3.92 (0.98, 6.86)	—
Almutairi, 2016 (38)	Saudi Arabia	76.20 (72.47, 79.93)	68.90 (56.50, 81.30)	76.20 (72.29, 80.12)	80.00 (75.56, 84.44)	84.10 (79.95, 88.25)	80.90 (76.52, 85.28)	59.30 (54.25, 64.35)	41.20 (35.93, 46.47)	52.00 (45.60, 58.40)
Najmeh, 2013 (39)	Iran	56.54 (51.30, 61.78)	26.23 (20.06, 32.40)	45.59 (40.22, 50.96)	24.02 (16.67, 31.37)	46.57 (38.69, 54.45)	35.78 (29.17, 42.39)	28.92 (20.72, 37.12)	15.69 (9.81, 21.57)	66.67 (59.17, 74.17)
Aishwarya, 2019 (40)	India	64.64 (56.47, 72.81)	11.82 (7.52, 16.12)	73.50 (67.08, 79.92)	39.78 (29.35, 50.21)	56.50 (44.93, 68.08)	24.42 (17.15, 31.69)	11.22 (5.55, 16.89)	2.64 (0.16, 5.12)	35.64 (29.35, 41.94)
Ganesh, 2016 (41)	Malaysia	28.90 (26.29, 31.51)	11.70 (9.26, 14.14)	18.80 (16.14, 21.46)	10.01 (7.57, 12.45)	21.30 (17.74, 24.86)	18.98 (15.62, 22.34)	9.90 (7.08, 12.72)	7.70 (5.43, 9.97)	40.10 (35.95, 44.25)
Sajani, 2014 (42)	Nepal	37.10 (32.55, 41.65)	20.30 (15.60, 25.00)	39.80 (34.80, 44.80)	19.00 (13.26, 24.74)	40.70 (32.53, 48.87)	38.00 (30.51, 45.49)	17.70 (11.80, 23.60)	11.70 (6.51, 16.89)	67.70 (62.17, 73.23)
Azlina, 2013 (43)	Malaysia	29.69 (22.42, 36.96)	6.61 (2.60, 10.62)	25.29 (17.27, 33.31)	6.90 (2.43, 11.38)	28.16 (18.96, 37.36)	22.99 (14.77, 31.22)	19.54 (13.11, 25.97)	4.60 (1.21, 7.99)	28.16 (18.68, 37.64)
Ahmet, 2009 (44)	Turkey	49.30 (42.80, 55.80)	24.80 (17.03, 32.57)	38.10 (30.30, 45.90)	19.20 (10.64, 27.76)	38.50 (29.62, 47.38)	33.00 (24.25, 41.75)	24.00 (16.18, 31.82)	16.20 (9.28, 23.12)	28.20 (20.03, 36.37)
Huang, 2017 (45)	China	19.27 (17.08, 21.46)	3.84 (2.58, 5.10)	11.57 (9.36, 13.78)	8.20 (6.21, 10.19)	26.06 (23.13, 28.99)	6.22 (4.14, 8.30)	15.08 (12.30, 17.86)	5.82 (4.13, 7.51)	13.89 (11.01, 16.77)

(Continued)

10.3389/fonc.2022.954179

TABLE 3 Continued

Study ID, Year	Country	Symptom subscales									
		FatigueES (95% CI)	Nausea and vomitingES (95% CI)	PainES (95% CI)	DyspneaES (95% CI)	InsomniaES (95% CI)	Appetite lossES (95% CI)	ConstipationES (95% CI)	DiarrheaES (95% CI)	Financial difficultiesES (95% CI)
Syarifah, 2022 (46)	Malaysia	20.80 (19.04, 22.56)	0.20 (-0.21, 0.61)	14.20 (12.23, 16.17)	2.10 (0.60, 3.60)	7.50 (4.73, 10.28)	0.60 (-0.10, 1.30)	0.80 (-0.01, 1.61)	0.60 (-0.10, 1.30)	11.00 (8.36, 13.64)
Huda, 2012 (47)	Lebanon	34.58 (28.70, 40.46)	9.50 (5.78, 13.22)	32.40 (25.22, 39.58)	7.86 (3.81, 11.91)	33.71 (25.33, 42.10)	24.34 (16.27, 32.41)	10.86 (5.56, 16.16)	9.74 (4.40, 15.08)	37.45 (29.18, 45.73)
Shafika, 2009 (48)	Kuwait	38.90 (36.65, 41.15)	30.20 (27.62, 32.79)	43.80 (41.51, 46.09)	42.40 (39.47, 45.33)	42.70 (39.72, 45.68)	37.50 (34.55, 40.46)	27.80 (24.82, 30.78)	22.10 (19.20, 25.00)	31.20 (28.54, 33.84)
Fatemeh, 2017 (49)	Iran	14.42 (10.42, 18.43)	5.67 (3.03, 8.31)	12.41 (9.33, 15.49)	8.16 (4.93, 11.39)	14.89 (10.10, 19.49)	7.80 (3.80, 11.81)	10.28 (6.20, 14.36)	2.84 (0.72, 4.97)	—
Saleha, 2010 (50)	Pakistan	73.55 (70.02, 77.08)	28.00 (24.50, 31.50)	51.00 (46.23, 55.77)	62.67 (56.50, 68.84)	34.67 (29.65, 39.69)	42.67 (36.77, 48.58)	35.00 (30.33, 39.67)	45.33 (3.17, 47.49)	50.00 (44.38, 55.62)
Fahimeh, 2018 (51)	Iran	68.10 (65.06, 71.14)	63.20 (59.67, 66.73)	22.90 (14.44, 31.36)	74.70 (70.03, 79.37)	65.10 (60.72, 69.48)	78.50 (74.32, 82.68)	84.30 (80.62, 87.98)	92.40 (89.46, 95.34)	52.40 (48.73, 56.07)
Random pool ES (95% CI)		42.17 (34.46, 49.88)	18.47 (14.26, 22.67)	32.51 (25.61, 39.42)	24.46 (17.02, 31.91)	34.96 (26.93, 42.99)	26.80 (18.50, 35.10)	22.52 (15.24, 29.81)	15.46 (8.60, 22.33)	39.07 (29.06, 49.07)

displayed in Table 4. The QLQ-BR23 tool also has two subscales to assess the functions and symptoms of BC patients specifically. As can be seen in Table 4, all 13 studies reported the evaluated results of body image, future perspective, breast symptoms, and arm symptoms; and 12 reported the results of sexual functioning (29, 30, 32–34, 36, 38, 39, 42, 43, 48, 50), sexual enjoyment (29, 30, 32–34, 36, 38, 39, 42, 43, 48, 50), systemic side effects (29, 30, 32–34, 36, 38–40, 42, 48, 50), and upset by hair loss (30, 32–34, 36, 38–40, 42, 43, 48, 50). The pooled scores in the functional subscales ranged from 49 to 66: sexual enjoyment (pooled mean=49.31; 95% CI: 31.97–63.36), sexual functioning (pooled mean=50.77; 95% CI: 28.00–73.54), future perspective (pooled mean=53.81; 95% CI: 44.26–63.81), and body image (pooled mean=66.15; 95% CI: 61.08–71.21). Additionally, the pooled scores of the symptom subscale ranged from 29 to 49: breast symptoms (pooled mean=29.26; 95% CI: 20.12–38.40), arm symptoms (pooled mean=34.02; 95% CI: 24.91–43.13), systemic side effects (pooled mean=35.88; 95% CI: 25.76–46.00), and upset by hair loss (pooled mean=48.38; 95% CI: 36.64–60.12). The pooled results suggest that sexual enjoyment and being upset by hair loss are the most frequent problems faced by Asian BC patients.

Heterogeneity, publication bias, and sensitivity analysis

As illustrated in Table 5, the heterogeneity between the studies included was high, with all I^2 values above 90%. According to the results of Begg's tests, there was no obvious publication bias for the meta-analysis, except for the results for nausea and vomiting (p=0.048), dyspnea (p=0.032), and diarrhea (p=0.011) in the QLQ-C30 tool (Table 5). Moreover, the outcomes of the trim-and-fill method showed that the publication bias may have exerted an influence on the results for nausea and vomiting (p=0.914), dyspnea (p=0.057), and diarrhea (p=0.361, Table 5). Moreover, the sensitivity-analysis results indicated that no single study essentially changed the pooled mean of all the outcomes, and we take the sensitivity analysis plot of global health status as an example (Figure 3).

Meta-regression results based on the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire C30 and Quality of Life Questionnaire Breast Cancer module 23

We took the region of the country (in East Asia, Southeast Asia, South Asia, and in West Asia), the publication year (in 2008–2012, 2013–2017, and 2018–2022), the mean age of patients (age < 50; age ≥ 50), the sample size (1–200, 201–

TABLE 4 Functional and symptom subscales of EORTC QLQ-BR23.

Study ID, Year	Country	Functional subscales				Symptom subscales			
		Body imageES (95% CI)	Sexual functioningES (95% CI)	Sexual enjoymentES (95% CI)	Future perspectiveES (95% CI)	Systemic side effectsES (95% CI)	Breast symptomsES (95% CI)	Arm symptomsES (95% CI)	Upset by hair lossES (95% CI)
Dubashi, 2010 (29)	India	80.44 (72.58, 88.30)	61.54 (51.34, 71.74)	58.15 (47.49, 68.81)	72.62 (63.34, 81.90)	13.04 (9.77, 16.31)	8.98 (4.10, 12.96)	15.52 (9.94, 21.11)	—
Ghufran, 2013 (30)	Bahrain	75.64 (72.45, 78.83)	25.92 (22.74, 29.10)	48.56 (45.13, 51.99)	61.29 (57.09, 65.49)	19.27 (17.37, 21.17)	13.66 (11.73, 15.5)	36.58 (33.19, 39.97)	46.33 (41.75, 50.91)
Chen, 2018 (32)	China	64.90 (62.91, 66.89)	89.00 (87.74, 90.26)	88.30 (86.74, 89.86)	51.50 (49.00.54.00)	24.70 (23.36, 26.04)	17.10 (15.53, 18.67)	20.20 (18.64, 21.76)	38.60 (36.19, 41.01)
Muna, 2018 (33)	Nepal	74.62 (71.71, 77.53)	2.95 (1.17, 4.73)	27.77 (25.31, 30.23)	80.36 (77.24, 83.48)	7.07 (5.95, 8.19)	12.69 (11.44, 13.94)	10.87 (9.34, 12.41)	12.66 (9.31, 16.01)
Huang, 2019 (34)	China	78.73 (75.51, 81.96)	15.51 (12.98, 18.04)	28.63 (24.92, 32.34)	57.64 (53.64, 61.64)	23.75 (21.25, 26.25)	18.30 (16.06, 20.55)	23.15 (20.25, 26.05)	34.46 (29.82, 39.10)
Fatemeh, 2021 (36)	Iran	59.70 (54.58, 64.82)	20.60 (17.61, 23.59)	19.70 (16.47, 22.93)	40.50 (5.65, 45.35)	38.90 (34.45, 43.35)	1.60 (1.50, 1.70)	19.80 (16.62, 22.99)	57.70 (52.55, 62.85)
Almutairi, 2016 (38)	Saudi Arabia	64.70 (58.89, 70.51)	52.30 (48.44, 56.16)	22.50 (17.99, 27.01)	76.30 (70.55, 82.05)	64.40 (59.91, 68.89)	65.10 (60.85, 69.35)	62.90 (58.98, 66.82)	64.40 (59.03, 69.77)
Najmeh, 2013 (39)	Iran	43.63 (37.34, 49.92)	14.46 (10.72, 18.20)	14.71 (7.04, 22.38)	57.84 (49.45, 66.24)	46.01 (40.60, 51.42)	27.57 (22.67, 32.47)	41.18 (34.97, 47.39)	60.29 (52.28, 68.30)
Aishwarya, 2019 (40)	India	53.12 (49.94, 56.31)	—	—	32.40 (24.78, 40.02)	60.78 (56.17, 65.40)	45.36 (40.16, 50.56)	49.50 (45.88, 53.12)	76.90 (72.13, 81.67)
Sajani, 2014 (42)	Nepal	56.00 (48.28, 63.72)	87.70 (84.23, 91.17)	79.30 (74.15, 84.46)	43.30 (36.07, 50.53)	37.60 (33.58, 41.62)	35.60 (31.29, 39.91)	37.40 (32.19, 42.61)	40.30 (31.83, 48.77)
Azlina, 2013 (43)	Malaysia	75.57 (68.74, 82.40)	77.30 (70.69, 83.91)	50.00 (44.52, 55.48)	44.25 (36.48, 52.02)	—	27.16 (21.46, 32.86)	21.84 (15.17, 28.51)	21.21 (15.43, 26.99)
Shafika, 2009 (48)	Kuwait	61.80 (59.34, 64.26)	69.90 (67.41, 72.39)	61.50 (59.07, 63.93)	59.50 (56.13, 62.87)	40.10 (38.25, 41.95)	35.60 (32.92, 38.28)	38.20 (35.73, 40.67)	44.80 (41.68, 47.92)
Saleha, 2010 (50)	Pakistan	70.5 (66.15, 74.86)	92.33 (89.52, 95.14)	92.33 (89.53, 95.13)	22.00 (18.08, 25.92)	55.90 (53.48, 58.32)	73.00 (68.69, 77.31)	65.33 (60.98, 69.68)	83.33 (78.46, 88.20)
Random pool ES (95% CI)		66.15 (61.08, 71.21)	50.77 (28.00, 73.54)	49.31 (31.97, 63.36)	53.81 (44.26, 63.81)	35.88 (25.76, 46.00)	29.26(20.12, 38.40)	34.02 (24.91, 43.13)	48.38 (36.64, 60.12)

400, and 401–608), and the medical level worldwide (ranked below 50, ranked between 50 and 100,; and ranked above 100) (52) as covariates in the meta-regression. The results demonstrated that the five factors above could not explain the heterogeneity between studies of almost all the dimensions of HRQoL significantly through the univariate meta-regression method (p-value > 0.05), except the physical functioning dimension of the QLQ-C30, in which its high heterogeneity may be associated with the regions of countries (p=0.02, Table 6).

TABLE 5 Results of the publication bias and trim-and-fill method.

Tool		HRQoL	I^2 (%)	Studies included	Begg's test		Trim-and-fill method	
					z	p	z	p
QLQ-C30								
	Functional subscales	Global health status	98.5	23	0.21	0.833	–	–
		Physical functioning	99.5	23	1.74	0.081	–	–
		Role functioning	99.1	23	0.11	0.916	–	–
		Emotional functioning	99.2	23	1.58	0.113	–	–
		Cognitive functioning	98.4	23	0.11	0.916	–	–
		Social functioning	98.7	22	0.73	0.463	–	–
	Symptom subscales	Fatigue	99.3	23	1.06	0.291	–	–
		Nausea and vomiting	99.3	22	1.97	0.048	1.298	0.194
		Pain	99.1	23	0.90	0.369	–	–
		Dyspnea	99.3	22	2.14	0.032	1.905	0.057
		Insomnia	98.9	23	0.85	0.398	–	–
		Appetite loss	99.4	23	0.95	0.342	–	–
		Constipation	99.4	22	0.23	0.822	–	–
		Diarrhea	99.6	22	2.54	0.011	0.914	0.361
		Financial difficulties	99.4	21	0.03	0.976	–	–
QLQ-BR23	Functional subscales	Body image	95.8	13	0.06	0.951	–	–
		Sexual functioning	99.9	12	0.21	0.837	–	–
		Sexual enjoyment	99.7	12	0.07	0.945	–	–
		Future perspective	98.3	13	0.18	0.855	–	–
	Symptom subscales	Systemic side effects	99.6	12	1.17	0.244	–	–
		Breast symptoms	99.7	13	0.31	0.760	–	–
		Arm symptoms	99.2	13	1.16	0.246	–	–
		Upset by hair loss	98.9	12	0.75	0.451	–	–

Begg's test: z: statistic; p: p value, the statistic significance of Begg's test. p<0.05 indicates than there is publication bias.
Trim-and-fill method: z: statistic; p: p value, the statistic significance of the pooled results after trim-and- fill method.

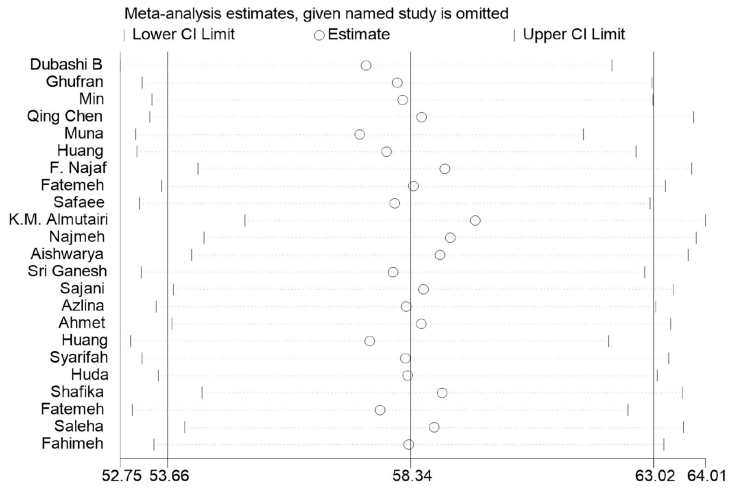


FIGURE 3
Results of sensitivity analysis plot of the global health status.

Discussion

The overall health-related quality of life of Asian breast cancer patients

This study shows that Asian BC patients have a global health status score of 58.34, similar to the results of research conducted by Hashemi (53) in the Middle East. However, compared with BC patients studied from other regions, Asian BC patients have a lower overall quality of life, especially when compared to those in Spain (54) or Germany (55). The literature review revealed that global health status is associated with many factors, such as the operation method (mastectomy or breast preservation) (29), presence of metastases (30), chemotherapy (56), and radiotherapy (57). In addition, the more comorbidities that BC patients have, the lower their quality of life. The survey conducted by Fu et al. (58) revealed that 20%–30% of BC patients present with comorbidities that were pre-existing or that developed after BC diagnosis, such as hypertension, arthritis, and diabetes (59). The research conducted by Miller et al., which addressed BC survivors in African-American and Latina groups, reached the same conclusion, i.e., that having fewer comorbidities means a better HRQoL (60). Therefore, it is necessary to help Asian BC patients to manage and control their comorbidities to enhance their HRQoL.

The functional status of Asian breast cancer patients

QLQ-C30 and QLQ-BR23 evaluate the functional status of BC patients from different perspectives. The meta-analysis demonstrated that for Asian BC patients, emotional functioning and sexual enjoyment were the most severely impaired elements of quality of life during the progression of BC. In terms of emotional functioning, Asian BC patients' scores were lower than those of Brazilian (61) and Mexican patients (62). This may be attributed to the culture of collectivism in Asia, which encourages the suppression of emotions to preserve interpersonal harmony (63). With the understanding that "sharing personal problems with others are [*sic*] regarded as unacceptable since it may make inappropriate demands on the group," Asian BC patients tend to restrain their emotional disclosure (64). This suppression of emotions, however, leads to more negative moods on the one hand, and on the other hand, decreases social functioning by discouraging patients from seeking help from family and society. Conversely, the differences in physical functioning, role functioning, and cognitive functioning are more significantly affected by treatment. Yue Li et al. once conducted a meta-analysis to compare patients' HRQoL status between breast conservation treatment (BCT) and mastectomy, with the results showing significant differences in the levels of the physical, role, and cognitive functioning of patients who received different treatments (65). Moreover, Eman et al. (66) also demonstrated that hormonotherapy could maintain a better functional status for patients than could chemotherapy and radiotherapy.

Although sexual enjoyment presented the lowest score in our study, the score of sexual functioning was similar to that of sexual enjoyment, and both had a negative status, which is different from the studies conducted in Latin America and the Caribbean (67), where patients had different scores for sexual enjoyment and sexual functioning. The decline of the sexual functioning of BC patients could be caused by various treatments, such as the commonly used drugs tamoxifen and aromatase inhibitors in adjuvant endocrine therapy, whose side effects of vaginal dryness and dyspareunia are often reported in reviews (68). Research has also demonstrated that for cancer patients, conserving the breast or not has a significant impact on their sexual wellbeing and satisfaction since the breast is regarded as a secondary sexual characteristic (69). In addition, patients from different cultural backgrounds have quite different attitudes toward sexual knowledge and sexual life. Compared to Western patients, Asian patients treat their sexual life as a private topic and do not like to discuss it publicly; moreover, they even choose random answers when responding to questionnaires to avoid exposing their privacy (32, 40). Thus, healthcare professionals should consider the possibility of inconsistency between the outcomes evaluated and the actual HRQoL status when they provide medical and nursing care for Asian BC patients.

With respect to the assessment of body image, our study's results are almost indistinguishable from those of Polish scholars (70): BC patients' perception of their body image decreases after surgery. Montazeri et al. and Arora et al. stated that a deterioration in women's perception of their body may worsen, even after their general physical condition has recovered and symptoms have been alleviated after surgery and subsequent systemic treatments (71, 72). Influenced by the negative emotion of anxiety, patients are usually pessimistic about their future, body image, and sexual functioning (73). Agnieszka showed that a higher level of emotional, cognitive, and social functioning cannot only help prevent negative assessment of scars in women but can also ensure a better perception of both their body image and future prospects (70). Therefore, paying long-term attention to the BC patients' functional status and providing them with dynamic interventions according to updated psychological data are essential to integrally improving and maintaining their quality of life.

TABLE 6 Meta-regression results.

Tool		HRQoL	Meta-regression (covariates) Region of the country [*p*, Coefficient (95% CI)]	Publication year [*p*, Coefficient (95% CI)]		Mean age [*p*, Coefficient (95% CI)]	Sample size [*p*, Coefficient (95% CI)]	Medical level [P, Coefficient (95% CI)]
QLQ-C30		Global health status	[0.27, -2.49 (-7.01, 2.04)]	[0.66, 1.49 (-5.47, 8.46)]		[0.35, 4.82 (-5.70, 15.34)]	[0.60, -2.36 (-12.62, 6.90]	[0.65, 1.65 (-5.83, 9.12)]
	Functional scales	Physical functioning	[0.02, -5.20 (-9.32, -1.07)]	[0.17, 4.67 (-2.13, 11.46)]		[0.38, 5.14 (-6.88, 17.16)]	[0.90, -0.58 (-10.06, 8.90)]	[0.75, -1.20 (-8.79, 6.39)]
		Role functioning	[0.06, -4.63 (-9.46, 0.20)]	[0.63, 1.87 (-6.02, 9.77)]		[0.83, -1.48 (-15.35, 12.39)]	[0.58, 2.85 (-7.64, 13.35)]	[0.51, -2.73 (-11.14, 5.69)]
		Emotional functioning	[0.16, -4.37 (-10.55, 1.81)]	[0.52, 3.02 (-6.64.12.68)]		[0.42, 6.55 (-9.90, 23.00)]	[0.65, 2.91 (-10.03, 15.85)]	[0.61, -2.60 (-13.01, 7.82)]
		Cognitive functioning	[0.17, -2.61 (-6.38, 1.16)]	[0.31, 2.94 (-2.88, 8.76)]		[0.55, 2.94 (-7.24, 13.12)]	[0.62, -1.93 (-9.81, 5.94)]	[0.79, 0.82 (-5.57, 7.22)]
		Social functioning	[0.17, -3.37 (-8.34, 1.60)]	[0.51, 2.55 (-5.45, 10.55)]		[0.06, 12.09 (-0.39, 24.57)]	[0.94, 0.36 (-10.23, 10.96)]	[0.93, 0.36 (-8.39, 9.11)]
	Symptom scales	Fatigue	[0.06, 6.62 (-0.30, 13.55)]	[0.77, 1.63 (-9.68, 12.94)]		[0.38, -7.62 (-25.38, 10.13)]	[0.60, -0.02 (-0.08.0.05)]	[0.20, 7.41 (-4.27, 19.09)]
		Nausea and vomiting	[0.10, 5.32 (-1.03, 11.66)]	[0.54, -3.18 (-13.78, 7.42)]		[0.46, -5.21 (-19.53, 9.12)]	[0.59, 3.59 (-10.18.17.35)]	[0.74, -1.83 (-13.25, 9.59)]
		Pain	[0.07, 5.81 (-0.59, 12.20)]	[0.42, -4.04 (-14.27, 6.19)]		[0.36, -6.79 (-21.80, 8.21)]	[0.72, 2.45 (-11.35.16.25)]	[0.64, 2.55 (-8.53, 13.62)]
		Dyspnea	[0.10, 6.77 (-1.37, 14.90)]	[0.53, -3.84 (-17.44, 9.77)]		[0.89, -1.36 (-21.00, 18.23)]	[0.86, 1.53 (-16.20, 19.26)]	[0.72, 2.60 (-12.07, 17.26)]
		Insomnia	[0.18, 4.51 (-2.22, 11.24)]	[0.90, -0.66 (-11.24, 9.91)]		[0.90, 0.92 (-14.17, 16.00)]	[0.40.5.67 (-8.17, 19.50)]	[0.51, -3.61 (-14.84, 7.61)]
		Appetite loss	[0.09, 6.43 (-1.00, 13.86)]	[0.65, -2.64 (-14.56, 9.27)]		[0.58, -4.57 (-21.76, 12.63)]	[0.86, 1.39 (-14.57, 17.35)]	[0.85, -1.20 (-14.04, 11.63)]
		Constipation	[0.13, 5.04 (-1.66, 11.75)]	[0.95, 0.33 (-10.82, 11.48)]		[0.80, 2.08 (15.07, 19.22)]	[0.84, 1.42 (-12.99, 15.83)]	[0.86, -1.05 (-12.98, 10.89)]
		Diarrhea	[0.12, 5.89 (-1.62, 13.40)]	[0.86, -1.10 (-13.64, 11.43)]		[0.81, 2.37 (-18.09, 22.83)]	[0.69, -3.09 (-19.27, 13.08)]	[0.98, 0.15 (-13.28, 13.59)]
		Financial difficulties	[0.22, 4.36 (-2.85, 11.57)]	[0.57, -3.13 (-14.39, 8.13)]		[0.12, -13.88 (-31.62, 3.85)]	[0.83, -1.54 (-16.43, 13.35)]	[0.44, 4.27 (-7.17, 15.70)]
QLQ-BR23	Functional scales	Body image	[0.24 -3.43 (-9.48, 2.63)]	[0.70, -1.62 (-10.63, 7.39)]		[0.37, 6.68 (-9.01, 22.38)]	[0.92, 0.56 (-10.85, 11.96)]	[0.91, -0.45, (-9.51, 8.60)]
		Sexual functioning	[0.66, -4.12 (-24.00, 15.78)]	[0.09, -21.22 (-46.73, 4.29)]		[0.55, 15.32 (-70.40, 39.77)]	[0.35, 15.20 (-19.09, 49.48)]	[0.84, 2.75 (-26.73, 32.23)]
		Sexual enjoyment	[0.51, -4.93 (-21.07, 11.21)]	[0.19, -14.03 (-36.22, 8.15)]		[0.51, -12.90 (-55.78, 29.98)]	[0.13, 19.21 (-6.97, 45.39)]	[0.70, 4.24 (-19.85, 28.34)]
		Future perspective	[0.10, 0.03 (-10.17, 10.22)]	[0.96, -0.34 (-13.90, 14.58)]		[0.70, -4.27 (-27.79, 19.26)]	[0.82, 1.78 (-16.17, 19.74)]	[0.61, -3.34 (-17.39, 10.71)]
	Symptom scales	Systemic side effects	[0.18, 6.99 (-3.78, 17.75)]	[0.64, -3.41 (-19.04, 12.21)]		[0.90, 1.62 (-26.25, 29.48)]	[0.42, -7.47 (-27.02, 12.08)]	[0.80, 1.80 (-13.96, 17.56)]
		Breast symptoms	[0.30, 5.95 (-6.16, 18.06)]	[0.18, -10.60 (-26.88, 5.69)]		[0.97, -0.41 (-28.12, 27.30)]	[0.49, -7.09 (-29.10, 14.9)]	[0.46, 5.94 (-11.36, 23.24)]
		Arm symptoms	[0.07, 8.10 (-0.82, 17.02)]	[0.20, -8.35 (-21.74, 5.05)]		[0.86, 1.84 (-20.88, 24.57)]	[0.60, -4.41 (-22.57, 13.74)]	[0.57, 3.77 (-10.51, 18.05)]
		Upset by hair loss	[0.08, 9.42 (-1.53, 20.36)]	[0.34, -8.50 (-27.31, 10.30)]		[0.82, 3.43 (-35.54, 28.69)]	[0.60, -5.42 (-27.92, 17.08)]	[0.42, 6.80 (-11.36, 24.96)]

The experience of symptoms of Asian breast cancer patients

Along with a decline in functioning comes a battery of symptoms caused by drugs, surgeries, and other treatments that damage BC patients' quality of life. Fatigue and being upset by hair loss are the common symptoms of BC patients in Asia. Cancer-related fatigue (CRF)—a persistent state of severe exhaustion—impairs BC patients' quality of life. It not only makes patients' functioning abnormal but also gives rise to an increase in BC incidence and mortality. Nevertheless, CRF is underestimated and underreported by physicians and patients (74–76). Currently, the pathophysiological mechanism of CRF remains unclear, except that surgery, chemotherapy, and hormonotherapy are associated with CRF. Also potentially contributing to different cancer outcomes are the following: less or no access to quality care, differences in tumor biology, and socioeconomic factors influencing treatment options that may be affected by racial differences (77, 78). These may help explain why, when we compared our results to those of Lucas, the degree of fatigue of patients in Asia is more severe than those in Latin America and the Caribbean (67). The etiology and pathogenesis of fatigue in BC patients are complicated and multicausal. Many studies have demonstrated that CRF and other symptom clusters, such as sleep disturbance, mood disorder, and pain, are simultaneous (79). The results of these studies are consistent with our conclusion that fatigue, pain, and insomnia affect BC patients' quality of life to a similar extent. Moreover, inflammation may be the key biological mechanism underlying this symptom cluster since a prior study found that the coexistence of arthralgia, fatigue, and insomnia was associated with an increased level of inflammatory biomarkers among women on endocrine therapy (80).

As evaluated by the symptom scale of EORTC QLQ-C30, financial difficulty is also a serious problem that negatively impacts Asian patients' quality of life. In comparison, Asian BC patients experience greater financial pressure than their American counterparts (67) but less than Egyptians (66). In addition to age, gender, marital status, monthly net income, educational level, and self-reported health status, national income level and health insurance coverage are also associated with financial hardship among BC patients (81–85), which suggests that with all relevant factors taken into consideration, designing a matching benefit package is essential to reducing the financial burden on patients.

Being upset by hair loss is another serious symptom of Asian BC patients according to this meta-analysis. Hair loss is one of the distressing side effects for BC patients who undergo chemotherapy. Hence, we here allude to the term chemotherapy-induced alopecia (CIA), which is usually an unavoidable but transient side effect that can be dealt with by wearing wigs. BC patients are mostly women, and they regard hair as an integral part of their identity. CIA, however, affects their social life, bringing them physical and psychological distress. More seriously, some BC patients may bear the agony of hair loss even after 6 months of chemotherapy, leading to low self-esteem and lower HRQoL (86). Chemotherapy-induced irreversible alopecia (CIIA) usually occurs after high-dose chemotherapy, and married women seem to be more upset about hair loss than others (86, 87). Therefore, some preventive measures, such as scalp cooling or psychological intervention, should be implemented for high-risk CIIA patients to prevent or reduce the degree of their hair loss and emotional distress. Nausea and vomiting, dyspnea, loss of appetite, constipation, and breast and arm symptoms may still affect patients' quality of life. However, the impact is much lighter than those symptoms mentioned above, perhaps because these symptoms usually appear during the acute phase of treatments and can be controlled by specific traditional medicines in Asia (88, 89).

This review used two tools to evaluate the HRQoL of BC patients, namely, EORTC QLQ-C30 and QLQ-BR23. Both tools represent patient-reported outcomes (PRO), which demonstrate the patients' physical, psychological, and social response to disease and therapy from their own perspective but not from that of the physician or anyone else. The evaluation clearly shows that the patients' functioning and symptoms interact with each other, jointly leading to the decline of HRQoL in BC patients. We therefore suggest that a holistic assessment of BC patients is necessary to provide targeted psychological and medical treatments and enhance their quality of life.

Nevertheless, there are still some limitations to this article. First, likely due to the specific methodological limitations of cross-sectional studies included in the meta-analysis, considerable heterogeneity was observed among the studies that evaluated the HRQoL of BC patients. Second, only studies published in English and Chinese were searched, and research projects that did not provide merged data were excluded. Third, gray literature was excluded from our review because of the difficulty of conducting a quality assessment without a detailed description of the methodology and peer review.

Conclusion

BC patients in Asia have a lower HRQoL under the high prevalence, growing incidence, and advanced breast cancer diagnosis at an earlier age. As assessed by EORTC QLQ-C30 and QLQ-BR23, Asian BC patients have different degrees of impairment in physical, social, sexual, and other functioning and suffer from various symptoms, including, but not limited to, fatigue, pain, insomnia, and the influence of financial difficulties, resulting to decline in their quality of life. Therefore, raising awareness of routine breast cancer screening in the Asian population is the first fundamental measure to curb BC deterioration and avoid s adverse impact on HRQoL. Since the

symptoms of BC affect the body functions of BC patients, and serious symptoms of this illness can lead to psychological dilemma and mental disorder and vice versa, the mutual effects change over time; thus, and a dynamic assessment of BC patients and a corresponding treatment plan are essential and should be ensured. Psychological interventions with one-on-one psychotherapy and cognitive behavioral therapy (CBT) can also be introduced to relieve patients' negative emotions caused by poor body image perceptions, such as embarrassment about hair loss and uneasiness about visible scarring. In addition, encouraging patients to seek help from their social networks could be an important approach to improving their psychological state. For pain, insomnia, arm dysfunction, and other symptoms, it is recommended that caregivers use traditional Asian medicine to help BC patients alleviate those symptoms in a cost-effective way. Finally, in view of the high prevalence of breast cancer in Asia, each country in Asia should do its best to increase the coverage of their healthcare systems so that BC patients' financial difficulties may be alleviated.

Data availability statement

The original contributions presented in the study are included in the article and Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

All authors contributed to the article and approved the version submitted. JG and CH proposed the concept and monitored the progress of the work. CH provided the ideas for article revisions. CW and DB designed the work and provided solutions to the inconsistencies. XC, CW, and TC performed the literature search, study selection, and data extraction, and then wrote the manuscript. XC and CW undertook data analysis and interpretation. XC, CW, and DB wrote the manuscript and made the equal contributions to the article. Meanwhile, LZ and HL helped to review and check the manuscript. CW and DB contributed to the revision of the manuscript. All the authors approved the submitted version and agreed to be personally accountable to their own contributions and to ensure that questions related to the accuracy or integrity of any part of the work are addressed.

Funding

This study was supported by grants from the Sichuan Federation of Social Science Associations (NO. SC22B149 and NO. SC22B150), the Health Commission of Sichuan Province (NO. 21PJ109), and the Sichuan Mental Health Education Research Center (NO. XLJKJY2203A).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.954179/full#supplementary-material

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. EBCTCG (Early Breast Cancer Trialists' Collaborative Group), McGale P, Taylor C, Correa C, Cutter D, Duane F, et al. Effect of radiotherapy after mastectomy and axillary surgery on 10-year recurrence and 20-year breast cancer mortality: Meta-analysis of individual patient data for 8135 women in 22 randomised trials. *Lancet* (2014) 383(9935):2127–35. doi: 10.1016/S0140-6736(14)60488-8

3. Wong IO, Schooling CM, Cowling BJ, Leung GM. Breast cancer incidence and mortality in a transitioning Chinese population: Current and future trends. *Br J Cancer.* (2015) 112(1):167–70. doi: 10.1038/bjc.2014.532

4. Sung H, Rosenberg PS, Chen WQ, Hartman M, Lim WY, Chia KS, et al. Female breast cancer incidence among Asian and Western populations: More similar than expected. *J Natl Cancer Inst* (2015) 107(7):djv107. doi: 10.1093/jnci/djv107

5. Porter P. Westernizing" women's risks? Breast cancer in lower-income countries. *N Engl J Med* (2008) 358(3):213–6. doi: 10.1056/NEJMp0708307

6. Hossain MS, Ferdous S, Karim-Kos HE. Breast cancer in south Asia: A Bangladeshi perspective. *Cancer Epidemiol.* (2014) 38(5):465–70. doi: 10.1016/j.canep.2014.08.004

7. Sinnadurai S, Kwong A, Hartman M, Tan EY, Bhoo-Pathy NT, Dahlui M, et al. Breast-conserving surgery versus mastectomy in young women with breast cancer in Asian settings. *BJS Open* (2018) 3(1):48–55. doi: 10.1002/bjs5.50111

8. Lazovich D, Solomon CC, Thomas DB, Moe RE, White E. Breast conservation therapy in the united states following the 1990 national institutes of health consensus development conference on the treatment of patients with early stage invasive breast carcinoma. *Cancer* (1999) 86(4):628–37. doi: 10.1002/(SICI)1097-0142(19990815)86:4<628::AID-CNCR11>3.0.CO;2-L

9. Pathy NB, Yip CH, Taib NA, Hartman M, Saxena N, Iau P, et al. Breast cancer in a multi-ethnic Asian setting: results from the Singapore-Malaysia hospital-based breast cancer registry. *Breast* (2011) 20 Suppl 2:S75–80. doi: 10.1016/j.breast.2011.01.015

10. Kwong A, Mang OW, Wong CH, Chau WW, Law SCHong Kong Breast Cancer Research Group. Breast cancer in Hong Kong, southern China: the first population-based analysis of epidemiological characteristics, stage-specific, cancer-specific, and disease-free survival in breast cancer patients: 1997-2001. *Ann Surg Oncol* (2011) 18(11):3072–8. doi: 10.1245/s10434-011-1960-4

11. Raina V, Bhutani M, Bedi R, Sharma A, Deo SV, Shukla NK, et al. Clinical features and prognostic factors of early breast cancer at a major cancer center in north India. *Indian J Cancer.* (2005) 42(1):40–5. doi: 10.4103/0019-509x.15099

12. Kummerow KL, Du L, Penson DF, Shyr Y, Hooks MA. Nationwide trends in mastectomy for early-stage breast cancer. *JAMA Surg* (2015) 150(1):9–16. doi: 10.1001/jamasurg.2014.2895

13. Garcia-Etienne CA, Tomatis M, Heil J, Friedrichs K, Kreienberg R, Denk A, et al. Mastectomy trends for early-stage breast cancer: A report from the EUSOMA multi-institutional European database. *Eur J Cancer* (2012) 48(13):1947–56. doi: 10.1016/j.ejca.2012.03.008

14. Kosmidis P. Quality of life as a new end point. *Chest* (1996) 109(5 Suppl):110S–2S. doi: 10.1378/chest.109.5_supplement.110s

15. Triberti S, Savioni L, Sebri V, Pravettoni G. eHealth for improving quality of life in breast cancer patients: A systematic review. *Cancer Treat Rev* (2019) 74:1–14. doi: 10.1016/j.ctrv.2019.01.003

16. Jacobs DHM, Charaghvandi RK, Horeweg N, Maduro JH, Speijer G, Roeloffzen EMA, et al. Health-related quality of life of early-stage breast cancer patients after different radiotherapy regimens. *Breast Cancer Res Treat* (2021) 189 (2):387–98. doi: 10.1007/s10549-021-06314-4

17. Trusson D, Pilnick A, Roy S. A new normal?: women's experiences of biographical disruption and liminality following treatment for early stage breast cancer. *Soc Sci Med* (2016) 151:121–9. doi: 10.1016/j.socscimed.2016.01.011

18. Woertman L, van den Brink F. Body image and female sexual functioning and behavior: A review. *J Sex Res* (2012) 49(2-3):184–211. doi: 10.1080/00224499.2012.658586

19. Canada AL, Schover LR. The psychosocial impact of interrupted childbearing in long-term female cancer survivors. *Psychooncology* (2012) 21 (2):134–43. doi: 10.1002/pon.1875

20. Ljungman L, Ahlgren J, Petersson LM, Flynn KE, Weinfurt K, Gorman JR, et al. Sexual dysfunction and reproductive concerns in young women with breast cancer: Type, prevalence, and predictors of problems. *Psychooncology* (2018) 27 (12):2770–7. doi: 10.1002/pon.4886

21. Bártolo A, Santos IM, Valério E, Monteiro S. Depression and health-related quality of life among young adult breast cancer patients: The mediating role of reproductive concerns. *J Adolesc Young Adult Oncol* (2020) 9(3):431–5. doi: 10.1089/jayao.2019.0144

22. McGinty HL, Small BJ, Laronga C, Jacobsen PB. Predictors and patterns of fear of cancer recurrence in breast cancer survivors. *Health Psychol* (2016) 35(1):1–9. doi: 10.1037/hea0000238

23. Cella DF. Quality of life: concepts and definition. *J Pain Symptom. Manage* (1994) 9(3):186–92. doi: 10.1016/0885-3924(94)90129-5

24. Chui PL, Abdullah KL, Wong LP, Taib NA. Quality of life in CAM and non-CAM users among breast cancer patients during chemotherapy in Malaysia. *PloS One* (2015) 10(10):e0139952. doi: 10.1371/journal.pone.0139952

25. Kang E, Yang EJ, Kim SM, Chung IY, Han SA, Ku DH, et al. Complementary and alternative medicine use and assessment of quality of life in Korean breast cancer patients: A descriptive study. *Support Care Cancer.* (2012) 20 (3):461–73. doi: 10.1007/s00520-011-1094-z

26. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *BMJ* (2009) 339: b2535. doi: 10.1136/bmj

27. Peters M., Godfrey C., McInerney P., Soares C. B., Khalil H., Parker D. Methodology for JBI scoping reviews. In: *The Joanna Briggs institute reviewers' manual 2015 (Joanna Briggs Institute)* (2015).

28. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: A tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* (2016) 12:355:i4919. doi: 10.1136/bmj.i4919

29. Dubashi B, Vidhubala E, Cyriac S, Sagar TG. Quality of life among younger women with breast cancer: study from a tertiary cancer institute in south India. *Indian J Cancer.* (2010) 47(2):142–7. doi: 10.4103/0019-509X.63005

30. Jassim GA, Whitford DL. Quality of life of bahraini women with breast cancer: A cross sectional study. *BMC Cancer.* (2013) 28:13:212. doi: 10.1186/1471-2407-13-212

31. Naung MT, Panza A. Quality of life and relationship between functioning and symptoms of female patients with breast cancer before chemotherapy in a cancer clinic At Yangon,Myanmar. *J Ayub Med Coll Abbottabad.* (2020) 32(4):540–5.

32. Chen Q, Li S, Wang M, Liu L, Chen G. Health-related quality of life among women breast cancer patients in Eastern China. *BioMed Res Int* (2018) 2018:1452635. doi: 10.1155/2018/1452635

33. Maharjan M, Thapa N, Adhikari RD, Petrini MA, Amatya KS. Quality of life of Nepalese women post mastectomy. *Asian Pac J Cancer Prev* (2018) 19(4):1005–12. doi: 10.22034/APJCP.2018.19.4.1005

34. Ou HT, Chung WP, Su PF, Lin TH, Lin JY, Wen YC, et al. Health-related quality of life associated with different cancer treatments in Chinese breast cancer survivors in Taiwan. *Eur J Cancer Care (Engl).* (2019) 28(4):e13069. doi: 10.1111/ecc.13069

35. Najafi F, Nedjat S, Zendehdel K, Mirzania M, Montazeri A. Self-reported versus proxy reported quality of life for breast cancer patients in the Islamic republic of Iran. *East Mediterr. Health J* (2017) 22(11):786–93. doi: 10.26719/2016.22.11.786

36. Mirzaei F, Farshbaf-Khalili A, Nourizadeh R, Zamiri RE. Quality of life and its predictors in Iranian women with breast cancer undergoing chemotherapy and radiotherapy. *Indian J Cancer.* (2021) 58(1):76–83. doi: 10.4103/ijc.IJC_750_18

37. Safaee A, Moghimi-Dehkordi B, Zeighami B, Tabatabaee H, Pourhoseingholi M. Predictors of quality of life in breast cancer patients under chemotherapy. *Indian J Cancer.* (2008) 45(3):107–11. doi: 10.4103/0019-509x.44066

38. Almutairi KM, Mansour EA. Vinluan JM. *A cross-sectional Assess Qual Life Breast Cancer patients Saudi Arabia. Public Health* (2016) 136:117–25. doi: 10.1016/j.puhe.2016.03.008

39. Jafari N, Farajzadegan Z, Zamani A, Bahrami F, Emami H, Loghmani A. Spiritual well-being and quality of life in Iranian women with breast cancer undergoing radiation therapy. *Support Care Cancer.* (2013) 21(5):1219–25. doi: 10.1007/s00520-012-1650-1

40. Kshirsagar AS, Wani SK. Health-related quality of life in patients with breast cancer surgery and undergoing chemotherapy in ahmednagar district. *J Cancer Res Ther* (2021) 17(6):1335–8. doi: 10.4103/jcrt.JCRT_154_19

41. Ganesh S, Lye MS, Lau FN. Quality of life among breast cancer patients in Malaysia. *Asian Pac J Cancer Prev* (2016) 17(4):1677–84. doi: 10.7314/apjcp.2016.17.4.1677

42. Manandhar S, Shrestha DS, Taechaboonsermsk P, Siri S, Suparp J. Quality of life among breast cancer patients undergoing treatment in national cancer centers in Nepal. *Asian Pac J Cancer Prev* (2014) 15(22):9753–7. doi: 10.7314/apjcp.2014.15.22.9753

43. Yusuf A, Ahmad Z, Keng SL. Quality of life in Malay and Chinese women newly diagnosed with breast cancer in kelantan, Malaysia. *Asian Pac J Cancer Prev* (2013) 14(1):435–40. doi: 10.7314/apjcp.2013.14.1.435

44. Alacacioglu A, Yavuzsen T, Dirioz M, Yilmaz U. Quality of life, anxiety and depression in Turkish breast cancer patients and in their husbands. *Med Oncol* (2009) 26(4):415–9. doi: 10.1007/s12032-008-9138-z

45. Huang HY, Tsai WC, Chou WY, Hung YC, Liu LC, Huang KF, et al. Quality of life of breast and cervical cancer survivors. *BMC Womens Health* (2017) 17(1):30. doi: 10.1186/s12905-017-0387-x

46. Syed Alwi SM, Narayanan V, Mohd Taib NA, Che Din N. Predictors of health-related quality of life after completion of chemotherapy among Malaysian early-stage breast cancer survivors. *Support Care Cancer.* (2022) 30(3):2793–801. doi: 10.1007/s00520-021-06686-9

47. Abu-Saad Huijer H, Abboud S. Health-related quality of life among breast cancer patients in Lebanon. *Eur J Oncol Nurs.* (2012) 16(5):491–7. doi: 10.1016/j.ejon.2011.11.003

48. Alawadi SA, Ohaeri JU. Health - related quality of life of Kuwaiti women with breast cancer: A comparative study using the EORTC quality of life questionnaire. *BMC Cancer.* (2009) 9:222. doi: 10.1186/1471-2407-9-222

49. Homaee Shandiz F, Karimi FZ, Khosravi Anbaran Z, Abdollahi M, Rahimi N, Ghasemi M, et al. Investigating the quality of life and the related factors in Iranian women with breast cancer. *Asian Pac J Cancer Prev* (2017) 18(8):2089–92. doi: 10.22034/APJCP.2017.18.8.2089

50. Saleha SB, SHAKEEL A, Shumaila E, Shazia R., Rashid R., Ibaham M, et al. An assessment of quality of life in breast cancer patients using EORTC QLQ C30/+

Br23 questionnaire. *International Journal Of Cancer Management* (2010) 3:98–104. doi: 10.1186/1471-2288-11-56

51. Sehati Shafaee F, Mirghafourvand M, Harischi S, Esfahani A, Amirzehni J. Self-confidence and quality of life in women undergoing treatment for breast cancer. *Asian Pac J Cancer Prev* (2018) 19(3):733–40. doi: 10.22034/APJCP.2018.19.3.733

52. GBD 2016 Healthcare Access and Quality Collaborators. Measuring performance on the healthcare access and quality index for 195 countries and territories and selected subnational locations: A systematic analysis from the global burden of disease study 2016. *Lancet* (2018) 391(10136):2236–71. doi: 10.1016/S0140-6736(18)30994-2

53. Hashemi SM, Balouchi A, Al-Mawali A, Rafiemanesh H, Rezaie-Keikhaie K, Bouya S, et al. Health-related quality of life of breast cancer patients in the Eastern Mediterranean region: A systematic review and meta-analysis. *Breast Cancer Res Treat* (2019) 174(3):585–96. doi: 10.1007/s10549-019-05131-0

54. Villar RR, Fernández SP, Garea CC, Pillado MTS, Barreiro VB, Martín CG. Quality of life and anxiety in women with breast cancer before and after treatment. *Rev Lat Am Enfermagem.* (2017) 25:e2958. doi: 10.1590/1518-8345.2258.2958

55. Engel J, Schlesinger-Raab A, Emeny R, Hölzel D, Schubert-Fritschle G. Quality of life in women with localised breast cancer or malignant melanoma 2 years after initial treatment: A comparison. *Int J Behav Med* (2014) 21(3):478–86. doi: 10.1007/s12529-013-9334-x

56. Li Y, Zhou Z, Ni N, Luan Z, Peng X. Quality of life and hope of women in China receiving chemotherapy for breast cancer. *Clin Nurs Res* (2021) 14. doi: 10.1177/10547738211046737

57. Hauth F, De-Colle C, Weidner N, Heinrich V, Zips D, Gani C. Quality of life and fatigue before and after radiotherapy in breast cancer patients. *Strahlenther. Onkol.* (2021) 197(4):281–7. doi: 10.1007/s00066-020-01700-1

58. Fu MR, Axelrod D, Guth AA, et al. Comorbidities and quality of life among breast cancer survivors: A prospective study. *J Pers Med* (2015) 5(3):229–42. doi: 10.3390/jpm5030229

59. Søgaard M, Thomsen RW, Bossen KS, Sørensen HT, Nørgaard M. The impact of comorbidity on cancer survival: A review. *Clin Epidemiol.* (2013) 5(Suppl 1):3–29. doi: 10.2147/CLEP.S47150

60. Miller AM, Ashing KT, Modeste NN, Herring RP, Sealy DA. Contextual factors influencing health-related quality of life in African American and latina breast cancer survivors. *J Cancer Surviv.* (2015) 9(3):441–9. doi: 10.1007/s11764-014-0420-0

61. Marcelo Castro E Silva I, Lúcia Penteado Lancellotti C. Health-related quality of life in women with breast cancer undergoing chemotherapy in Brazil. *Int J Gen Med* (2021) 14:10265–70. doi: 10.2147/IJGM.S343804

62. Cortés-Flores AO, Morgan-Villela G, Zuloaga-Fernández del Valle CJ, et al. Quality of life among women treated for breast cancer: A survey of three procedures in Mexico. *Aesthetic Plast Surg* (2014) 38(5):887–95. doi: 10.1007/s00266-014-0384-5

63. Wei M, Su JC, Carrera S, Lin SP, Yi F. Suppression and interpersonal harmony: A cross-cultural comparison between Chinese and European americans. *J Couns Psychol* (2013) 60(4):625–33. doi: 10.1037/a0033413

64. Chu Q, Wong CCY, Lu Q. Social constraints and PTSD among Chinese American breast cancer survivors: not all kinds of social support provide relief. *J Behav Med* (2021) 44(1):29–37. doi: 10.1007/s10865-020-00165-y

65. Li Y, Guo J, Sui Y, Chen B, Li D, Jiang J. Quality of life in patients with breast cancer following breast conservation surgery: A systematic review and meta-analysis. *J Healthc. Eng.* (2022) 2022:3877984. doi: 10.1155/2022/3877984

66. Mortada EM, Salem RA, Elseifi OS, Khalil OM. Comparing health-related quality of life among breast cancer patients receiving different plans of treatment, Egypt. *J Community Health* (2018) 43(6):1183–91. doi: 10.1007/s10900-018-0538-5

67. Gonzalez L, Bardach A, Palacios A, Peckaitis C, Ciapponi A, Pichón-Riviere A, et al. Health-related quality of life in patients with breast cancer in Latin America and the Caribbean: A systematic review and meta-analysis. *Oncologist* (2021) 26(5):e794–806. doi: 10.1002/onco.13709

68. Cella D, Fallowfield LJ. Recognition and management of treatment-related side effects for breast cancer patients receiving adjuvant endocrine therapy. *Breast Cancer Res Treat* (2008) 107(2):167–80. doi: 10.1007/s10549-007-9548-1

69. Stein MJ, Karir A, Arnaout A, Roberts A, Cordeiro E, Zhang T, et al. Quality-of-Life and surgical outcomes for breast cancer patients treated with therapeutic reduction mammoplasty versus mastectomy with immediate reconstruction. *Ann Surg Oncol* (2020) 27(11):4502–12. doi: 10.1245/s10434-020-08574-8

70. Słowik AJ, Jabłoński MJ, Michałowska-Kaczmarczyk AM, Jach R. Evaluation of quality of life in women with breast cancer, with particular emphasis on sexual satisfaction, future perspectives and body image, depending on the method of surgery. *Psychiatr Pol* (2017) 51(5):871–88. doi: 10.12740/PP/OnlineFirst/63787

71. Montazeri A, Vahdaninia M, Harirchi I. Quality of life in patients with breast cancer before and after diagnosis: An eighteen months follow-up study. *BMC Cancer.* (2008) 8:330. doi: 10.1186/1471-2407-8-330

72. Arora NK, Gustafson DH, Hawkins RP, McTavish F, Cella DF, Pingree S, et al. Impact of surgery and chemotherapy on the quality of life of younger women with breast carcinoma: A prospective study. *Cancer* (2001) 92(5):1288–98. doi: 10.1002/1097-0142(20010901)92:5<1288::aid-cncr1450>3.0.co;2-e

73. Van Esch L, Roukema JA, van der Steeg AF, De Vries J. Trait anxiety predicts disease-specific health status in early-stage breast cancer patients. *Qual. Life Res* (2011) 20(6):865–73. doi: 10.1007/s11136-010-9830-2

74. Wang XS, Woodruff JF. Cancer-related and treatment-related fatigue. *Gynecol. Oncol* (2015) 136(3):446–52. doi: 10.1016/j.ygyno.2014.10.013

75. Ebede CC, Jang Y, Escalante CP. Cancer-related fatigue in cancer survivorship. *Med Clin North Am* (2017) 101(6):1085–97. doi: 10.1016/j.mcna.2017.06.007

76. Mohandas H, Jaganathan SK, Mani MP, Ayyar M, Rohini Thevi GV. Cancer-related fatigue treatment: An overview. *J Cancer Res Ther* (2017) 13 (6):916–29. doi: 10.4103/jcrt.JCRT_50_17

77. Grimison PS, Stockler MR. Quality of life and adjuvant systemic therapy for early-stage breast cancer. *Expert Rev Anticancer Ther* (2007) 7(8):1123–34. doi: 10.1586/14737140.7.8.1123

78. Coughlin SS, Yoo W, Whitehead MS, Smith SA. Advancing breast cancer survivorship among African-American women. *Breast Cancer Res Treat* (2015) 153 (2):253–61. doi: 10.1007/s10549-015-3548-3

79. Kenne Sarenmalm E, Browall M, Gaston-Johansson F. Symptom burden clusters: A challenge for targeted symptom management. a longitudinal study examining symptom burden clusters in breast cancer. *J Pain Symptom. Manage* (2014) 47(4):731–41. doi: 10.1016/j.jpainsymman.2013.05.012

80. Bauml J, Chen L, Chen J, Boyer J, Kalos M, Li SQ, et al. Arthralgia among women taking aromatase inhibitors: is there a shared inflammatory mechanism with co-morbid fatigue and insomnia? *Breast Cancer Res* (2015) 17(1):89. doi: 10.1186/s13058-015-0599-7

81. Shankaran V, Jolly S, Blough D, Ramsey SD. Risk factors for financial hardship in patients receiving adjuvant chemotherapy for colon cancer: A population-based exploratory analysis. *J Clin Oncol* (2012) 30(14):1608–14. doi: 10.1200/JCO.2011.37.9511

82. Dee EC, Nipp RD, Muralidhar V, Yu Z, Butler SS, Mahal BA, et al. Financial worry and psychological distress among cancer survivors in the united states, 2013-2018. *Support Care Cancer.* (2021) 29(9):5523–35. doi: 10.1007/s00520-021-06084-1

83. Knight TG, Deal AM, Dusetzina SB, Muss HB, Choi SK, Bensen JT, et al. Financial toxicity in adults with cancer: Adverse outcomes and noncompliance. *J Oncol Pract* (2018) 24:JOP1800120. doi: 10.1200/JOP.18.00120

84. Yabroff KR, Dowling EC, Guy GPJr, Banegas MP, Davidoff A, Han X, et al. Financial hardship associated with cancer in the united states: Findings from a population-based sample of adult cancer survivors. *J Clin Oncol* (2016) 34(3):259–67. doi: 10.1200/JCO.2015.62.0468

85. Kong YC, Wong LP, Ng CW, Taib NA, Bhoo-Pathy NT, Yusof MM, et al. Understanding the financial needs following diagnosis of breast cancer in a setting with universal health coverage. *Oncologist* (2020) 25(6):497–504. doi: 10.1634/theoncologist.2019-0426

86. Kim GM, Kim S, Park HS, Kim JY, Nam S, Park S, et al. Chemotherapy-induced irreversible alopecia in early breast cancer patients. *Breast Cancer Res Treat* (2017) 163(3):527–33. doi: 10.1007/s10549-017-4204-x

87. Konieczny M, Cipora E, Sygit K, Fal A. Quality of life of women with breast cancer and socio-demographic factors. *Asian Pac J Cancer Prev* (2020) 21(1):185–93. doi: 10.31557/APJCP.2020.21.1.185

88. Shi G, Yu D, Wu J, Liu Y, Huang R, Zhang CS. A systematic review and meta-analysis of traditional Chinese medicine with chemotherapy in breast cancer. *Gland Surg* (2021) 10(5):1744–55. doi: 10.21037/gs-21-284

89. Karia P, Patel KV, Rathod SSP. Breast cancer amelioration by butea monosperma *in-vitro* and *in-vivo*. *J Ethnopharmacol* (2018) 217:54–62. doi: 10.1016/j.jep.2017.12.026

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 07 November 2022
DOI 10.3389/fonc.2022.1037831

OPEN ACCESS

EDITED BY
Maria Rosaria De Miglio,
University of Sassari, Italy

REVIEWED BY
Daisuke Takahashi,
Keio University, Japan
Alejandra de Moreno de LeBlanc,
CONICET Centro de Referencia para Lactobacilos (CERELA), Argentina

*CORRESPONDENCE
Guoshuang Shen
Guoshuangshen@126.com

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 07 September 2022
ACCEPTED 19 October 2022
PUBLISHED 07 November 2022

CITATION
Zhang J, Xie Q, Huo X, Liu Z, Da M, Yuan M, Zhao Y and Shen G (2022) Impact of intestinal dysbiosis on breast cancer metastasis and progression. *Front. Oncol.* 12:1037831. doi: 10.3389/fonc.2022.1037831

Impact of intestinal dysbiosis on breast cancer metastasis and progression

Jin Zhang, Qiqi Xie, Xingfa Huo, Zhilin Liu, Mengting Da, Mingxue Yuan, Yi Zhao and Guoshuang Shen*

Affiliated Hospital of Qinghai University, Affiliated Cancer Hospital of Qinghai University, Xining, China

Breast cancer has a high mortality rate among malignant tumors, with metastases identified as the main cause of the high mortality. Dysbiosis of the gut microbiota has become a key factor in the development, treatment, and prognosis of breast cancer. The many microorganisms that make up the gut flora have a symbiotic relationship with their host and, through the regulation of host immune responses and metabolic pathways, are involved in important physiologic activities in the human body, posing a significant risk to health. In this review, we build on the interactions between breast tissue (including tumor tissue, tissue adjacent to the tumor, and samples from healthy women) and the microbiota, then explore factors associated with metastatic breast cancer and dysbiosis of the gut flora from multiple perspectives, including enterotoxigenic Bacteroides fragilis, antibiotic use, changes in gut microbial metabolites, changes in the balance of the probiotic environment and diet. These factors highlight the existence of a complex relationship between host-breast cancer progression-gut flora. Suggesting that gut flora dysbiosis may be a host-intrinsic factor affecting breast cancer metastasis and progression not only informs our understanding of the role of microbiota dysbiosis in breast cancer development and metastasis, but also the importance of balancing gut flora dysbiosis and clinical practice.

KEYWORDS

gastrointestinal flora, intestinal dysbiosis, breast cancer, metastasis, progression

Abbreviations: TNBC, Triple-negative breast tumors; LPS, Lipopolysaccharide; iNKT, Invariant natural killer T; GLP-1, glucagon-like peptide-1; BBB, blood-brain barrier; BC, Breast cancer; VNMA, vancomycin, neomycin, metronidazole, and amphotericin; SCFAs, short-chain fatty acids; LCA, lithophanic acid; HR+, hormone receptor–positive; FM, fermented milk; FMT, fecal microbiota transplantation; ETBF, enterotoxigenic B. Fragilis; BFT, B. fragilis toxin.

1 Background

1.1 Epidemiology and staging of breast cancer

The 2020 Global Cancer Statistics report shows that female breast cancer is the most common cancer worldwide, with the highest number of new cases annually (approximately 11.7% of all new cases in both men and women), having overtaken lung cancer (11.4%) (1). There are four main subtypes of breast cancer, approximately 75% of them are positive for ER and/or PR (2). The luminal A (ER and PR positive, HER2 negative, low Ki67) subtype accounts for approximately 40% of all cases; it is characterized by low invasiveness, a low recurrence rate, a high survival rate, and the best response to hormonal therapy (3). In turn, the luminal B (ER and PR positive, HER2 positive or HER2 negative, with high Ki67) subtype is responsible for 10–20% of all cancer cases, has a higher relapse rate, proliferative index, and lower recurrence survival (4–6). HER2 positive (non-luminal) were defined as HER2 overexpression or amplification, ER and PR absence, and survival rate significantly improvement with targeted therapy (7). Triple-negative breast tumors (TNBC) are defined as ER, PR, and HER2 negative. TNBC which makes up approximately 15% of all breast tumors and have a high risk of distant relapse in the first 3 to 5 years following diagnosis (8, 9).

With advances in early diagnosis and comprehensive treatment, the prognosis for patients with breast cancer has improved; however, the incidence of metastasis is also increasing (10). It has been reported that 20%–30% of patients with breast cancer can develop metastases after diagnosis and treatment of the primary tumor, with metastases being the cause of approximately 90% of deaths (11). Breast cancer shows a tendency to metastasize to a variety of organs, including bone, lung, liver, and brain, which is termed metastatic heterogeneity. Bone metastases account for approximately 75% of metastases (12), with an overall 5-year survival rate of 22.8% (13). Lung is the second most common site of breast cancer metastasis (14), with an overall 5-year survival rate of 16.8%. The liver is second only to lung as a metastasis site, but survival is poor relative to local, bone, and lung recurrences, with an expected 5-year overall survival rate of 8.5% (15). Brain accounts for approximately 15%–30% of metastatic sites in patients with metastatic breast cancer, limiting quality of life and a very short life expectancy (16–18).

The priority of metastasis varies from organ to organ, resulting in differences in prognosis and treatment response. A widely accepted model of metastasis is the "seed and soil" hypothesis proposed by Paget (19), which initially revealed that successful colonization of second organs depends on the intrinsic properties of the tumor cells and the compatibility and support of the microenvironment.

1.2 Intestinal flora dysbiosis

1.2.1 Gut microbiota composition in human health

A dynamic balance is maintained between the microbiota and the host, and this balance plays an important role in human health by influencing the physiological functions of the organism. A healthy intestinal microbiota is composed mainly of the *phyla Firmicutes* and *Bacteroidetes*, followed by *the phyla Actinobacteria* and *Verrucomicrobia* (20). The distribution of microorganisms in the gastrointestinal tract varies longitudinally from the esophagus to the rectum, Helicobacter is the dominant species in the stomach and determines the microbial status of the entire gastric flora. While H. pylori inhabits the stomach as a commensal, other genera constitute the rich diversity of the gastric flora (21, 22). Conversely, this diversity is reduced when *H. pylori* cause disease. *Firmicutes* and *Actinobacteria* are the most dominant phylum in the duodenum (23). The jejunum is dominated by the growth of Gram-positive aerobic and facultative anaerobes, including *Lactobacilli, Enterococci* and *Streptococci*. In the ileum, with predominance of aerobic species, while the distal ileum has a similar bacterial body to the colon, with anaerobes and Gram-negative organisms (23). The bacteriophage in the large intestine is dominated by *the phyla Firmicutes* and *Bacteroidetes*. Furthermore, there are other important pathogens in the human colon, such as *Campylobacter jejuni*, *Salmonella enterica*, *Vibrio cholera and Escherichia coli (E. coli)*, and *Bacteroides fragilis* (24, 25). The abundance of the Proteobacteria phylum is significantly lower in normal humans, and its absence along with the high abundance of genera such as *Bacteroides*, *Prevotella* and *Ruminococcus* indicates a healthy gut microbiota (26).

1.2.2 Gut microbiota function

Intestinal flora homeostasis has an important role in maintaining normal body function, The gut microflora creates a stable mucosal barrier for the intestine to prevent the invasion of pathogenic microorganisms (27). Gut microbes break down non-digestible compounds through anaerobic fermentation to produce compounds of short-chain fatty acids (SCFAs), which have good anti-inflammatory and chemopreventive properties and act as barrier protectors (28, 29) and are considered as tumor suppressors (30). Microorganisms containing Lipopolysaccharide (LPS), such as Salmonella and Escherichia coli, activate antigen presenting cells through pattern recognition receptors to produce cytokines, which together with endogenous glycolipid antigens and the major histocompatibility complex (MHC) class I-related glycoprotein CD1d activate Invariant natural killer T (iNKT) cells and participate in various immunomodulatory responses (31, 32). In addition, many intestinal microbiota are involved in bone

remodeling processes as immunomodulators, such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus GG*, *Lactobacillus reuteri*, *Lactobacillus paracasei* and *Bacillus clausii* (33). The gut microbiota regulates nutrient metabolism by regulating lipid metabolism, propionic acid in short-chain fatty acids reduces fatty acid levels in liver and plasma and reduces food intake (34), and the gut microbiota regulates intestinal and plasma Lipopolysaccharide (LPS) levels by modulating the intestinal endocannabinoid (eCB) system (35), which affects adipose tissue metabolism. Intestinal flora are involved in the production of gastrin, insulin (36) and glucagon-like peptide-1 (GLP-1) (37, 38) through a paracrine pathway produced by enterocytes, and it is also involved in the synthesis of bile acids, cholesterol, bound fatty acids (39) and vitamin (40), thereby regulation of endocrine levels and metabolic changes in the host. The gut microbiota synthesizes a number of neurochemicals, (e.g., gamma amino butyric acid (GABA): an inhibitory neurotransmitter), which influence central nervous and gut function (41). A gut-brain microbial axis exists between gut microbes, the gastrointestinal tract and the central nervous system (42), which links brain emotional centers to mechanisms such as gut function, gut neural reflexes and gut endocrine signaling to jointly coordinate organismal changes (43, 44). Circulating SCFAs produced by gut microbiota metabolism affect the integrity of the blood-brain barrier (BBB) by increasing the production of tight junction proteins, and increased BBB integrity reduces the entry of undesirable metabolites into brain tissue and strengthens the defense mechanisms of the blood-brain barrier (45). Compounds produced by the metabolism of the gut microbiota, such as lipoproteins and lipopolysaccharides, affect autoimmune function by stimulating the release of cytokines from immune cells. These cytokines can cross the BBB and activate neurons, altering neurological function and leading to changes in mood and behavior (46), providing new ideas for the treatment of brain dysfunction.

1.2.3 Dysbiosis

Dysbiosis refers to a state in which the intestinal flora loses its normal "beneficial" function and is continuously disturbed, causing disease. It is associated with a large proportional change in the composition of the microbiota beyond the normal range caused by host-related and environmental factors (47). Dysbiosis is usually characterized by the following feature: Bloom of pathobionts (48), Loss of commensals (49) and Loss of alpha diversity (50–52), which can be present individually or simultaneously and mutually exclusive. Currently, dysbiosis has a causal relationship with the manifestation, diagnosis or treatment of specific diseases, from the perspective of the composition of the intestinal microflora, mainly originating from Infection and inflammation (53, 54); Diet and xenobiotics (55, 56); Genetics (57) and Familial transmission (58–60) etc.

1.2.4 Link between dysbiosis and cancer

Dysbiosis states may negatively affect the organism leading to various disease states. The microbiota may have some tumor suppressive effects on the host, and deviations in flora balance may be associated with cancer development (61). Studies (62–67) have identified direct and indirect roles of the gut microbiota in carcinogenesis, cancer treatment and prevention. including colon (66, 68, 69), gastric (70–73), lung (74, 75), prostate (76–78) and breast cancers (79) (Tables 1, 2), and suggest that the gut microbiota and these cancers are interlinked through tumor suppression and tumor initiation factors. Modification of the composition and activity of the intestinal flora through the administration of prebiotics, probiotics and synthetics, providing benefits to patients with colorectal cancer, such as: modulation of immunity, improvement of bile acid metabolism and restoration of intestinal microbial diversity (68). *H. pylori* is one of the major causative factors of gastric cancer. Probiotics against *H. pylori* through various mechanisms, including: secretion of antibacterial compounds; inhibition of *H. pylori* colonization; action through stimulation of mucin synthesis; and modulation of host immune response, which provides new perspectives on gastric cancer prevention and treatment (100). It was found that memory T and NK cell profiles were increased in peripheral blood samples from patients with beneficial and diversity-rich gut microbes. This has important implications for predicting the response to anti-PD-1 immunotherapy in Chinese non-small-cell lung cancer patients (101). Gram-positive bacteria stimulate the production of specific subpopulations of "pathogenic" T helper 17 (pTh17) cells and memory Th1 immune responses, and the absence of these bacteria leads to reduced pTh17 responses and cyclophosphamide tumor resistance, demonstrating that the gut microbiota contributes to the formation of anti-cancer immune responses in lung cancer patients (102). However, the symbiotic gut microbiota promotes endocrine resistance in castration-resistant prostate cancer by providing an alternative source of androgens, implying that the gut flora may play a negative role in this process (103). Gut bacteria can regulate insulin-like growth factor-1 (IGF1) levels in the host *via* short-chain fatty acids, thereby promoting the proliferation of prostate cancer cells, then modulating the gut microbiota to influence the gut microbiota-IGF1-prostate axis may be beneficial in the prevention and treatment of prostate cancer (104). In addition, the use of gut microbiota analysis to predict patient response to immune check inhibition sites has emerged in cancer treatment, e.g., breast cancer (105). Currently, the role of gut microbes in the development of various cancers varies, and their variation may have implications for achieving more personalized precision medicine in oncology.

TABLE 1 A summary of studies addressing changes in microbiota between breast cancer tissue, non-cancerous adjacent tissue and healthy breast tissue.

REF	Mian methodology	Sample type	Microbiome related results		
			Normal breast tissue	Non-cancerous adjacent tissues	Breast cancer (BC)
(80)	Pyrosequencing V4 16S rDNA Pipeline: QIIME	20 BC patients		↑*Sphingomonas yanoikuyae*	↑*Methylobacterium radiotolerans*
(81)	V3-V4 16S rRNA sequencing (Illumina) Pipeline: UCLUST	57 women with invasive breast carcinoma and 21 healthy women	↑*Methylobacterium*		↑*Alcaligenacea*
(82)	V3-V5 16S rRNA amplified sequencing data	668 tumor tissues and 72 normal adjacent tissues from The Cancer Genome Atlas (TCGA)		↑*Actinobacteria and Firmicutes*	↑*Proteobacteria*, *Mycobacterium fortuitum* and *Mycobacterium phlei*
(83)	V1-V2 16S rRNA sequencing (Illumina HiSeq)	22 Chinese patients with benign tumor and 72 malignant BC patients			↑*Propionicimonas, Micrococcaceae, Caulobacteraceae, Rhodobacteraceae, Nocardioidaceae* and *Methylobacteriaceae* (Ethnicity-related); ↓*Bacteroidaceae* and ↑ *Agrococcus* (with malignancy)
(84)	Pathochips array	20 normal breast tissue and 148 BC tissue			↑*Actinomyces, Aerococcus, Arcanobacterium, Bifidobacterium, Bordetella, Cardiobacterium, Corynebacterium, Eikenella, Fusobacterium, Geobacillus, Helicobacter, Kingella, Orientia, Pasteurella, Peptinophilus, Prevotella, Rothia, Salmonella, and Treponema*
(85)	Pathochips array	100 women with triple negative BC (TNBC), 17 matched controls and 20 non-matched controls			↑*Arcanobacterium* (75%), *Brevundimonas*, *Sphingobacteria, Providencia*, *Prevotella*, *Brucella, Eschherichia*, *Actinomyces, Mobiluncus*, *Propiniobacteria, Geobacillus*, *Rothia, Peptinophilus*, and *Capnocytophaga* (Canimorsus) ↑*Herpesviridae*, *Retroviridae, Parapoxviridae, Polyomaviridae, Papillomaviridae* (virus)
(86)	V3 16S-rRNA gene amplicons sequencing (Ion Torrent)	16 Mediterranean patients with BC	↓*Methylobacterium* (↑*Ralstonia)*		↑*Sphingomonas*
(87)	V6 16S rRNA gene sequencing (Illumina MiSeq) Pipeline: QIIME	58 women after surgery:13 benign, 45 cancerous tumors and 23 healthy women	↓ *Prevotella*, *Lactococcus*, *Streptococcus, Corynebacterium and Staphylococcus*		↑*Bacillus, Staphylococcus*, *Enterobacteriaceae* (unclassified), *Comamondaceae* (unclassified) *and Bacteroidetes* (unclassified)
(88)	V3-V5 16S rDNA hypervariable taq sequencing (Illumina MiSeq) Pipeline: IM-TORNADO	28 women undergoing non-mastectomy breast surgery: 13 benign breast disease and 15 invasive BC			↓*Fusobacterium*, *Atopobium, Gluconacetobacter*, *Hydrogenophaga* and *Lactobacillus*
(89)	V4 16S rRNA gene sequencing (Illumina MiSeq) Pipeline: Mothur	25 women with breast cancer and 23 healthy women	↓unclassified genus of the *Sphingomonadaceae* family in NAF		↑*Alistipes*
(90)	V4 16S rRNA gene sequencing (Illumina Miseq)	32 women with BC stage 0 to II			↓*Akkermansia muciniphila* (AM) in BC patients with elevated body fat.
(91)	V3-V4 and V7-V9 16S rRNA gene sequencing	221 patients with breast cancer, 18 individuals predisposed to breast cancer, and 69 controls.	↑*Stenotrophomonas* and *Caulobacter*		↓*Propionibacterium* and *Staphylococcus*
(92)	16s rRNA gene sequencing; Quantitative Insights into Microbial Ecology (QIIME) tool;RStudio	Bilateral normal breast tissue samples (n = 36) and breast tumor samples (n = 10)	↑(OUT) [*Mogibacteriaceae*] family, and *Flavobacterium*, *Acinetobacter*,		↑(OUT) *Ruminococcaceae*, *Rikenellaceae*, genera *Butyricimonas*, *Sutterella*, and *Akkermansia*.

(Continued)

TABLE 1 Continued

REF	Mian methodology	Sample type	Microbiome related results		
			Normal breast tissue	Non-cancerous adjacent tissues	Breast cancer (BC)
			and *Brevibacillus* genera		
(93)	Kraken2 and Metaphlan3	breast tumours and normal tissues (from cancer-free women) of 23 individuals (Slovak); 91 samples obtained from SRA database (China)	↑*Proteobacteria* (47%), *Bacteroidetes*, *Firmicutes and Actinobacteria* (12%) (Slovak women); ↑*Proteobacteria* (42%), *Firmicutes* (42%), *Actinobacteria* (5%), *Cyanobacteria* (4%)		↑*Acinetobacter*, *Rhodobacter, Micrococcus*, order *Corynebacteriales* and *Priestia megaterium* (Slovak patients) ↑*Streptomyces*, *viruses Siphoviridea* and *Myoviridae* (China patient)
(94)	Illumina MiSeq sequencing	Tumor tissue and normal tissue in 34 women	↑*Actinobacteria*, ↓*Proteobacteria*,		↑*Firmicutes* and *Alpha-proteobacteria*

↑ means up, ↓ means down.

1.3 The role of microbiota in breast tumourigenesis

1.3.1 Estrogen and metabolism

The gastrointestinal microbiome regulates systemic estrogen, and the development of postmenopausal breast cancer is associated with disordered (high) levels of estrogen in the body (106). The metabolism of estrogen occurs in the liver, where the metabolites are conjugated and excreted into the gastrointestinal lumen within the bile. They are de-conjugated by β-glucuronidase-producing bacteria in gastrointestinal lumen, and then they are reabsorbed as free estrogens through the enterohepatic circulation to reach breast (107). All the genes in the gut flora that metabolize estrogen are collectively known

TABLE 2 A summary of studies addressing changes in gut flora between breast cancer patients and non-breast cancer patients.

REF	Mian methodology	Sample type	Gut flora related results	
			Non-breast cancer patients	Breast cancer patients
(95)	Real-time qPCR targeting specific 16S rRNA sequences	31 women with early-stage BC: 15 stage 0, 7 stage I, 7 stage II and 2 stage III.		↑*Bacteroidetes*, *Clostridium coccoides* cluster, *Clostridium leptum* cluster, *Faecalibacterium prausnitzii*, and *Blautia* spp. in patients with stage II/III BC compared to patients in stage 0/I.
(96)	V3-V4 16S rRNA sequencing (Illumina) Pipeline: QIIME	48 postmenopausal women with BC and 48 paired control women		↑*Clostridiaceae*, *Faecalibacterium*, and *Ruminococcaceae* and ↓ *Dorea* and *Lachnospiraceae* in BC patients compared to controls.
(97)	Illumina sequencing	18 premenopausal BC patients, 25 premenopausal healthy patients, 44 postmenopausal BC patients and 46 postmenopausal healthy patients		↑*Escherichia coli*, *Citrobacter koseri*, *Acinetobacter radioresistens*, *Enterococcus gallinarum*, *Shewanella putrefaciens*, *Erwinia amylovora*, *Actinomyces* spp. *HPA0247*, *Salmonella enterica*, and *Fusobacterium nucleatum* and ↓*Eubacterium eligens* and *Roseburia inulinivorans* in postmenopausal BC patients.
(98)	16s rRNA gene sequencing	54 premenopausal women with breast cancer and 28 normal premenopausal women	↑*Photobacterium*, *Pseudobutyrivibrio*	↑*Firmicutes/Bacteroidetes* (F/B) Ratio; ↑*Parasutterella* and *Campylobacter*
(99)	V3–V4 16S rRNA Gene Sequencing	30 healthy women controls and 25 breast cancer patients	↑*Bacteroidetes*	↑*Firmicutes*

↑ means up, ↓ means down.

as the estrobolome (108). A study found that difference s in urinary estrogen levels were associated with beta-glucuronidase activity in pre- and post-menopausal women, and that gastrointestinal flora could influence non-ovarian estrogen levels *via* the enterohepatic circulation (109). In addition, urinary estrogen levels in men and postmenopausal women were strongly correlated with all indicators of microbiota richness and diversity in faeces, with non-ovarian-acting systemic estrogens significantly associated with fecal Clostridium perfringens (including non-clostridial and three genera of *the family Rhizobiaceae*), and Gut microbiota may influence estrogen-related diseases in the elderly (109), such as Postmenopausal Breast Cancer. Many of the microbes associated with breast cancer have the β-glucuronidase enzymatic activity mentioned above, which prevents the binding of estrogen and other compounds and makes them biologically active, thus affecting local and systemic levels of estrogen and its metabolites (79, 110). During estrogen metabolism, the gut acts as an important site for estrogen reactivation and microorganisms act locally or distally to regulate disease development and homeostasis (111). When the balance of the intestinal environment is disrupted and the structure and ratio of the flora are imbalanced, excess intestinal bacteria, Lipopolysaccharides and pro-inflammatory cytokines are produced, and this change disrupts the integrity of the intestinal mucosa, which in turn triggers inflammation after bacterial translocation (112). In addition to those involving hormone metabolism (estrogen and progesterone),Studies in growing numbers are exploring the relationship between the gut microbiome and breast cancer risk *via* a non-estrogen-dependent pathway. Obesity, insulin resistance, dyslipidemia, leukocytosis, and elevated C-reactive protein (113) are associated with reduced gut microbial diversity, some of which are associated with breast cancer. Studies have demonstrated that metabolic health status (as defined by the homeostasis model assessment of insulin resistance [HOMA-IR] index, or fasting insulin level), but not obesity per se, may be an associated factor in the risk of postmenopausal breast cancer development, suggesting that hyper insulinemia is an important risk factor for breast cancer (114). Karen L Margolis et al. demonstrated an increased risk of invasive breast cancer in postmenopausal women with higher white blood cell counts (115), Nicholas J Ollberding et al. concluded that circulating C-reactive protein levels44 reflecting adipokines and systemic inflammation were associated with the risk of postmenopausal breast cancer, independent of Body fat rate (116), These further support the possibility that inflammation may be associated with the initiation, promotion and progression of breast cancer. In addition, breast cancer in postmenopausal women is significantly associated with the immune-recognised (IgA-positive) and -unrecognised (IgA-negative) gut microbiota, the former possibly through immune-mediated pathways and the latter possibly through the enterohepatic circulation effects of estrogen (117). It was shown that the microbiota of breast tissue is different from that of mammary skin tissue, where bacterial species are more abundant than in skin tissue, and more operative taxonomic units (mostly low abundance) were observed in the breast tissue microbiota. These taxa with different abundance were from *the phyla Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* (88). A comparison of breast tissue from breast cancer patients and normal women revealed higher levels of Enterobacteriaceae and Staphylococcus and increased numbers of Bacillus in breast cancer patients (87). In contrast, Lactobacillus and Streptococcus were higher in healthy women and have anticancer properties that may play a role in the prevention of breast cancer (118). *Prevotella*, which produces SCFAs propionic acid and exerts benefits in the intestine, was higher in healthy women compared with breast cancer patients (119). Further study of bacterial metabolites and bacterially induced host metabolites would provide insight into the role of bacteria in the role of breast disease will provide important information.

1.3.2 The role of antibiotics

Indirect evidence suggests that the development of breast cancer is strongly associated with alterations in specific microbiota when taking antibiotics or probiotics. Through a large-scale analysis of nearly 4 million women, Simin et al. (120) showed a specific dose-dependent relationship between antibiotic use and breast cancer, with a different correlation between the type of antibiotic and breast cancer risk, such as β-lactams, macrolide (121). Irregular use or overuse of antibiotics may increase the risk of gut dysbiosis and decrease microbial diversity, and this effect may be long-lasting (122, 123), For example: co-amoxiclav and clarithromycin, Cefprozil, Amoxicillin, etc. Also, overuse of antibiotics (*penicillins*, *streptomycin*, *chloramphenicol*, *tetracyclines*, *erythromycin*, *cephalosporins* and their analogues) decreases plasma levels of lignans-enterolactone, which can increase the risk of breast cancer by affecting the microbiota (124). A study has shown that the increased excretion of bound estrogens in the feces of patients treated with ampicillin suggests that the gut microbiota are actively involved in estrogen metabolism and can have some effect on the pathogenesis of breast cancer by altering the individual's microbial status (106). Antibiotics have been shown to disrupt the microbiota, leading to a reduced response by tumor cells to platinum-based chemotherapy and immunotherapy (106, 125, 126), suggesting that a stable microbiota is necessary for an optimal response to antitumor therapy.

1.3.3 Regulation of chronic inflammation and immunity

Microbiota may promote the risk of malignancy by inducing the persistence of chronic inflammation, disrupting the balance between cell proliferation and death in the body, and triggering

uncontrolled innate and adaptive immune responses (127, 128). A putative inflammatory mechanism associated with breast carcinogenesis has been demonstrated to be the upregulation of cyclooxygenase 2 (COX2) and its product prostaglandin E2 (PGE), which would increase the expression of aromatase in adipose tissue, thereby promoting the conversion of androgen precursors to estrogens (129, 130) and increasing the risk of breast carcinogenesis. Studies have demonstrated that a potential inflammatory biomarker, mucosal secretory immunoglobulin A (IgA) (131), can maintain the integrity of the mucosal barrier by regulating the composition of the intestinal microbial community, thereby attenuating the host's innate immune response. The link between breast cancer and the mucosal secretory IgA has been established (117). This mechanism places some limits on the participation of intestinal microbial antigens in the circulation of the body, and some limits on the invasiveness of potentially dangerous microorganisms (132). Certain specific microbiota may also maintain breast health by stimulating the host inflammatory response. For example, specific bacteria S. yanoikuyae are present in the breast tissue of healthy women and their abundance is significantly reduced in the corresponding tumor tissue. An increase in its abundance may lead to a decrease in bacterial-dependent immune cell stimulation in the body, resulting in a reduced environmental risk level for the development of breast tumors (80). Studies have also confirmed the role of microorganisms in regulating specific immune processes in the development of cancer (133), For example, *Lactococcus* spp. can activate important cells associated with tumor growth (murine splenic NK cells), maintain their cytotoxicity, and enhance cellular immunity (134). In another case-control study, Goedert and colleagues (117) investigated the role of immunity and inflammation in breast cancer risk and whether the gut microbiota differed in the composition of the immune recognition microbiota and found significant differences in the composition, abundance and alpha diversity of the microbiota between the IgA+ and control IgA- groups in cancer cases and correlated with changes in high and low estrogen levels. This suggests a significant association with IgA+ and IgA- gut microbiota in postmenopausal women with breast cancer, suggesting that the gut microbiota may influence breast cancer risk through altered metabolism, estrogen cycling and immune pathways.

1.3.4 Genomic stability and DNA damage

DNA damage may not be sufficient to promote cancer development, but microbes can trigger transformation by destabilizing genes, cell proliferation and death, and it has been demonstrated that microbes cause cancer development by damaging host DNA in order to survive (135). Urbaniak et al. (87) found that *Escherichia coli* (a member of the *Enterobacteriaceae family*) isolates and a *Staphylococcus* epidermidis isolate from normal adjacent tissues of breast cancer patients had the ability to induce DNA double-strand breaks, thus causing genomic instability (136). In addition, some bacterial species may eventually lead to genotoxicity by increasing the production of reactive oxygen species (137).

2 Current status on breast cancer progression, metastasis and microbiology

2.1 Enterotoxigenic bacteroides fragilis

Bacteroides fragilis is a common colonic colonizing enterobacterium (138) whose virulence is attributed to a 20 kDa zinc metalloprotease toxin known as B. fragilis toxin (BFT) (139). With reference to the effect of enterotoxigenic B. fragilis (ETBF) intestinal or ductal colonization on breast cancer progression in the mammary intraductal model, Parida S et al. (140) colonized BALB/c mice *via* the teats with ETBF or a non-toxic mutant B. fragilis (086Mut) that does not secrete BFT. The presence of BFT was found to be detected in the mammary glands of ETBF-carrying mice compared to controls, with a 3.9-fold higher tumor volume than 086Mut controls, enhanced lung and liver metastases, and more proliferative tumors forming in the ETBF group, exhibiting a mesenchymal phenotype. Moreover, trichrome staining showing significantly higher stromal infiltration, demonstrating that ETBF intestinal or ductal colonization was associated with breast cancer progression and distant metastasis. Furthermore, significant differences in breast tissue structure were found in the ETBF group compared with the 086Mut control group (140), including extensive local inflammation and tissue fibrosis, Ki-67 and proliferating cell nuclear antigen staining showed increased epithelial cell proliferation, CD3 staining showed increased T-cell infiltration, and significantly altered expression of pan-keratin, all indicating that BFT was associated with a significant increase in oncogenic cell activity and growth rate. The study also found that RNA-seq analysis of secondary tumors arising from breast cancer cells treated with BFT showed enrichment of the β-catenin pathway. The expression of several Notch-responsive genes was enriched in breast cancer cells suggesting that BFT also triggered activation of the Notch1 pathway. The results advance our understanding of the molecular mechanisms associated with ETBF/BFT and breast cancer progression (140), and point to a hypothesis that dysbiosis or disruption of the gut flora might be associated with breast cancer metastasis and progression, and that inhibition of manipulable key molecules or pathways could potentially reduce the impact of ETBF infection on breast cancer.

In looking at whether BFT affects the tumorigenicity of breast cancer cells, the team found that, compared with cells

from the control group, BFT-pretreated MCF-7 and MCF-10A cell groups showed greater invasion and migration, with local tumor expansion and the formation of multifocal tumors resembling local metastases (140).However, it was not clear whether ETBF spread from within the gut to the breast or whether gut-infected mice acquired the mammary gland infection through environmental factors. Data from RNA-seq analysis of secondary tumors with limited *in vivo* formation showed higher expression of genes associated with migration, homing, and metastasis in the BFT pre-treatment group, suggesting that BFT production by ETBF intestinal colonization might be associated with the initiation of breast cancer metastasis; and breast cells exposed to BFT showed dramatic changes supporting cell motility, embryonic pluripotency pathways, expression of metastatic genes, and molecular mechanisms. However, it cannot be demonstrated that ETBF can be the sole driver directly triggering the transformation of human breast cells into tumor cells or interacting with other microbiota to show oncogenic activity.

2.2 Antibiotic-induced intestinal flora dysbiosis and the progression of breast cancer metastasis

To assess the effect of pre-established dysbiosis on the metastasis of hormone receptor–positive (HR+) breast cancer in a more aggressive and metastatic tumor model, Parida S et al. (141) evaluated tumor spread to the lung and axillary lymph nodes in a highly metastatic MMTV-PyMT mouse model with reference to the poorly metastatic HR+ mouse breast cancer cell line BRPKp110. The results were similar to those observed in the BRPKp110 cell line: where the spread of tumor cells to the lung was significantly increased after commensal dysregulated of the intestinal flora due to antibiotic treatment, independent of tumor volume. Moreover, the tumors progressed with the same kinetics regardless of the symbiotic dysregulation status in the experimental mice, suggesting that symbiotic dysregulation has a significant and sustained effect on HR+ breast cancer dissemination and that the enhanced ability of cancer cells to spread in symbiotically dysregulated mice is independent of tumor growth kinetics. To confirm the impact of the flora-dysregulation-driven host-intrinsic differences in inducing propagation in a mammary tumor model, they tested the symbiotic dysregulation using the L-Stop-L-KRasG12Dp53flx/flxL-Stop-L-Myristoylated p110α-GFP+ induced mouse model of breast cancer (141), and found that consistent with that observed in the homozygous model, the lungs of mice with dysbiosis of the intestinal flora showed a higher frequency of disseminated tumor cells. No significant increase in GFP+ tumor cells was observed in the distal lymph nodes. Those results confirmed that dysbiosis is independent of primary tumor growth and is associated with enhanced tumor cell dissemination; they also suggest that the tumor dissemination enhancement is the result of host dysbiosis rather than of intrinsic differences in tumor aggressiveness. Macrophages in the mammary gland may promote the metastasis of mammary tumors in experimental animals (142). Parida S et al. (141) found that commensal dysbiosis influenced the frequencies and numbers of macrophages during early or advanced stages of mammary tumor progression. Macrophages are one of the most abundant cell types within the breast tumor-microenvironment (143) and are a significant prognostic indicator of reduced survival for patients diagnosed with HR+ breast cancer (144). They observed that the majority of myeloid infiltrates within the mammary tumor microenvironment were M2-like macrophages during at early and advanced stages of tumor progression based upon CD206 expression. Importantly, the number of infiltrating tumor-promoting M2-like macrophages was significantly increased in advanced tumors of mice in the dysbiotic mice compared to non-dysbiotic controls with equal tumor burden. These data suggest that systemic expression of inflammatory mediators is increased in mice with dysbiosis tumors and that commensal dysbiosis acts synergistically with developing tumors to enhance myeloid recruitment into mammary tumors. Enhanced interstitial density or dense breast tissue is a recognized risk factor for the development of breast cancer metastases (145) and intra-mammary pro-tumor inflammation (146). They found that pre-established dysbiosis was associated with significantly enhanced collagen deposition in normal adjacent mammary glands and in tumors, and that collagen accumulation was slightly increased in the lungs of advanced tumor-bearing mice with dysbiosis, suggests that enhanced local and distal fibrosis is a long-term consequence of dysbiosis during breast cancer. Parida S et al. to determine whether gastrointestinal dysbiosis is sufficient to enhance mammary tumor cell dissemination (141), and a fecal microbiota transplantation (FMT) method was used, Both the experimental and control group and control groups were BRPKp110 breast tumor cells, Mice receiving flora-dysregulated cecal contents by FMT also showed enhanced infiltration of inflammatory myeloid cells into the mammary tissue and increased accumulation of myeloid cells into tumor tissue. Similar effects were observed in the mammary gland and tumor tissue during the advanced stages of tumor progression—that is, mammary gland tissue and tumors showed enhanced tissue fibrosis. Importantly, the spread of tumor cells to peripheral blood, lung, and distal axillary lymph nodes was also significantly increased in mice receiving dysbiosis flora (rather than “normal” FMT) by FMT, considering that a dynamically imbalanced microbiome is sufficient to enhance the metastatic spread of breast cancer. Moreover, it may be an independent correlate of the distant spread of tumor cells. Further supporting the idea that dysbiosis contributes to the evolution of breast tissue and/or tumors toward more aggressive and high-grade disease. regardless of the metastatic potential of

the HR+ breast tumor model used in the study, dysbiosis of the gut flora was associated with enhanced dissemination and metastasis of breast tumor cells.

Changes in the gut microbiota also to effects in metabolites, and inflammatory signaling pathways can be amplified or inhibited. Using an *in-situ* mouse model of breast cancer, Kirkup et al. (147, 148) found significant differences in metabolic regulatory pathways across the tumor transcriptome in animals treated with broad-spectrum antibiotics, and single-cell transcriptomics revealed that the stromal cell population was altered in breast tumors from antibiotic-treated mice. The main form of the alteration was an increased number of mast cells, which accelerate tumor progression. The breast cancer model used a PyMT-derived ductal lumen cell line (PyMT-BO1) to investigate the role of gut microbiota in regulating the growth of primary mammary tumors (149). Disruption of intestinal microbiota by gavage administration of oral antibiotics (vancomycin, neomycin, metronidazole, amphotericin, and ampicillin [VNMAA]) prior to administration of tumor cells to animals, producing severe intestinal microbial changes (150, 151), and although no significant differences in tumor tissue structure were observed in those animals compared with a control group receiving plain water, significantly accelerated tumor growth was observed. Under a similar treatment regimen (152), enhanced growth resembling basal-like breast cancers was observed when spontaneously derived basal cells (EO771) were implanted *in situ*, suggesting that antibiotic-induced microbiota disruption can drive disease progression in multiple breast cancer subtypes. To determine the effect of the VNMAA mixture on the microbiota, microbial DNA was isolated from the cecum of control and VNMAA-treated animals on day 18 and subjected to birdshot macro-genomics analysis. The analysis revealed dramatic changes in the populations and overall diversity of the bacteria obtained from the animals that received VNMAA treatment, with the Shannon diversity index showing that the abundance of several microbial communities in the gut of antibiotic-treated mice was significantly reduced. In parallel, some communities (e.g., *Fusobacterium nucleatum*) persisted or overgrew. The composition of the gut microbiota was significantly altered in terms of species, abundance and overall diversity following the use of antibiotics, which was associated with accelerated tumor growth and an increase in mast cells in the tumor stroma. To determine whether mast cells affected tumor growth, Kirkup et al. (147) treated control and VNMAA-treated tumor-bearing mice with cromolyn (a mast cell stabilizer) and found that cromolyn inhibited tumor growth in the antibiotic-treated animals. Notably, the VNMAA-treated group without cromolyn treatment showed a significant increase in tumor size when compared to the control animals treated with cromolyn, and an increase in the number of mast cells was observed in sections of the EO771 tumor stroma taken from VNMAA-treated mice (147, 148). Those data suggested the key role that mast cells play in tumor progression after antibiotic-induced microbiota disruption in mouse breast cancer: when vancomycin alone was used to induce microbiota disruption, effects similar to those already described were observed in a completely different model of breast cancer. Possibly, microbiota disruption was associated with increased homing of mast cells to, and/or increased proliferation within, tumor. However, given that mast cells in the control animals did not affect tumor progression, the pro-tumor function observed was shown to be specifically regulated by the microbiota. Given confirmation that antibiotic disruption of the gut microbiota has a detrimental effect on breast cancer, antibiotic-induced dysbiosis of the flora and dysregulation of the associated metabolites could be hypothesized to promote tumor growth by reprogramming mast cell homing and/or function. Future studies might consider determining the changes that occur in mast cells and breast tumor cells in response to gut dysbiosis. Kirkup et al. (147, 148) used a mixture of vancomycin, neomycin, metronidazole, and amphotericin (VNMA) to assess DNA concentrations in feces after microbiota dysbiosis and found very low DNA concentrations in the feces of the experimental group compared to the control group (water treatment). Importantly, the rate of PyMT-BO1 and EO771 breast tumor growth was significantly increased after disruption of the gut microbiota in the treated animals compared with the control animals (water treatment). Transcriptomic analysis also revealed dramatic differences in the regulation of metabolic pathways after antibiotic-induced dysbiosis of the intestinal flora, suggesting that accelerated breast cancer tumor growth might be associated with metabolic reprogramming. Fecal metabolomics was confirmed by 1H NMR spectroscopy analysis, which showed that 8 metabolites were elevated and 9 were significantly reduced in the major components of fecal samples from antibiotic-treated animals compared to fecal samples from control animals (147). Several of these amino acids (among them alanine, histidine and aspartic acid) were significantly increased in the antibiotic-treated animals. In contrast, the SCFAs butyrate and acetate, but not the branched-chain fatty acid isovalerate, were significantly reduced. Microbiota-derived butyrate is readily absorbed from the gut and can play a role in inhibiting histone deacetylases (153) in a variety of diseases, including cancer. Inhibition by butyrate can sensitize cancer cells to reactive oxygen species–induced apoptosis, thereby inhibiting the proliferation of breast cancer cells (154), but its role in the organism is yet to be confirmed in clinical trials. The authors hypothesized that a decrease in the bioavailability of the intestinal flora metabolite butyrate plays a role in enhanced tumor metabolism. Metabolites from the gut can reach distant tissues and organs such as the breast *via* the circulation, where they might play a role in regulating cancer cell function. Kirkup et al. noticed that antibiotics associated with breast cancer (e.g., cefadroxil, which is widely used in the USA after mastectomy). C57BL/6 mice carrying PyMT-BO1 tumor cells and receiving a cefadroxil dose equivalent to that in human patients experienced a significant acceleration in tumor growth. Analysis of the gut microbiota of the animals showed that the

microbiota aggregates in samples from the experimental and control animals were independent and clustered differently before and after treatment. The relative abundance of *Lactobacillus* decreased over time in the control and experimental groups, and this appeared to be replaced mainly by fecal genera in the control animals, however, this did not occur in the antibiotic-treated animals. The genus with the most significant change in the microbial composition of the animals in the experimental group compared to the pre-treatment samples was *Lactobacillus*, but there was no significant difference before and after the control group. We presume that the disappearance of *Lactobacillus* might be driven by tumor cells, tumor–microbiota interactions, or natural maturation of the microbiome rather than by cefadroxil administration. Further analysis revealed differences in the abundance of 11 genera after cefadroxil treatment: *Mucispirillum*, *Marvinbryantia*, *Parabacteroides*, *Anaeroplasma*, *Bacteroides*, and *Paraprevotella* were significantly higher, and *Alloprevotella*, *Alistipes*, *Odoribacter*, *Faecalibaculum*, and *Anaerotruncus* were significantly lower. When multiple comparisons were made, 8 genera were significantly altered after antibiotic treatment. the genera that were significantly lesser abundant in treated animals relative to the controls, several are known butyrate-producing bacteria (e.g., *Odoribacter* and *Anaerotruncus*) or genera carrying the genes required for butyric acid production (e.g., *Faecalibaculum* and *Alistipes*) (147), consistent with the significant reduction in butyrate production observed in the metabolomic analysis of feces. That observation suggests that the use of a single antibiotic associated with breast cancer causes significant changes in microbiota genera and aggregation, potentially correlating with the tumor growth rate, but without a direct link to accelerated growth of breast tumors.

2.3 Effect of changes in metabolites following microbial perturbation on breast cancer metastasis

A major signaling route between the microbiome and the host is the secretion of Microbial metabolites that enter the circulation and reach their target cells (155–158). microbial metabolites synthesized in organs or glands (in this study, in the microbiome) function much like human hormones, in that they transfer to other anatomic locations and exert biologic effects (159). Microbial metabolites can enter the circulation and interfere with the steady-state of the intestinal and other local environments, acting as signaling mediators that influence the progression of breast cancer. SCFAs (160, 161), Lithocholic acid (LCA) (162–165), cadaverine (166), and de-conjugated estrogens (96, 109, 167), these metabolites have the ability to inhibit tumor-cell proliferation, the conversion of epithelial cells to mesenchymal cells, tumor metastasis, and cell migration and metastasis, and to induce antitumor immunity, to restructure cell metabolism, to induce senescence, and lower the number of tumor stem cells (164, 166, 168, 169).

The finding that perturbations in the gut microbiome are associated with tumor propagation at a distance supports the idea that the gut microbiome can be considered to be an endocrine gland (159, 170). Some metabolites associated with the activity of gut bacteria can enter the bloodstream and have been shown *in vitro* to affect the functioning of breast cancer and immune cells. Members of the microbiota can digest certain indigestible components of the human diet (e.g., dietary fiber), and SCFAs —for example, acetate, propionate, and butyrate—are components of metabolized dietary fiber (30, 171) and act as modulators of the host's immune response. Bioactive compounds such as metabolic polyphenols (172) promote the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* and produce SCFAs (173, 174). Some studies have shown that microbially derived homologous receptors for SCFAs were associated with a reduction in the invasive potential of breast cancer cells, with the homologous receptor FFAR2 inhibiting the Hippo-Yap pathway and increasing the expression of the adhesion protein E-cadherin, and FFAR3 inhibiting MAPK signalling (175), particularly butyrate, which has anticancer effects, as demonstrated in cancer cell cultures (176, 177) and animal models (160). Crucially, those microbial metabolites are produced after fermentation and/or metabolism of dietary components, and one of the key roles of the microbiota is to break down complex foods into simple bioactive compounds.

In the gut, disruption of the microbiota breaches the biologic barrier between it and the underlying tissue, leading to adverse physical contact between microbes and host cells, inducing paracrine production of bacterial metabolites (135). Changes in the microbiome have been associated with metabolic diseases such as obesity and type II diabetes (178), which are risk factors for certain cancers, including breast cancer (80, 179). The intestinal flora is responsible for the conversion of primary bile acids to secondary bile acids (180), and changes in the intestinal microbiota can therefore directly affect changes in secondary bile acids. Edit Mikóah et al. (169) studied three secondary bile acids—LCA, deoxycholic acid, and ursodeoxycholic acid. Of those three, LCA was found to exert a tumor-suppressive effect by reducing the growth of MCF7, SKBR3, and 4T1 breast cancer cells. They tested the cytostatic properties of LCA in mice transplanted with 4T1 breast cancer cells and found that the ability of the primary tumor to infiltrate surrounding tissues and metastasize was significantly reduced after LCA treatment. This study was the first to provide evidence for a mechanism of interaction between the microbiome and breast cancer by describing that LCA, a metabolite of microorganisms in the gut, is transferred to the breast *via* the bloodstream and might play an important role in promoting antiproliferative effects in breast cancer. However, LCA might be produced by the breast's own microbiota and not only by the gut microbiota. The ratio of those two sources (breast and gut) in

terms of LCA abundance is unknown and requires substantial research and continued trials.

Cadaverine is produced through lysine decarboxylation by lysine decarboxylase (181). *Shigella felis*, *Shigella sonnei*, *Escherichia coli*, and *Streptococcus* are all capable of expressing it (182). Kovács et al. (166) explored the effects of cadaverine supplementation (500 nmol/kg) on mice homozygously transplanted with 4T1 breast cancer cells and found a reduction in the aggressiveness of the primary tumor. Histologic examination of the primary tumors after cadaverine treatment showed a reduced mitotic rate and heterogeneity of nuclear morphology in the mammary tumor cells. To assess whether cadaverine treatment could convert mesenchymal-like carcinoma cells into epithelial-like cells, increased cadaverine resistance was measured using ECIS (Electric Cell-substrate Impedance Sensing), which showed better cell adhesion. To verify that finding, cells stained with Texas Red-X phalloidin and observed under microscopy showed that, after cadaverine treatment, the fibroblast-like morphology of 4T1, MDA-MB-231 and SKBR-3 breast cancer cells had changed to a cobblestone-like morphology that is characteristic of epithelial cells, and the inhibition of matrix metalloproteinase 9 expression also confirms the decrease in tumour cell migratory properties. A cellular flux analyser assessed the metabolic changes induced by necrotropism and found a reduction in glycolytic flux, which is characteristic of breast cancer mesenchymal cells (183). Cadaverine exerts its anticancer effects by inhibiting epithelial–mesenchymal transition, cell motility, chemotaxis, and metastasis. A further assessment of the "stemness" of 4T1 cells using an aldehyde dehydrogenase assay found that "stemness" was also slightly reduced (166). Dysbiosis of the intestinal flora (i.e., a change in the basal environment) leads to a change in the level and type of metabolites produced, which might have no effect on reducing the proportion of stem cells in breast cancer and slowing the rate of metastasis or might have the opposite effect, promoting malignant progression of the tumor. In the early stages of breast cancer in dysbiosis mice, bacterial cadaverine biosynthesis in the gut is reduced, leading to lower production of anti-cancer bacterial metabolites. We can speculate that in the presence of disturbed or slightly disturbed gastrointestinal flora, the metabolites produced act as signaling mediators and a specific crosstalk reaction may occur with the host, and this process may be directly or indirectly linked to the metastasis, migration and invasion of mammary tumors in mice.

2.4 Role of probiotics to block breast cancer spreading

A few studies have found that probiotic preparations are gaining in popularity for the improvement of health conditions such as antibiotic-induced diarrhea, irritable bowel syndrome, and obesity (184, 185). The use of probiotics can reduce or inhibit tumor growth, reduce tumor angiogenesis, tumor cell extravasation and lung metastasis (186). Long-term disturbance of the gut microbiome, which disrupts the probiotic structure and composition, may conversely increase the risk of breast cancer metastasis (187, 188).

Lactobacillus casei, a type of probiotic, is a Gram-positive bacterium that is resistant to the body's defense mechanisms. After entering the human body, *L. casei* can survive in large numbers in the intestinal tract and can play a role in regulating the balance of intestinal flora, promoting digestion and absorption, among other processes (189). It is highly effective in lowering blood pressure (190) and cholesterol (191), promoting cell division and antibody immunity, enhancing human immunity, preventing cancer, and inhibiting tumor growth. Aragón et al. (186) used milk fermented with *L. casei* CRL431 to evaluate its possible effects on tumor growth, tumor cell extravasation and lung metastasis in a mouse model. By comparing mice fed fermented milk (FM), mice fed regular milk and mice not fed any special food, it was found that the group fed FM showed an inhibition of tumor growth and a decrease in tumor vascular filling, tumor cell extravasation and lung metastasis. Khoury et al. (192) used kefir water, a fermented milk product containing probiotics, to treat BALB/c mice that had been transplanted with 4T1 mammary cancer cells and, in the treated mice, detected a significant reduction in tumor size and weight, a significant enhancement of helper T cells and cytotoxic T cells, a significant reduction in lung and bone marrow metastases. Zamberi et al. (193) found that kefir water (mix of *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactococcus lactis*) exerted an anti- angiogenic effect on mouse mammary tumors by down-regulating the tumor-promoting invasive interleukin 1β and vascular endothelial growth factor (a key mediator of angiogenesis). In the above model, levels of the pro-angiogenic factor interleukin 6 were found to have declined (186, 189, 194, 195) after probiotic treatment, suggesting that downregulation by Lactobacillus might affect the metastatic potential of cancer cells. some study (186, 196, 197) demonstrated that milk fermented with *Lactobacillus casei* CRL431 (probiotic fermented milk (PFM)) reduced the side effects of capecitabine and reduced intestinal mucositis and mortality in a mouse model of breast cancer by modulating the immune response, this suggests the potential of PFM as a probiotic as an immune adjuvant that may reduce tumor growth and metastasis without compromising the anti-tumor/anti-metastatic effects of chemotherapy. They differentially regulate cancer-related signaling pathways in a cell-type-specific manner and play a suppressive role in the pro-tumor microenvironment (198–200). Conversely, disruptions in the intestinal flora might simultaneously or subsequently affect the probiotic

environment, which could cause probiotics to lose their "dominant" role in the tumor environment, negatively affecting the control or inhibition of breast tumor cell growth or even accelerating the growth of tumor cells and promoting angiogenesis, becoming an indirect contributor to tumor metastasis. Yazdi et al. (201) demonstrated that selenium-nanoparticle-enriched L. brevis administered to mammary tumor-bearing BALB/c mice induced an effective immune response, resulting in reduced liver metastases and an increased lifespan, included increases in the T helper cytokines, interferon-gamma and interleukin 17, and enhanced natural killer cell activity. Hassan Z et al. Demonstrated that (202) *Enterococcus faecalis* and *Staphylococcus hominis* can significantly inhibit cell proliferation, induce apoptosis, and cell cycle arrest, and that they have no cytotoxic effect on normal cells, making them a good alternative drug for breast cancer treatment (Figure 1).

Figure 1. The linkage between probiotic environmental homeostasis and breast cancer metastasis

Probiotics have specific anticancer properties, and studies have shown that they can alter the expression of various genes involved in apoptosis (203), invasion and metastasis (204), maintenance of cancer stem cells (205), and control of the cell cycle (206). Probiotics have been highlighted as superior in the treatment of cancer. however, more pre-clinical and clinical studies are needed to determine which strains are beneficial during specific treatments before probiotic administration is considered safe and customisable for all individuals.

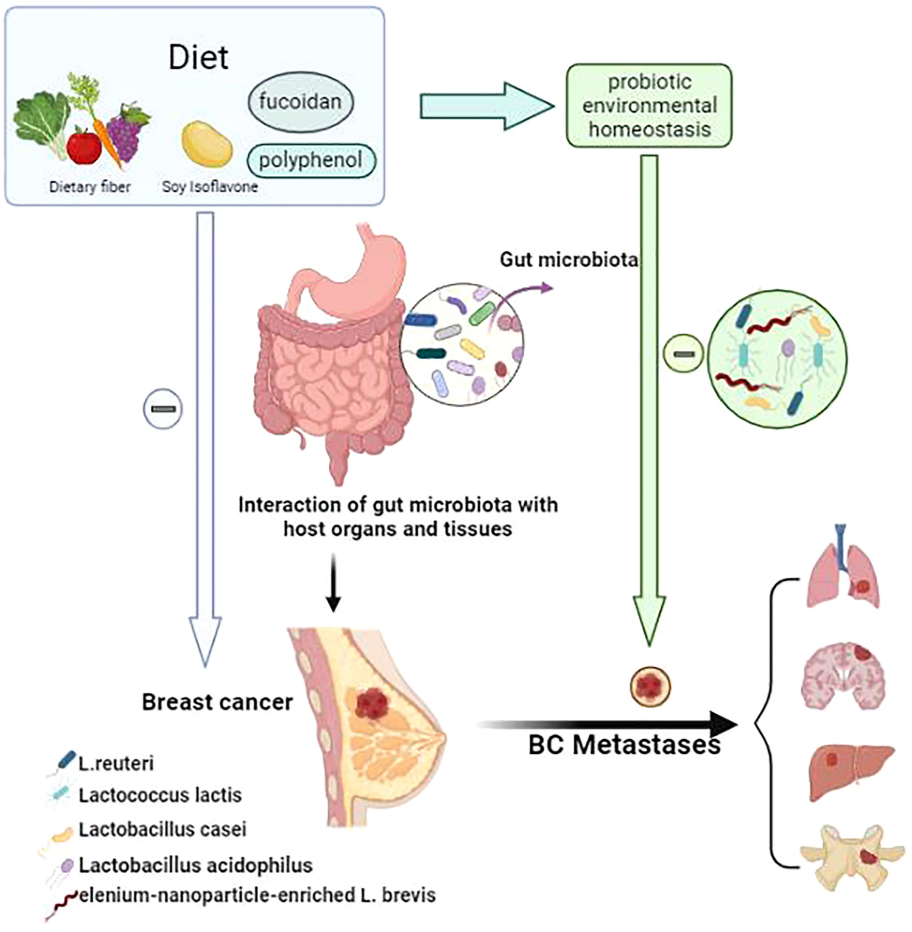


FIGURE 1
The linkage between probiotic environmental homeostasis and breast cancer metastasis. A good diet (e.g. foods rich in dietary fiber, soy isoflavones, fucoxanthin and polyphenols) can reduce intestinal flora dysbiosis and thus harmonize the body to reduce the incidence and metastasis of breast cancer. Diet as an important factor in the stable composition of the host probiotic environment, through intestinal flora regulation. probiotic environmental homeostasis can play an adjuvant anti-cancer role in the progression and metastasis of breast cancer (lung, brain, liver, bone).

10.3389/fonc.2022.1037831

TABLE 3 Correlation between factors that disturb the intestinal flora and breast cancer metastasis and progression.

Factor	Study model	Regulation	Key biologic function
ETBF			
BFT	BALB/C, MCF-7, MCF-10A	Up	ETBF produces BFT, which is highly invasive in breast cells and expresses migration- and metastasis-related genes (137–139)
Antibiotics			
	MMTV-PyMT	Up	Dysbiosis was associated with enhanced distant metastasis and dissemination of breast tumor cells (140)
	BRPKP110	Up	Infiltration of myeloid cells in breast tissue was enhanced after FMT perfusion (140)
	GFP+ tumor cells	Up	Dysbiosis was associated with increased breast tumor cell dissemination (140)
M2-like macrophages		Up	Dysbiosis was associated with enhanced infiltration of myeloid cells into the breast tissue (140, 141)
VNMAA or vancomycin	PyMT-BO1	Up	Significant reduction in gut microbiota abundance and accelerated tumor growth were observed after VNMAA treatment (146)
	E0771	Uncertain	Increased homing/value-added of mast cells in breast cancer tumors where gut microbiota were disturbed after antibiotic treatment (146)
VNMA	PyMT-BO1, E0771	Up	Antibiotic-induced dysbiosis of microflora was associated with reduced expression of pro-apoptotic genes and increased expression of pro-survival genes (153)
	PyMT-BO1, E0771	Up	Antibiotic administration was associated with dramatic differences in the regulation of microbial metabolic pathways and increased tumor growth rates in laboratory animals (146, 148)
cefadroxil	PyMT-BO1	Up	Gut microbial aggregation, genus differences, and accelerated tumor growth were observed in cefadroxil-treated animals (146, 148)
Probiotics			
CRL431		Down	FM was associated with inhibited mammary tumor growth and metastasis in mice (185)
Kefir water	4T1, BALB/C	Down	Administration of kefir water was associated with inhibition of tumor size and distant metastasis with downregulatory effect (190, 191)
L. brevis	BALB/C	Down	*L. brevis* administration was associated with immune response and reduced liver metastases from mammary carcinoma in mice (195)
Microbial metabolite			
SCFAs	SCFAs	Down	Butyrate has anti-cancer properties (159, 175, 176)
LCA	MCF-7, SKBR3, 4T1	Down	LCA was associated with inhibition in the growth of breast cancer cells (180) and reduction in infiltration by the primary tumor into the surrounding tissue and metastasis (168)
Cadaverine	4T1, MDA-MB-231, SKBR3	Down	Cadaverine can fight breast cancer progression by inhibiting EMT, cell motility, chemotaxis, and metastasi (182)
Diet			
Lactobacillus casei Shirota and Soy isoflavones from puberty onwards (207)		Uncertain	
polyphenol (173)		Uncertain	
Fucoidan (209)		Uncertain	

ETBF, Enterotoxigenic Bacteroides fragilis; BFT, B. fragilis; BALB/C, experimental mouse; MCF-7, human breast cancer cells; MCF-10A, epithelial cell line; MMTV-PyMT, mouse model of highly metastatic breast cancer; BRPKP110, HR+ mouse breast cancer cell line model; FMT, fecal microbiota transplantation; GFP+, Green fluorescent protein; VNMAA, vancomycin, neomycin, metronidazole, amphotericin, ampicillin; PyMT-BO1, PyMT-derived ductal cell line *in situ* mammary fat pad injection model; E0771, spontaneously derived basal cells; VNMA, vancomycin, neomycin, metronidazole, amphotericin; FM, fermented milk; CRL431, type of L. casei used to ferment milk; 4T1, breast cancer cells; SCFAs, short-chain fatty acids; TLR4, Toll-like receptor 4;LCA, lithophanic acid; MDA-MB-231, breast cancer cells; SKBR3, breast cancer cells; EMT, epithelial–mesenchymal transition.

2.5 Diet affects the likelihood of breast cancer progression

Although the correlations between BRCA risk and dietary intake have been intensively studied, the underlying associations or effector mechanisms remain poorly understood. Historically, increased risk of BRCA has been tied to high intake of red meat and animal fat (207, 208), with decreased risk being concurrently linked to fruit and vegetables consumption (209). Changing dietary patterns affects the microbiome and Indirect affects the development of breast cancer. A case-control study in Japan showed that regular consumption of Lactobacillus casei Shirota and soy isoflavones from puberty onwards reduced the incidence of breast cancer in Japanese women (210); Newman TM et al. also indicated that the Mediterranean diet could prevent breast cancer, because of its inclusion of an abundance of plant-based foods and the lack of processed foods (211). Xue M et al. confirmed through experiments (212) that fucoidan increases the diversity of intestinal flora and can promote the intestinal barrier function, and he suggested fucoidan as a preventive agent for breast cancer. studies have shown that increased polyphenol intake is associated with higher levels of beneficial bacteria (such as *Bifidobacterium* and *Lactobacillus*) and SCFAs in humans (174), while also decreasing levels of bacteria that have been associated with disease, so-called pathobionts. Diet is an important factor in all microbiota studies and can help maintain the stability of gut microbes, which can influence the development of breast cancer. If dietary interventions are to be successfully used in future treatments, studies of diet and microbiota metabolites might have to be conducted in parallel. Indeed, recent studies have highlighted the personalised response to individuals (and the microbiota) to the same diet (213), which highlights the limitations and challenges for next-stage studies of this kind.

Alcohol consumption increases the risk of breast cancer, although alcohol itself is not a direct carcinogen, acetaldehyde, a product of alcohol metabolism, is a mutagen which can form adducts with protein and DNA, inducing gene mutation, DNA crosslinks and chromosomal aberrations (214–216). Many studies have also confirmed that alcohol consumption not only induces breast cancer development (217, 218) but also promotes the progression of existing breast tumors and induces a more aggressive phenotype (219–222). There are no clear reports to confirm the correlation between alcohol, microorganisms and breast cancer metastasis, but there is no doubt that alcohol causes dysbiosis of the intestinal flora (223–225). It is not difficult to guess that there may be an alcohol-gut flora-breast cancer axis, which means that changing lifestyle habits could have profound implications for the prevention and prognosis of the disease, but the role of gut flora in this needs to be studied in depth.

Details of the following studies included in this review are summarized in Table 3.

3 Conclusions and future prospects

Globally, the number of factors affecting gastrointestinal dysbiosis is increasing and the gastrointestinal microbiome is emerging as an important player in the risk and progression of breast cancer. This provides an exciting new perspective on breast cancer metastasis, namely that the causes of intestinal dysbiosis are complex and variable, and that there may be a complex causal relationship between progression and metastasis of breast cancer. Therefore, treating the gut flora to stabilize the microenvironment may reduce pro-tumorigenic factors and their propagation in the tissue microenvironment, and establishing new strategies to balance these deleterious fluctuations is of interest in the treatment and prognosis of breast cancer. Given that several intrinsic and extrinsic factors are known and that the gut microbiota and breast cancer have an interactive relationship, future sequencing of the microbiota to capture metadata about dysbiosis and the selection of *in vivo* models are expected. Those steps will be informative and positive in reducing the risk of breast cancer progression and metastasis, and in guiding therapy for gastrointestinal symptoms or prognosis in patients with breast cancer. Future studies analyzing the gastrointestinal microbiota in patients with breast cancer should consider definitive stratification by histology and molecular science, which could require longer experience and a longer time frame. In addition, because of the large number of complex resident gut flora species, the difficulty of data collection and the unclear specific mechanisms of microenvironmental changes due to dysbiosis, studies and evidence linking the gastrointestinal microbiota to breast cancer metastasis and progression are currently relatively scarce and need to be validated by more specific and high-quality clinical trials and data, and there is an urgent need to combine different disciplines and microbiome studies and design new technical approaches.

Data availability statement

The current state of research and references in this article (review) are cited from the relevant references and the data are authentic and publicly available.

Author contributions

JZ, ZL, MD, XH and MY contributed to the conception and the drafting of manuscripts. GS, QX and YZ are responsible for coordinating and participating in the article revision. All authors contributed to the article and approved the submitted version.

Funding

The project was supported by the 2022 Qinghai Province Central Guide to Local Science and Technology Development Fund, the Breast Disease Treatment Centre of the Affiliated Hospital of Qinghai University manages this funding.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71:209–49. doi: 10.3322/caac.21660

2. Yersal O, Barutca S. Biological subtypes of breast cancer: Prognostic and therapeutic implications. *World J Clin Oncol* (2014) 5:412–24. doi: 10.5306/wjco.v5.i3.412

3. Gao JJ, Swain SM. Luminal a breast cancer and molecular assays: A review. *Oncologist* (2018) 23:556–65. doi: 10.1634/theoncologist.2017-0535

4. Ades F, Zardavas D, Bozovic-Spasojevic I, Pugliano L, Fumagalli D, de Azambuja E, et al. Luminal b breast cancer: molecular characterization, clinical management, and future perspectives. *J Clin Oncol* (2014) 32:2794–803. doi: 10.1200/JCO.2013.54.1870

5. Cheang MCU, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 index, HER2 status, and prognosis of patients with luminal b breast cancer. *J Natl Cancer Inst* (2009) 101:736–50. doi: 10.1093/jnci/djp082

6. Inic Z, Zegarac M, Inic M, Markovic I, Kozomara Z, Djurisic I, et al. Difference between luminal a and luminal b subtypes according to ki-67, tumor size, and progesterone receptor negativity providing prognostic information. *Clin Med Insights Oncol* (2014) 8:107–11. doi: 10.4137/CMO.S18006

7. Arciero CA, Guo Y, Jiang R, Behera M, O'Regan R, Peng L, et al. ER+/HER2+ breast cancer has different metastatic patterns and better survival than ER-/HER2+ breast cancer. *Clin Breast Cancer* (2019) 19:236–45. doi: 10.1016/j.clbc.2019.02.001

8. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St gallen international expert consensus on the primary therapy of early breast cancer 2013. *Ann Oncol* (2013) 24:2206–23. doi: 10.1093/annonc/mdt303

9. Waks AG, Winer EP. Breast cancer treatment: A review. *JAMA* (2019) 321:288–300. doi: 10.1001/jama.2018.19323

10. Liang Y, Zhang H, Song X, Yang Q. Metastatic heterogeneity of breast cancer: Molecular mechanism and potential therapeutic targets. *Semin Cancer Biol* (2020) 60:14–27. doi: 10.1016/j.semcancer.2019.08.012

11. Guller A, Kuschnerus I, Rozova V, Nadort A, Yao Y, Khabir Z, et al. Chick embryo experimental platform for micrometastases research in a 3D tissue engineering model: Cancer biology, drug development, and nanotechnology applications. *Biomedicines* (2021) 9:1578. doi: 10.3390/biomedicines9111578

12. Tulotta C, Ottewell P. The role of IL-1B in breast cancer bone metastasis. *Endocr Relat Cancer* (2018) 25:R421–34. doi: 10.1530/ERC-17-0309

13. Xiong Z, Deng G, Huang X, Li X, Xie X, Wang J, et al. Bone metastasis pattern in initial metastatic breast cancer: A population-based study. *Cancer Manag Res* (2018) 10:287–95. doi: 10.2147/CMAR.S155524

14. Smid M, Wang Y, Zhang Y, Sieuwerts AM, Yu J, Klijn JGM, et al. Subtypes of breast cancer show preferential site of relapse. *Cancer Res* (2008) 68:3108–14. doi: 10.1158/0008-5472.CAN-07-5644

15. Pentheroudakis G, Fountzilas G, Bafaloukos D, Koutsoukou V, Pectasides D, Skarlos D, et al. Metastatic breast cancer with liver metastases: A registry analysis of clinicopathologic, management and outcome characteristics of 500 women. *Breast Cancer Res Treat* (2006) 97:237–44. doi: 10.1007/s10549-005-9117-4

16. Lin NU, Amiri-Kordestani L, Palmieri D, Liewehr DJ, Steeg PS. CNS metastases in breast cancer: old challenge, new frontiers. *Clin Cancer Res* (2013) 19:6404–18. doi: 10.1158/1078-0432.CCR-13-0790

17. Altundag K. Primary breast cancer phenotypes associated with propensity for central nervous system metastases. *Cancer* (2006) 107:2521–2. doi: 10.1002/cncr.22270

18. The shifting landscape of metastatic breast cancer to the CNS. (Accessed December 1, 2021). doi: 10.1007/s10143-012-0446-6

19. Paget S. The distribution of secondary growths in cancer of the breast. 1889. *Cancer Metastasis Rev* (1989) 8:98–101. doi: 10.1016/s0140-6736(00)49915-0

20. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the healthy gut microbiota composition? a changing ecosystem across age, environment, diet, and diseases. *Microorganisms* (2019) 7:E14. doi: 10.3390/microorganisms7010014

21. Blaser MJ. Hypothesis: the changing relationships of helicobacter pylori and humans: implications for health and disease. *J Infect Dis* (1999) 179:1523–30. doi: 10.1086/314785

22. Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L. Comparative analysis of human gut microbiota by barcoded pyrosequencing. *PloS One* (2008) 3:e2836. doi: 10.1371/journal.pone.0002836

23. El Aidy S, van den Bogert B, Kleerebezem M. The small intestine microbiota, nutritional modulation and relevance for health. *Curr Opin Biotechnol* (2015) 32:14–20. doi: 10.1016/j.copbio.2014.09.005

24. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT. Human microbiome project consortium. structure, function and diversity of the healthy human microbiome. *Nature* (2012) 486:207–14. doi: 10.1038/nature11234

25. Gillespie JJ, Wattam AR, Cammer SA, Gabbard JL, Shukla MP, Dalay O, et al. PATRIC: the comprehensive bacterial bioinformatics resource with a focus on human pathogenic species. *Infect Immun* (2011) 79:4286–98. doi: 10.1128/IAI.00207-11

26. Hollister EB, Gao C, Versalovic J. Compositional and functional features of the gastrointestinal microbiome and their effects on human health. *Gastroenterology* (2014) 146:1449–58. doi: 10.1053/j.gastro.2014.01.052

27. Karczewski J, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer R-JM, et al. Regulation of human epithelial tight junction proteins by lactobacillus plantarum *in vivo* and protective effects on the epithelial barrier. *Am J Physiol Gastrointest Liver Physiol* (2010) 298:G851–859. doi: 10.1152/ajpgi.00327.2009

28. Bron PA, Kleerebezem M, Brummer R-J, Cani PD, Mercenier A, MacDonald TT, et al. Can probiotics modulate human disease by impacting intestinal barrier function? *Br J Nutr* (2017) 117:93–107. doi: 10.1017/S0007114516004037

29. Singh RK, Chang H-W, Yan D, Lee KM, Ucmak D, Wong K, et al. Influence of diet on the gut microbiome and implications for human health. *J Transl Med* (2017) 15:73. doi: 10.1186/s12967-017-1175-y

30. Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* (2016) 7:189–200. doi: 10.1080/19490976.2015.1134082

31. Matsuda JL, Mallevaey T, Scott-Browne J, Gapin L. CD1d-restricted iNKT cells, the "Swiss-army knife" of the immune system. *Curr Opin Immunol* (2008) 20:358–68. doi: 10.1016/j.coi.2008.03.018

32. Tupin E, Kinjo Y, Kronenberg M. The unique role of natural killer T cells in the response to microorganisms. *Nat Rev Microbiol* (2007) 5:405–17. doi: 10.1038/nrmicro1657

33. Parvaneh M, Karimi G, Jamaluddin R, Ng MH, Zuriati I, Muhammad SI. Lactobacillus helveticus (ATCC 27558) upregulates Runx2 and Bmp2 and modulates bone mineral density in ovariectomy-induced bone loss rats. *Clin Interv Aging* (2018) 13:1555–64. doi: 10.2147/CIA.S169223

34. Al-Lahham SH, Peppelenbosch MP, Roelofsen H, Vonk RJ, Venema K. Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. *Biochim Biophys Acta* (2010) 1801:1175–83. doi: 10.1016/j.bbalip.2010.07.007

35. Muccioli GG, Naslain D, Bäckhed F, Reigstad CS, Lambert DM, Delzenne NM, et al. The endocannabinoid system links gut microbiota to adipogenesis. *Mol Syst Biol* (2010) 6:392. doi: 10.1038/msb.2010.46

36. Neuman H, Debelius JW, Knight R, Koren O. Microbial endocrinology: the interplay between the microbiota and the endocrine system. *FEMS Microbiol Rev* (2015) 39:509–21. doi: 10.1093/femsre/fuu010

37. Ranganath LR, Beety JM, Morgan LM, Wright JW, Howland R, Marks V. Attenuated GLP-1 secretion in obesity: cause or consequence? *Gut* (1996) 38:916–9. doi: 10.1136/gut.38.6.916

38. Vrieze A, Holleman F, Zoetendal EG, de Vos WM, Hoekstra JBL, Nieuwdorp M. The environment within: how gut microbiota may influence metabolism and body composition. *Diabetologia* (2010) 53:606–13. doi: 10.1007/s00125-010-1662-7

39. Abdollahi-Roodsaz S, Abramson SB, Scher JU. The metabolic role of the gut microbiota in health and rheumatic disease: mechanisms and interventions. *Nat Rev Rheumatol* (2016) 12:446–55. doi: 10.1038/nrrheum.2016.68

40. LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D, Ventura M. Bacteria as vitamin suppliers to their host: A gut microbiota perspective. *Curr Opin Biotechnol* (2013) 24:160–8. doi: 10.1016/j.copbio.2012.08.005

41. Forsythe P, Sudo N, Dinan T, Taylor VH, Bienenstock J. Mood and gut feelings. *Brain Behav Immun* (2010) 24:9–16. doi: 10.1016/j.bbi.2009.05.058

42. Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nat Rev Gastroenterol Hepatol* (2009) 6:306–14. doi: 10.1038/nrgastro.2009.35

43. Chen X, Eslamfam S, Fang L, Qiao S, Ma X. Maintenance of gastrointestinal glucose homeostasis by the gut-brain axis. *Curr Protein Pept Sci* (2017) 18:541–7. doi: 10.2174/1389203717666160627083604

44. Soty M, Gautier-Stein A, Rajas F, Mithieux G. Gut-brain glucose signaling in energy homeostasis. *Cell Metab* (2017) 25:1231–42. doi: 10.1016/j.cmet.2017.04.032

45. Mohajeri MH, La Fata G, Steinert RE, Weber P. Relationship between the gut microbiome and brain function. *Nutr Rev* (2018) 76:481–96. doi: 10.1093/nutrit/nuy009

46. Sampson TR, Mazmanian SK. Control of brain development, function, and behavior by the microbiome. *Cell Host Microbe* (2015) 17:565–76. doi: 10.1016/j.chom.2015.04.011

47. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. *Nat Rev Immunol* (2017) 17:219–32. doi: 10.1038/nri.2017.7

48. Chow J, Mazmanian SK. A pathobiont of the microbiota balances host colonization and intestinal inflammation. *Cell Host Microbe* (2010) 7:265–76. doi: 10.1016/j.chom.2010.03.004

49. Korem T, Zeevi D, Suez J, Weinberger A, Avnit-Sagi T, Pompan-Lotan M, et al. Growth dynamics of gut microbiota in health and disease inferred from single metagenomic samples. *Science* (2015) 349:1101–6. doi: 10.1126/science.aac4812

50. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature* (2012) 486:222–7. doi: 10.1038/nature11053

51. Monaco CL, Gootenberg DB, Zhao G, Handley SA, Ghebremichael MS, Lim ES, et al. Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. *Cell Host Microbe* (2016) 19:311–22. doi: 10.1016/j.chom.2016.02.011

52. Kostic AD, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hämäläinen A-M, et al. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* (2015) 17:260–73. doi: 10.1016/j.chom.2015.01.001

53. Stecher B, Robbiani R, Walker AW, Westendorf AM, Barthel M, Kremer M, et al. Salmonella enterica serovar typhimurium exploits inflammation to compete with the intestinal microbiota. *PloS Biol* (2007) 5:2177–89. doi: 10.1371/journal.pbio.0050244

54. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of enterobacteriaceae. *Cell Host Microbe* (2007) 2:119–29. doi: 10.1016/j.chom.2007.06.010

55. Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-induced extinctions in the gut microbiota compound over generations. *Nature* (2016) 529:212–5. doi: 10.1038/nature16504

56. Norman JM, Handley SA, Baldridge MT, Droit L, Liu CY, Keller BC, et al. Disease-specific alterations in the enteric virome in inflammatory bowel disease. *Cell* (2015) 160:447–60. doi: 10.1016/j.cell.2015.01.002

57. Levy M, Thaiss CA, Elinav E. Metagenomic cross-talk: the regulatory interplay between immunogenomics and the microbiome. *Genome Med* (2015) 7:120. doi: 10.1186/s13073-015-0249-9

58. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U.S.A.* (2010) 107:11971–5. doi: 10.1073/pnas.1002601107

59. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, et al. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U.S.A.* (2011) 108(Suppl 1):4578–85. doi: 10.1073/pnas.1000081107

60. Ts S, Hw V. Accounting for reciprocal host-microbiome interactions in experimental science. *Nature* (2016) 534(7606):191–9. doi: 10.1038/nature18285

61. Scott AJ, Alexander JL, Merrifield CA, Cunningham D, Jobin C, Brown R, et al. International cancer microbiome consortium consensus statement on the role of the human microbiome in carcinogenesis. *Gut* (2019) 68:1624–32. doi: 10.1136/gutjnl-2019-318556

62. Fessler J, Matson V, Gajewski TF. Exploring the emerging role of the microbiome in cancer immunotherapy. *J Immunother Cancer* (2019) 7:108. doi: 10.1186/s40425-019-0574-4

63. Wong SH, Kwong TNY, Wu C-Y, Yu J. Clinical applications of gut microbiota in cancer biology. *Semin Cancer Biol* (2019) 55:28–36. doi: 10.1016/j.semcancer.2018.05.003

64. Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat Rev Gastroenterol Hepatol* (2019) 16:690–704. doi: 10.1038/s41575-019-0209-8

65. Gopalakrishnan V, Helmink BA, Spencer CN, Reuben A, Wargo JA. The influence of the gut microbiome on cancer, immunity, and cancer immunotherapy. *Cancer Cell* (2018) 33:570–80. doi: 10.1016/j.ccell.2018.03.015

66. Fong W, Li Q, Yu J. Gut microbiota modulation: A novel strategy for prevention and treatment of colorectal cancer. *Oncogene* (2020) 39:4925–43. doi: 10.1038/s41388-020-1341-1

67. Xavier JB, Young VB, Skufca J, Ginty F, Testerman T, Pearson AT, et al. The cancer microbiome: Distinguishing direct and indirect effects requires a systemic view. *Trends Cancer* (2020) 6:192–204. doi: 10.1016/j.trecan.2020.01.004

68. Kaźmierczak-Siedlecka K, Daca A, Fic M, van de Wetering T, Folwarski M, Makarewicz W. Therapeutic methods of gut microbiota modification in colorectal cancer management - fecal microbiota transplantation, prebiotics, probiotics, and synbiotics. *Gut Microbes* (2020) 11:1518–30. doi: 10.1080/19490976.2020.1764309

69. Gagnière J, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, et al. Gut microbiota imbalance and colorectal cancer. *World J Gastroenterol* (2016) 22:501–18. doi: 10.3748/wjg.v22.i2.501

70. Noto JM, Peek RM. The gastric microbiome, its interaction with helicobacter pylori, and its potential role in the progression to stomach cancer. *PloS Pathog* (2017) 13:e1006573. doi: 10.1371/journal.ppat.1006573

71. Handa O, Naito Y, Yoshikawa T. Redox biology and gastric carcinogenesis: the role of helicobacter pylori. *Redox Rep* (2011) 16:1–7. doi: 10.1179/174329211X12968219310756

72. Williams L, Jenkins GJS, Doak SH, Fowler P, Parry EM, Brown TH, et al. Fluorescence *in situ* hybridisation analysis of chromosomal aberrations in gastric tissue: the potential involvement of helicobacter pylori. *Br J Cancer* (2005) 92:1759–66. doi: 10.1038/sj.bjc.6602533

73. Aviles-Jimenez F, Vazquez-Jimenez F, Medrano-Guzman R, Mantilla A, Torres J. Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer. *Sci Rep* (2014) 4:4202. doi: 10.1038/srep04202

74. Carbone C, Piro G, Di Noia V, D'Argento E, Vita E, Ferrara MG, et al. Lung and gut microbiota as potential hidden driver of immunotherapy efficacy in lung cancer. *Mediators Inflammation* (2019) 2019:7652014. doi: 10.1155/2019/7652014

75. Feng Q, Chen W-D, Wang Y-D. Gut microbiota: An integral moderator in health and disease. *Front Microbiol* (2018) 9:151. doi: 10.3389/fmicb.2018.00151

76. Amirian ES, Petrosino JF, Ajami NJ, Liu Y, Mims MP, Scheurer ME. Potential role of gastrointestinal microbiota composition in prostate cancer risk. *Infect Agent Cancer* (2013) 8:42. doi: 10.1186/1750-9378-8-42

77. Golombos DM, Ayangbesan A, O'Malley P, Lewicki P, Barlow L, Barbieri CE, et al. The role of gut microbiome in the pathogenesis of prostate cancer: A prospective, pilot study. *Urology* (2018) 111:122–8. doi: 10.1016/j.urology.2017.08.039

78. Liss MA, White JR, Goros M, Gelfond J, Leach R, Johnson-Pais T, et al. Metabolic biosynthesis pathways identified from fecal microbiome associated with prostate cancer. *Eur Urol* (2018) 74:575–82. doi: 10.1016/j.eururo.2018.06.033

79. Fuhrman BJ, Feigelson HS, Flores R, Gail MH, Xu X, Ravel J, et al. Associations of the fecal microbiome with urinary estrogens and estrogen metabolites in postmenopausal women. *J Clin Endocrinol Metab* (2014) 99:4632–40. doi: 10.1210/jc.2014-2222

80. Xuan C, Shamonki JM, Chung A, Dinome ML, Chung M, Sieling PA, et al. Microbial dysbiosis is associated with human breast cancer. *PloS One* (2014) 9: e83744. doi: 10.1371/journal.pone.0083744

81. Wang H, Altemus J, Niazi F, Green H, Calhoun BC, Sturgis C, et al. Breast tissue, oral and urinary microbiomes in breast cancer. *Oncotarget* (2017) 8:88122–38. doi: 10.18632/oncotarget.21490

82. Thompson KJ, Ingle JN, Tang X, Chia N, Jeraldo PR, Walther-Antonio MR, et al. A comprehensive analysis of breast cancer microbiota and host gene expression. *PloS One* (2017) 12:e0188873. doi: 10.1371/journal.pone.0188873

83. Meng S, Chen B, Yang J, Wang J, Zhu D, Meng Q, et al. Study of microbiomes in aseptically collected samples of human breast tissue using needle biopsy and the potential role of *in situ* tissue microbiomes for promoting malignancy. *Front Oncol* (2018) 8:318. doi: 10.3389/fonc.2018.00318

84. Banerjee S, Tian T, Wei Z, Shih N, Feldman MD, Peck KN, et al. Distinct microbial signatures associated with different breast cancer types. *Front Microbiol* (2018) 9:951. doi: 10.3389/fmicb.2018.00951

85. Banerjee S, Wei Z, Tan F, Peck KN, Shih N, Feldman M, et al. Distinct microbiological signatures associated with triple negative breast cancer. *Sci Rep* (2015) 5:15162. doi: 10.1038/srep15162

86. Costantini L, Magno S, Albanese D, Donati C, Molinari R, Filippone A, et al. Characterization of human breast tissue microbiota from core needle biopsies through the analysis of multi hypervariable 16S-rRNA gene regions. *Sci Rep* (2018) 8:16893. doi: 10.1038/s41598-018-35329-z

87. Urbaniak C, Gloor GB, Brackstone M, Scott L, Tangney M, Reid G. The microbiota of breast tissue and its association with breast cancer. *Appl Environ Microbiol* (2016) 82:5039–48. doi: 10.1128/AEM.01235-16

88. Hieken TJ, Chen J, Hoskin TL, Walther-Antonio M, Johnson S, Ramaker S, et al. The microbiome of aseptically collected human breast tissue in benign and malignant disease. *Sci Rep* (2016) 6:30751. doi: 10.1038/srep30751

89. Chan AA, Bashir M, Rivas MN, Duvall K, Sieling PA, Pieber TR, et al. Characterization of the microbiome of nipple aspirate fluid of breast cancer survivors. *Sci Rep* (2016) 6:28061. doi: 10.1038/srep28061

90. Frugé AD, van der Pol W, Rogers LQ, Morrow CD, Tsuruta Y, Demark-Wahnefried W. Fecal akkermansia muciniphila is associated with body composition and microbiota diversity in overweight and obese women with breast cancer participating in a presurgical weight loss trial. *J Acad Nutr Diet* (2020) 120:650–9. doi: 10.1016/j.jand.2018.08.164

91. Tzeng A, Sangwan N, Jia M, Liu C-C, Keslar KS, Downs-Kelly E, et al. Human breast microbiome correlates with prognostic features and immunological signatures in breast cancer. *Genome Med* (2021) 13:60. doi: 10.1186/s13073-021-00874-2

92. Klann E, Williamson JM, Tagliamonte MS, Ukhanova M, Asirvatham JR, Chim H, et al. Microbiota composition in bilateral healthy breast tissue and breast tumors. *Cancer Causes Control* (2020) 31:1027–38. doi: 10.1007/s10552-020-01338-5

93. Hadzega D, Minarik G, Karaba M, Kalavska K, Benca J, Ciernikova S, et al. Uncovering microbial composition in human breast cancer primary tumour tissue using transcriptomic RNA-seq. *Int J Mol Sci* (2021) 22:9058. doi: 10.3390/ijms22169058

94. Esposito MV, Fosso B, Nunziato M, Casaburi G, D'Argenio V, Calabrese A, et al. Microbiome composition indicate dysbiosis and lower richness in tumor breast tissues compared to healthy adjacent paired tissue, within the same women. *BMC Cancer* (2022) 22:30. doi: 10.1186/s12885-021-09074-y

95. Luu TH, Michel C, Bard J-M, Dravet F, Nazih H, Bobin-Dubigeon C. Intestinal proportion of blautia sp. is associated with clinical stage and histoprognostic grade in patients with early-stage breast cancer. *Nutr Cancer* (2017) 69:267–75. doi: 10.1080/01635581.2017.1263750

96. Goedert JJ, Jones G, Hua X, Xu X, Yu G, Flores R, et al. Investigation of the association between the fecal microbiota and breast cancer in postmenopausal women: A population-based case-control pilot study. *J Natl Cancer Inst* (2015) 107: djv147. doi: 10.1093/jnci/djv147

97. Zhu J, Liao M, Yao Z, Liang W, Li Q, Liu J, et al. Breast cancer in postmenopausal women is associated with an altered gut metagenome. *Microbiome* (2018) 6:136. doi: 10.1186/s40168-018-0515-3

98. He C, Liu Y, Ye S, Yin S, Gu J. Changes of intestinal microflora of breast cancer in premenopausal women. *Eur J Clin Microbiol Infect Dis* (2021) 40:503–13. doi: 10.1007/s10096-020-04036-x

99. Bobin-Dubigeon C, Luu HT, Leuillet S, Lavergne SN, Carton T, Le Vacon F, et al. Faecal microbiota composition varies between patients with breast cancer and healthy women: A comparative case-control study. *Nutrients* (2021) 13:2705. doi: 10.3390/nu13082705

100. Ji J, Yang H. Using probiotics as supplementation for helicobacter pylori antibiotic therapy. *Int J Mol Sci* (2020) 21:E1136. doi: 10.3390/ijms21031136

101. Jin Y, Dong H, Xia L, Yang Y, Zhu Y, Shen Y, et al. The diversity of gut microbiome is associated with favorable responses to anti-programmed death 1 immunotherapy in Chinese patients with NSCLC. *J Thorac Oncol* (2019) 14:1378–89. doi: 10.1016/j.jtho.2019.04.007

102. Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillère R, Hannani D, et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* (2013) 342:971–6. doi: 10.1126/science.1240537

103. Pernigoni N, Zagato E, Calcinotto A, Troiani M, Mestre RP, Calì B, et al. Commensal bacteria promote endocrine resistance in prostate cancer through androgen biosynthesis. *Science* (2021) 374:216–24. doi: 10.1126/science.abf8403

104. Matsushita M, Fujita K, Hayashi T, Kayama H, Motooka D, Hase H, et al. Gut microbiota-derived short-chain fatty acids promote prostate cancer growth *via* IGF1 signaling. *Cancer Res* (2021) 81:4014–26. doi: 10.1158/0008-5472.CAN-20-4090

105. Vitorino M, Baptista de Almeida S, Alpuim Costa D, Faria A, Calhau C, Azambuja Braga S. Human microbiota and immunotherapy in breast cancer - a review of recent developments. *Front Oncol* (2022) 11:815772. doi: 10.3389/fonc.2021.815772

106. Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* (2013) 342:967–70. doi: 10.1126/science.1240527

107. Rea D, Coppola G, Palma G, Barbieri A, Luciano A, Del Prete P, et al. Microbiota effects on cancer: from risks to therapies. *Oncotarget* (2018) 9:17915–27. doi: 10.18632/oncotarget.24681

108. Plottel CS, Blaser MJ. Microbiome and malignancy. *Cell Host Microbe* (2011) 10:324–35. doi: 10.1016/j.chom.2011.10.003

109. Flores R, Shi J, Fuhrman B, Xu X, Veenstra TD, Gail MH, et al. Fecal microbial determinants of fecal and systemic estrogens and estrogen metabolites: A cross-sectional study. *J Transl Med* (2012) 10:253. doi: 10.1186/1479-5876-10-253

110. Yaghjyan L, Colditz GA. Estrogens in the breast tissue: A systematic review. *Cancer Causes Control* (2011) 22:529–40. doi: 10.1007/s10552-011-9729-4

111. Ervin SM, Li H, Lim L, Roberts LR, Liang X, Mani S, et al. Gut microbial β-glucuronidases reactivate estrogens as components of the estrobolome that reactivate estrogens. *J Biol Chem* (2019) 294:18586–99. doi: 10.1074/jbc.RA119.010950

112. Creely SJ, McTernan PG, Kusminski CM, Fisher ff M, Da Silva NF, Khanolkar M, et al. Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. *Am J Physiol Endocrinol Metab* (2007) 292:E740–747. doi: 10.1152/ajpendo.00302.2006

113. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* (2013) 500:541–6. doi: 10.1038/nature12506

114. Gunter MJ, Xie X, Xue X, Kabat GC, Rohan TE, Wassertheil-Smoller S, et al. Breast cancer risk in metabolically healthy but overweight postmenopausal women. *Cancer Res* (2015) 75:270–4. doi: 10.1158/0008-5472.CAN-14-2317

115. Margolis KL, Rodabough RJ, Thomson CA, Lopez AM, McTiernan AWomen's Health Initiative Research Group. Prospective study of leukocyte

count as a predictor of incident breast, colorectal, endometrial, and lung cancer and mortality in postmenopausal women. *Arch Intern Med* (2007) 167:1837–44. doi: 10.1001/archinte.167.17.1837

116. Ollberding NJ, Kim Y, Shvetsov YB, Wilkens LR, Franke AA, Cooney RV, et al. Prediagnostic leptin, adiponectin, c-reactive protein, and the risk of postmenopausal breast cancer. *Cancer Prev Res (Phila)* (2013) 6:188–95. doi: 10.1158/1940-6207.CAPR-12-0374

117. Goedert JJ, Hua X, Bielecka A, Okayasu I, Milne GL, Jones GS, et al. Postmenopausal breast cancer and oestrogen associations with the IgA-coated and IgA-noncoated faecal microbiota. *Br J Cancer* (2018) 118:471–9. doi: 10.1038/bjc.2017.435

118. van't Veer P, Dekker JM, Lamers JW, Kok FJ, Schouten EG, Brants HA, et al. Consumption of fermented milk products and breast cancer: A case-control study in the Netherlands. *Cancer Res* (1989) 49:4020–3. Available at: https://pubmed.ncbi.nlm.nih.gov/2736542/.

119. Hosseini E, Grootaert C, Verstraete W, Van de Wiele T. Propionate as a health-promoting microbial metabolite in the human gut. *Nutr Rev* (2011) 69:245–58. doi: 10.1111/j.1753-4887.2011.00388.x

120. Simin J, Tamimi RM, Engstrand L, Callens S, Brusselaers N. Antibiotic use and the risk of breast cancer: A systematic review and dose-response meta-analysis. *Pharmacol Res* (2020) 160:105072. doi: 10.1016/j.phrs.2020.105072

121. Koumarianou A, Manali ED, Fragou A, Katsaounis P, Bouga G, Karageorgopoulou S, et al. Antibiotic exposure and risk of breast cancer: A causal association or a skyfall? *JCO* (2013) 31:e12524–4. doi: 10.1200/jco.2013.31.15_suppl.e12524

122. Chng KR, Ghosh TS, Tan YH, Nandi T, Lee IR, Ng AHQ, et al. Metagenome-wide association analysis identifies microbial determinants of post-antibiotic ecological recovery in the gut. *Nat Ecol Evol* (2020) 4:1256–67. doi: 10.1038/s41559-020-1236-0

123. Ferrer M, Méndez-García C, Rojo D, Barbas C, Moya A. Antibiotic use and microbiome function. *Biochem Pharmacol* (2017) 134:114–26. doi: 10.1016/j.bcp.2016.09.007

124. Knekt P, Adlercreutz H, Rissanen H, Aromaa A, Teppo L, Heliövaara M. Does antibacterial treatment for urinary tract infection contribute to the risk of breast cancer? *Br J Cancer* (2000) 82:1107–10. doi: 10.1054/bjoc.1999.1047

125. Peterson SN, Bradley LM, Ronai ZA. The gut microbiome: An unexpected player in cancer immunity. *Curr Opin Neurobiol* (2020) 62:48–52. doi: 10.1016/j.conb.2019.09.016

126. Aarnoutse R, Hillege LE, Ziemons J, De Vos-Geelen J, de Boer M, Aerts EMER, et al. Intestinal microbiota in postmenopausal breast cancer patients and controls. *Cancers (Basel)* (2021) 13:6200. doi: 10.3390/cancers13246200

127. Crusz SM, Balkwill FR. Inflammation and cancer: advances and new agents. *Nat Rev Clin Oncol* (2015) 12:584–96. doi: 10.1038/nrclinonc.2015.105

128. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. *Nature* (2011) 473:174–80. doi: 10.1038/nature09944

129. Subbaramaiah K, Morris PG, Zhou XK, Morrow M, Du B, Giri D, et al. Retraction: Increased levels of COX-2 and prostaglandin E2 contribute to elevated aromatase expression in inflamed breast tissue of obese women. *Cancer Discovery* (2021) 11:1306. doi: 10.1158/2159-8290.CD-21-0224

130. Bowers LW, Brenner AJ, Hursting SD, Tekmal RR, deGraffenried LA. Obesity-associated systemic interleukin-6 promotes pre-adipocyte aromatase expression *via* increased breast cancer cell prostaglandin E2 production. *Breast Cancer Res Treat* (2015) 149:49–57. doi: 10.1007/s10549-014-3223-0

131. IgA response to symbiotic bacteria as a mediator of gut homeostasis. Accessed March 9, 2022). doi: 10.1016/j.chom.2007.09.013

132. Pabst O. New concepts in the generation and functions of IgA. *Nat Rev Immunol* (2012) 12:821–32. doi: 10.1038/nri3322

133. Carrega P, Bonaccorsi I, Di Carlo E, Morandi B, Paul P, Rizzello V, et al. CD56(bright)perforin(low) noncytotoxic human NK cells are abundant in both healthy and neoplastic solid tissues and recirculate to secondary lymphoid organs *via* afferent lymph. *J Immunol* (2014) 192:3805–15. doi: 10.4049/jimmunol.1301889

134. Kosaka A, Yan H, Ohashi S, Gotoh Y, Sato A, Tsutsui H, et al. Lactococcus lactis subsp. cremoris FC triggers IFN-γ production from NK and T cells *via* IL-12 and IL-18. *Int Immunopharmacol* (2012) 14:729–33. doi: 10.1016/j.intimp.2012.10.007

135. Garrett WS. Cancer and the microbiota. *Science* (2015) 348:80–6. doi: 10.1126/science.aaa4972

136. Koller VJ, Marian B, Stidl R, Nersesyan A, Winter H, Simić T, et al. Impact of lactic acid bacteria on oxidative DNA damage in human derived colon cells. *Food Chem Toxicol* (2008) 46:1221–9. doi: 10.1016/j.fct.2007.09.005

137. Microbiota in cancer development and treatment. (Accessed April 4, 2022). doi: 10.1007/s00432-018-2816-0

138. Sears CL, Pardoll DM. Perspective: alpha-bugs, their microbial partners, and the link to colon cancer. *J Infect Dis* (2011) 203:306–11. doi: 10.1093/jinfdis/jiq061

139. Sears CL. The toxins of bacteroides fragilis. *Toxicon* (2001) 39:1737–46. doi: 10.1016/s0041-0101(01)00160-x

140. Parida S, Wu S, Siddharth S, Wang G, Muniraj N, Nagalingam A, et al. A procarcinogenic colon microbe promotes breast tumorigenesis and metastatic progression and concomitantly activates notch and β-catenin axes. *Cancer Discovery* (2021) 11:1138–57. doi: 10.1158/2159-8290.CD-20-0537

141. Buchta Rosean C, Bostic RR, Ferey JCM, Feng T-Y, Azar FN, Tung KS, et al. Preexisting commensal dysbiosis is a host-intrinsic regulator of tissue inflammation and tumor cell dissemination in hormone receptor-positive breast cancer. *Cancer Res* (2019) 79:3662–75. doi: 10.1158/0008-5472.CAN-18-3464

142. Degnim AC, Hoskin TL, Arshad M, Frost MH, Winham SJ, Brahmbhatt RA, et al. Alterations in the immune cell composition in premalignant breast tissue that precede breast cancer development. *Clin Cancer Res* (2017) 23:3945–52. doi: 10.1158/1078-0432.CCR-16-2026

143. Cb W, Es Y, Ac S. Tumor-associated macrophages: unwitting accomplices in breast cancer malignancy. *NPJ Breast Cancer* (2016) 2:15025. doi: 10.1038/npjbcancer.2015.25

144. Jm G, Mh J, Di K, An S, Sy P. Prognostic value of tumor-associated macrophages according to histologic locations and hormone receptor status in breast cancer. *PloS One* (2015) 10(4):e0125728. doi: 10.1371/journal.pone.0125728

145. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, et al. Mammographic density and the risk and detection of breast cancer. *N Engl J Med* (2007) 356:227–36. doi: 10.1056/NEJMoa062790

146. Huo CW, Hill P, Chew G, Neeson PJ, Halse H, Williams ED, et al. High mammographic density in women is associated with protumor inflammation. *Breast Cancer Res* (2018) 20:92. doi: 10.1186/s13058-018-1010-2

147. Antibiotic-induced disturbances of the gut microbiota result in accelerated breast tumour growth *via* a mast cell-dependent pathway, in: *bioRxiv* . Available at: https://www.biorxiv.org/content/10.1101/2020.03.07.982108v1.abstract (Accessed August 22, 2021).

148. McKee AM, Kirkup BM, Madgwick M, Fowler WJ, Price CA, Dreger SA, et al. Antibiotic-induced disturbances of the gut microbiota result in accelerated breast tumor growth. *iScience* (2021) 24:103012. doi: 10.1016/j.isci.2021.103012

149. Su X, Esser AK, Amend SR, Xiang J, Xu Y, Ross MH, et al. Antagonizing integrin β3 increases immunosuppression in cancer. *Cancer Res* (2016) 76:3484–95. doi: 10.1158/0008-5472.CAN-15-2663

150. Croswell A, Amir E, Teggatz P, Barman M, Salzman NH. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric salmonella infection. *Infect Immun* (2009) 77:2741–53. doi: 10.1128/IAI.00006-09

151. Reikvam DH, Erofeev A, Sandvik A, Grcic V, Jahnsen FL, Gaustad P, et al. Depletion of murine intestinal microbiota: effects on gut mucosa and epithelial gene expression. *PloS One* (2011) 6:e17996. doi: 10.1371/journal.pone.0017996

152. Ewens A, Mihich E, Ehrke MJ. Distant metastasis from subcutaneously grown E0771 medullary breast adenocarcinoma. *Anticancer Res* (2005) 25:3905–15. Available at: https://pubmed.ncbi.nlm.nih.gov/16312045/.

153. Davie JR. Inhibition of histone deacetylase activity by butyrate. *J Nutr* (2003) 133:2485S–93S. doi: 10.1093/jn/133.7.2485S

154. Salimi V, Shahsavari Z, Safizadeh B, Hosseini A, Khademian N, Tavakoli-Yaraki M. Sodium butyrate promotes apoptosis in breast cancer cells through reactive oxygen species (ROS) formation and mitochondrial impairment. *Lipids Health Dis* (2017) 16:208. doi: 10.1186/s12944-017-0593-4

155. Burcelin R, Serino M, Chabo C, Garidou L, Pomié C, Courtney M, et al. Metagenome and metabolism: the tissue microbiota hypothesis. *Diabetes Obes Metab* (2013) 15(Suppl 3):61–70. doi: 10.1111/dom.12157

156. Puertollano E, Kolida S, Yaqoob P. Biological significance of short-chain fatty acid metabolism by the intestinal microbiome. *Curr Opin Clin Nutr Metab Care* (2014) 17:139–44. doi: 10.1097/MCO.0000000000000025

157. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U.S.A.* (2009) 106:3698–703. doi: 10.1073/pnas.0812874106

158. Dumas M-E. The microbial-mammalian metabolic axis: beyond simple metabolism. *Cell Metab* (2011) 13:489–90. doi: 10.1016/j.cmet.2011.04.005

159. Mikó E, Kovács T, Sebő É, Tóth J, Csonka T, Ujlaki G, et al. Microbiome–microbial metabolome–cancer cell interactions in breast cancer–familiar, but unexplored. *Cells* (2019) 8:293. doi: 10.3390/cells8040293

160. Tian Y, Xu Q, Sun L, Ye Y, Ji G. Short-chain fatty acids administration is protective in colitis-associated colorectal cancer development. *J Nutr Biochem* (2018) 57:103–9. doi: 10.1016/j.jnutbio.2018.03.007

161. Tao J, Li S, Gan R-Y, Zhao C-N, Meng X, Li H-B. Targeting gut microbiota with dietary components on cancer: Effects and potential mechanisms of action. *Crit Rev Food Sci Nutr* (2020) 60:1025–37. doi: 10.1080/10408398.2018.1555789

162. Goldberg AA, Beach A, Davies GF, Harkness TAA, LeBlanc A, Titorenko VI. Lithocholic bile acid selectively kills neuroblastoma cells, while sparing normal neuronal cells. *Oncotarget* (2011) 2:761–82. doi: 10.18632/oncotarget.338

163. Goldberg AA, Titorenko VI, Beach A, Sanderson JT. Bile acids induce apoptosis selectively in androgen-dependent and -independent prostate cancer cells. *PeerJ* (2013) 1:e122. doi: 10.7717/peerj.122

164. Luu TH, Bard J-M, Carbonnelle D, Chaillou C, Huvelin J-M, Bobin-Dubigeon C, et al. Lithocholic bile acid inhibits lipogenesis and induces apoptosis in breast cancer cells. *Cell Oncol (Dordr)* (2018) 41:13–24. doi: 10.1007/s13402-017-0353-5

165. Gafar AA, Draz HM, Goldberg AA, Bashandy MA, Bakry S, Khalifa MA, et al. Lithocholic acid induces endoplasmic reticulum stress, autophagy and mitochondrial dysfunction in human prostate cancer cells. *PeerJ* (2016) 4:e2445. doi: 10.7717/peerj.2445

166. Kovács T, Mikó E, Vida A, Sebő É, Toth J, Csonka T, et al. Cadaverine, a metabolite of the microbiome, reduces breast cancer aggressiveness through trace amino acid receptors. *Sci Rep* (2019) 9:1300. doi: 10.1038/s41598-018-37664-7

167. Chen J-Q, Yager JD. Estrogen's effects on mitochondrial gene expression: mechanisms and potential contributions to estrogen carcinogenesis. *Ann N Y Acad Sci* (2004) 1028:258–72. doi: 10.1196/annals.1322.030

168. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* (2013) 499:97–101. doi: 10.1038/nature12347

169. Mikó E, Vida A, Kovács T, Ujlaki G, Trencsényi G, Márton J, et al. Lithocholic acid, a bacterial metabolite reduces breast cancer cell proliferation and aggressiveness. *Biochim Biophys Acta Bioenerg* (2018) 1859:958–74. doi: 10.1016/j.bbabio.2018.04.002

170. Fernández MF, Reina-Pérez I, Astorga JM, Rodríguez-Carrillo A, Plaza-Díaz J, Fontana L. Breast cancer and its relationship with the microbiota. *Int J Environ Res Public Health* (2018) 15:E1747. doi: 10.3390/ijerph15081747

171. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol* (2014) 121:91–119. doi: 10.1016/B978-0-12-800100-4.00003-9

172. Teng NMY, Price CA, McKee AM, Hall LJ, Robinson SD. Exploring the impact of gut microbiota and diet on breast cancer risk and progression. *Int J Cancer* (2021) 149:494–504. doi: 10.1002/ijc.33496

173. Fraga CG, Croft KD, Kennedy DO, Tomás-Barberán FA. The effects of polyphenols and other bioactives on human health. *Food Funct* (2019) 10:514–28. doi: 10.1039/c8fo01997e

174. Sun H, Chen Y, Cheng M, Zhang X, Zheng X, Zhang Z. The modulatory effect of polyphenols from green tea, oolong tea and black tea on human intestinal microbiota *in vitro*. *J Food Sci Technol* (2018) 55:399–407. doi: 10.1007/s13197-017-2951-7

175. Thirunavukkarasan M, Wang C, Rao A, Hind T, Teo YR, Siddiquee AA-M, et al. Short-chain fatty acid receptors inhibit invasive phenotypes in breast cancer cells. *PloS One* (2017) 12:e0186334. doi: 10.1371/journal.pone.0186334

176. Li Q, Ding C, Meng T, Lu W, Liu W, Hao H, et al. Butyrate suppresses motility of colorectal cancer cells *via* deactivating Akt/ERK signaling in histone deacetylase dependent manner. *J Pharmacol Sci* (2017) 135:148–55. doi: 10.1016/j.jphs.2017.11.004

177. Li Q, Cao L, Tian Y, Zhang P, Ding C, Lu W, et al. Butyrate suppresses the proliferation of colorectal cancer cells *via* targeting pyruvate kinase M2 and metabolic reprogramming. *Mol Cell Proteomics* (2018) 17:1531–45. doi: 10.1074/mcp.RA118.000752

178. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet* (2014) 383:1068–83. doi: 10.1016/S0140-6736(13)62154-6

179. Sundaram S, Johnson AR, Makowski L. Obesity, metabolism and the microenvironment: Links to cancer. *J Carcinog* (2013) 12:19. doi: 10.4103/1477-3163.119606

180. Ridlon JM, Kang D-J, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res* (2006) 47:241–59. doi: 10.1194/jlr.R500013-JLR200

181. Miller-Fleming L, Olin-Sandoval V, Campbell K, Ralser M. Remaining mysteries of molecular biology: The role of polyamines in the cell. *J Mol Biol* (2015) 427:3389–406. doi: 10.1016/j.jmb.2015.06.020

182. de las Rivas B, Marcobal A, Carrascosa AV, Muñoz R. PCR detection of foodborne bacteria producing the biogenic amines histamine, tyramine, putrescine, and cadaverine. *J Food Prot* (2006) 69:2509–14. doi: 10.4315/0362-028x-69.10.2509

183. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, et al. The reverse warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. *Cell Cycle* (2009) 8:3984–4001. doi: 10.4161/cc.8.23.10238

184. Bafeta A, Koh M, Riveros C, Ravaud P. Harms reporting in randomized controlled trials of interventions aimed at modifying microbiota: A systematic review. *Ann Intern Med* (2018) 169:240–7. doi: 10.7326/M18-0343

185. Suez J, Zmora N, Segal E, Elinav E. The pros, cons, and many unknowns of probiotics. *Nat Med* (2019) 25:716–29. doi: 10.1038/s41591-019-0439-x

186. Aragón F, Carino S, Perdigón G, de Moreno de LeBlanc A. Inhibition of growth and metastasis of breast cancer in mice by milk fermented with lactobacillus casei CRL 431. *J Immunother* (2015) 38:185–96. doi: 10.1097/CJI.0000000000000079

187. Sharifi M, Moridnia A, Mortazavi D, Salehi M, Bagheri M, Sheikhi A. Kefir: A powerful probiotics with anticancer properties. *Med Oncol* (2017) 34:183. doi: 10.1007/s12032-017-1044-9

188. Ranjbar S, Seyednejad SA, Azimi H, Rezaeizadeh H, Rahimi R. Emerging roles of probiotics in prevention and treatment of breast cancer: A comprehensive review of their therapeutic potential. *Nutr Cancer* (2019) 71:1–12. doi: 10.1080/01635581.2018.1557221

189. Aragón F, Carino S, Perdigón G, de Moreno de LeBlanc A. The administration of milk fermented by the probiotic lactobacillus casei CRL 431 exerts an immunomodulatory effect against a breast tumour in a mouse model. *Immunobiology* (2014) 219:457–64. doi: 10.1016/j.imbio.2014.02.005

190. Yap WB, Ahmad FM, Lim YC, Zainalabidin S. Lactobacillus casei strain C1 attenuates vascular changes in spontaneously hypertensive rats. *Korean J Physiol Pharmacol* (2016) 20:621–8. doi: 10.4196/kjpp.2016.20.6.621

191. Kapila S, Vibha, Sinha PR. Antioxidative and hypocholesterolemic effect of lactobacillus casei ssp casei (biodefensive properties of lactobacilli). *Indian J Med Sci* (2006) 60:361–70. doi: 10.4103/0019-5359.27220

192. Khoury N, El-Hayek S, Tarras O, El-Sabban M, El-Sibai M, Rizk S. Kefir exhibits anti–proliferative and pro–apoptotic effects on colon adenocarcinoma cells with no significant effects on cell migration and invasion. *Int J Oncol* (2014) 45:2117–27. doi: 10.3892/ijo.2014.2635

193. Zamberi NR, Abu N, Mohamed NE, Nordin N, Keong YS, Beh BK, et al. The antimetastatic and antiangiogenesis effects of kefir water on murine breast cancer cells. *Integr Cancer Ther* (2016) 15:NP53–66. doi: 10.1177/1534735416642862

194. de Moreno de LeBlanc A, Matar C, Farnworth E, Perdigon G. Study of cytokines involved in the prevention of a murine experimental breast cancer by kefir. *Cytokine* (2006) 34:1–8. doi: 10.1016/j.cyto.2006.03.008

195. de Moreno de LeBlanc A, Matar C, LeBlanc N, Perdigón G. Effects of milk fermented by lactobacillus helveticus R389 on a murine breast cancer model. *Breast Cancer Res* (2005) 7:R477–486. doi: 10.1186/bcr1032

196. Méndez Utz VE, Pérez Visñuk D, Perdigón G, de Moreno de LeBlanc A. Milk fermented by lactobacillus casei CRL431 administered as an immune adjuvant in models of breast cancer and metastasis under chemotherapy. *Appl Microbiol Biotechnol* (2021) 105:327–40. doi: 10.1007/s00253-020-11007-x

197. Utz VEM, Perdigón G, de Moreno de LeBlanc A. Milk fermented by lactobacillus casei CRL431 modifies cytokine profiles associated to different stages of breast cancer development in mice. *Benef Microbes* (2019) 10:689–97. doi: 10.3920/BM2019.0011

198. Taherian-Esfahani Z, Abedin-Do A, Nouri Z, Mirfakhraie R, Ghafouri-Fard S, Motevaseli E. Lactobacilli differentially modulate mTOR and wnt/ β-catenin pathways in different cancer cell lines. *Iran J Cancer Prev* (2016) 9:e5369. doi: 10.17795/ijcp-5369

199. Esfandiary A, Taherian-Esfahani Z, Abedin-Do A, Mirfakhraie R, Shirzad M, Ghafouri-Fard S, et al. Lactobacilli modulate hypoxia-inducible factor (HIF)-1 regulatory pathway in triple negative breast cancer cell line. *Cell J* (2016) 18:237–44. doi: 10.22074/cellj.2016.4319

200. Esfandiary A, Abedin-Do A, Taherian-Esfahani Z, Ghafouri-Fard S, Motevaseli E. Intact expression of hypoxia-inducible factor 1α (HIF-1α) gene in HeLa cell line following treatment with lactobacilli supernatants. *Int J Cancer Manag* (2017) 10(2):e4774. doi: 10.5812/ijcp.4774

201. Yazdi MH, Mahdavi M, Setayesh N, Esfandyar M, Shahverdi AR. Selenium nanoparticle-enriched lactobacillus brevis causes more efficient immune responses *in vivo* and reduces the liver metastasis in metastatic form of mouse breast cancer. *Daru: J Faculty Pharmacy Tehran Univ Med Sci* (2013) 21(1):33. doi: 10.1186/2008-2231-21-33

202. Hassan Z, Mustafa S, Rahim RA, Isa NM. Anti-breast cancer effects of live, heat-killed and cytoplasmic fractions of enterococcus faecalis and staphylococcus hominis isolated from human breast milk. *In Vitro Cell Dev Biol Anim* (2016) 52:337–48. doi: 10.1007/s11626-015-9978-8

203. Motevaseli E, Shirzad M, Akrami SM, Mousavi A-S, Mirsalehian A, Modarressi MH. Normal and tumour cervical cells respond differently to vaginal lactobacilli, independent of pH and lactate. *J Med Microbiol* (2013) 62:1065–72. doi: 10.1099/jmm.0.057521-0

204. Nouri Z, Karami F, Neyazi N, Modarressi MH, Karimi R, Khorramizadeh MR, et al. Dual anti-metastatic and anti-proliferative activity assessment of two probiotics on HeLa and HT-29 cell lines. *Cell J* (2016) 18:127–34. doi: 10.22074/cellj.2016.4307

205. Azam R, Ghafouri-Fard S, Tabrizi M, Modarressi M-H, Ebrahimzadeh-Vesal R, Daneshvar M, et al. Lactobacillus acidophilus and lactobacillus crispatus

culture supernatants downregulate expression of cancer-testis genes in the MDA-MB-231 cell line. *Asian Pac J Cancer Prev* (2014) 15:4255–9. doi: 10.7314/apjcp.2014.15.10.4255

206. Matsuzaki T, Yokokura T, Mutai M. The role of lymph node cells in the inhibition of metastasis by subcutaneous injection of lactobacillus casei in mice. *Med Microbiol Immunol* (1988) 177:245–53. doi: 10.1007/BF00189410

207. Levi F, La Vecchia C, Gulie C, Negri E. Dietary factors and breast cancer risk in vaud, Switzerland. *Nutr Cancer* (1993) 19:327–35. doi: 10.1080/01635589309514263

208. Dietary factors and the risk of breast cancer. (Accessed October 7, 2022). doi: 10.1080/01635588709513958

209. Malin AS, Qi D, Shu X-O, Gao Y-T, Friedmann JM, Jin F, et al. Intake of fruits, vegetables and selected micronutrients in relation to the risk of breast cancer. *Int J Cancer* (2003) 105:413–8. doi: 10.1002/ijc.11088

210. Toi M, Hirota S, Tomotaki A, Sato N, Hozumi Y, Anan K, et al. Probiotic beverage with soy isoflavone consumption for breast cancer prevention: A case-control study. *Curr Nutr Food Sci* (2013) 9:194–200. doi: 10.2174/15734013113099990001

211. Newman TM, Vitolins MZ, Cook KL. From the table to the tumor: The role of Mediterranean and Western dietary patterns in shifting microbial-mediated signaling to impact breast cancer risk. *Nutrients* (2019) 11:E2565. doi: 10.3390/nu11112565

212. Xue M, Ji X, Liang H, Liu Y, Wang B, Sun L, et al. The effect of fucoidan on intestinal flora and intestinal barrier function in rats with breast cancer. *Food Funct* (2018) 9:1214–23. doi: 10.1039/c7fo01677h

213. Deehan EC, Yang C, Perez-Muñoz ME, Nguyen NK, Cheng CC, Triador L, et al. Precision microbiome modulation with discrete dietary fiber structures directs short-chain fatty acid production. *Cell Host Microbe* (2020) 27(3):389–404.e6. doi: 10.1016/j.chom.2020.01.006

214. Nakamura K, Iwahashi K, Furukawa A, Ameno K, Kinoshita H, Ijiri I, et al. Acetaldehyde adducts in the brain of alcoholics. *Arch Toxicol* (2003) 77:591–3. doi: 10.1007/s00204-003-0465-8

215. Liu Y, Nguyen N, Colditz GA. Links between alcohol consumption and breast cancer: A look at the evidence. *Womens Health (Lond)* (2015) 11:65–77. doi: 10.2217/whe.14.62

216. Dumitrescu RG, Shields PG. The etiology of alcohol-induced breast cancer. *Alcohol* (2005) 35(3):213–25. doi: 10.1016/j.alcohol.2005.04.005

217. Castro GD, Castro JA. Alcohol drinking and mammary cancer: Pathogenesis and potential dietary preventive alternatives. *World J Clin Oncol* (2014) 5:713–29. doi: 10.5306/wjco.v5.i4.713

218. Varela-Rey M, Woodhoo A, Martinez-Chantar M-L, Mato JM, Lu SC. Alcohol, DNA methylation, and cancer. *Alcohol Res* (2013) 35:25–35. Available at: https://pubmed.ncbi.nlm.nih.gov/24313162/.

219. Xu M, Wang S, Ren Z, Frank JA, Yang XH, Zhang Z, et al. Chronic ethanol exposure enhances the aggressiveness of breast cancer: the role of p38γ. *Oncotarget* (2016) 7:3489–505. doi: 10.18632/oncotarget.6508

220. Xu M, Ren Z, Wang X, Comer A, Frank JA, Ke Z-J, et al. ErbB2 and p38γ MAPK mediate alcohol-induced increase in breast cancer stem cells and metastasis. *Mol Cancer* (2016) 15:52. doi: 10.1186/s12943-016-0532-4

221. Zhao M, Howard EW, Parris AB, Guo Z, Zhao Q, Yang X. Alcohol promotes migration and invasion of triple-negative breast cancer cells through activation of p38 MAPK and JNK. *Mol Carcinog* (2017) 56:849–62. doi: 10.1002/mc.22538

222. Xu M, Chen G, Fu W, Liao M, Frank JA, Bower KA, et al. Ethanol disrupts vascular endothelial barrier: implication in cancer metastasis. *Toxicol Sci* (2012) 127:42–53. doi: 10.1093/toxsci/kfs087

223. Bode JC, Bode C, Heidelbach R, Dürr HK, Martini GA. Jejunal microflora in patients with chronic alcohol abuse. *Hepatogastroenterology* (1984) 31:30–4. Available at: https://pubmed.ncbi.nlm.nih.gov/6698486/.

224. Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, et al. Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol* (2012) 302:G966–978. doi: 10.1152/ajpgi.00380.2011

225. Elamin EE, Masclee AA, Dekker J, Jonkers DM. Ethanol metabolism and its effects on the intestinal epithelial barrier. *Nutr Rev* (2013) 71:483–99. doi: 10.1111/nure.12027

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 09 November 2022
DOI 10.3389/fonc.2022.1002360

OPEN ACCESS

EDITED BY
Maria Rosaria De Miglio,
University of Sassari, Italy

REVIEWED BY
Muhammad Asif,
Balochistan University of Information Technology, Engineering and Management Sciences, Pakistan
Luo Hai,
Chinese Academy of Medical Sciences and Peking Union Medical College, China

*CORRESPONDENCE
Tianhui Chen
chenth@zjcc.org.cn
Xiao-jia Wang
wangxj@zjcc.org.cn

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 25 July 2022
ACCEPTED 24 October 2022
PUBLISHED 09 November 2022

CITATION
Lei H, Zhang M, Zhang L, Hemminki K, Wang X-j and Chen T (2022) Overview on population screening for carriers with germline BRCA mutation in China.
Front. Oncol. 12:1002360.
doi: 10.3389/fonc.2022.1002360

Overview on population screening for carriers with germline BRCA mutation in China

Huijun Lei[1,2†], Min Zhang[3†], Luyao Zhang[4], Kari Hemminki[5,6], Xiao-jia Wang[2,7*] and Tianhui Chen[1,2,8*]

[1]Department of Cancer Prevention/Zhejiang Cancer Institute, Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Hangzhou, China, [2]Institute of Basic Medicine and Cancer (IBMC), Chinese Academy of Sciences, Hangzhou, China, [3]School of Public Health, Hangzhou Medical College, Hangzhou, Zhejiang, China, [4]Department of Cancer Epidemiology and Prevention, Henan Engineering Research Center of Cancer Prevention and Control, Henan International Joint Laboratory of Cancer Prevention, The Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou, China, [5]Biomedical Center, Faculty of Medicine, Charles University in Pilsen, Pilsen, Czechia, [6]Division of Cancer Epidemiology, German Cancer Research Center Deutsches Krebsforschungszentrum (DKFZ), Im Neuenheimer Feld, Heidelberg, Germany, [7]Department of Breast Medical Oncology, Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Hangzhou, China, [8]Department of Preventive Medicine, School of Medicine, Ningbo University, Ningbo, China

Carriers with *BRCA1/2* germline pathogenic variants are associated with a high risk of breast and ovarian cancers (also pancreatic and prostate cancers). While the spectrum on germline *BRCA* mutations among the Chinese population shows ethnic specificity, the identification of carriers with germline *BRCA* mutation before cancer onset is the most effective approach to protect them. This review focused on the current status of *BRCA1/2* screening, the surveillance and prevention measures, and discussed the issues and potential impact of *BRCA1/2* population screening in China. We conducted literature research on databases PubMed and Google Scholar, as well as Chinese databases CNKI and Wangfang Med Online database (up to 31 March 2022). Latest publications on germline *BRCA1/2* prevalence, spectrum, genetic screening as well as carrier counseling, surveillance and prevention were captured where available. While overall 15,256 records were retrieved, 72 publications using germline *BRCA1/2* testing were finally retained for further analyses. Germline *BRCA1/2* mutations are common in Chinese patients with hereditary breast, ovarian, prostate and pancreatic cancers. Within previous studies, a unique *BRCA* mutation spectrum in China was revealed. Next-generation sequencing panel was considered as the most common method for *BRCA1/2* screening. Regular surveillance and preventive surgeries were tailored to carriers with mutated-*BRCA1/2*. We recommend that all Chinese diagnosed with breast, ovarian, pancreatic or prostate cancers and also healthy family members, shall undergo *BRCA1/2* gene test to provide risk assessment. Subsequently, timely preventive measures for mutation carriers are recommended after authentic genetic counseling.

KEYWORDS

population screening, BRCA, germline mutation, China, familial risk

Introduction

Breast cancer genes *BRCA1* and *BRCA2* are tumor suppressor genes that function in DNA double-strand break repair in the homologous recombination pathway. Mutated *BRCA1/2* genes can cause *BRCA1/2* protein deficiency and genome instability (1). Since the identification of *BRCA1* and *BRCA2* genes in the 1990s as the landmarks of hereditary breast and ovarian cancer, human beings enter the era of cancer genetic testing. Female *BRCA* mutation carriers have 60-80% of lifetime risk of developing breast cancer and 20-40% of risk of ovarian cancer (2). Mutation in *BRCA* is also associated with an increased risk of prostate and pancreatic cancers (3). In addition, *BRCA* pathogenic mutation carriers are significantly associated with increased disease risk for three additional cancers, including biliary tract cancer, gastric cancer, and esophageal cancer (4). Notably, *BRCA1* pathogenic variants carriers have a 4.30, 2.36 and 2.17-fold elevated lifetime risk of the male breast, pancreatic and stomach cancers compared to non-carriers. *BRCA2* pathogenic variants carriers have 44.0, 3.69, 3.34 and 2.22-fold elevated lifetime risk of the male breast, stomach, pancreatic and prostate cancers compared to non-carriers, respectively (5).

Early detection and prevention have been proven to reduce cancer incidence and mortality (while increasing cancer survival) in mutation carriers (3, 6). Therefore, identifying *BRCA* mutation carriers is important to reduce cancer risk. In this review, we conducted literature research on PubMed, Google Scholar and Chinese databases about germline *BRCA1/2* mutation in the Chinese populations included literature published up to 31 March 2022. A total of 15,256 publications were obtained: PubMed (n=856), Google Scholar (n=6,153), CNKI (n=4,935) and Wangfang Med Online database (n=3,312). After removing duplicates, selecting the title and the abstract and carefully reading the whole paper, 72 publications related to germline *BRCA1/2* testing were finally included. Based on the comprehensive literature review, we discuss population screening approaches for comprehensive identification of the *BRCA* mutation carriers in the Chinese population and propose the ideal procedure for achieving the goals in China (Figure 1) (7–11).

Abbreviations: CFCSG-database, Chinese Familial & Hereditary Cancer Susceptibility Gene Mutation database; ESMO, the European Society of Medical Oncology; HBOC, hereditary breast and ovarian cancer syndrome; LGRs, large genomic rearrangements; MLPA, multiplex ligation-dependent probe amplification; MRI, magnetic resonance imaging; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; PARP, poly (ADP-ribose) polymerase.PSA, prostate-specific antigen; SGO, the Society of Gynecologic Oncology; VUS, variants of uncertain significance; WES, whole-exome sequencing; WGS, whole-genome sequencing.

Overall prevalence and spectrum of *BRCA* mutation carriers in China and elsewhere

In the general Chinese population, the prevalence of pathogenic *BRCA1/2* variation has been reported to range from 0.29 to 1.10% (0.02 to 0.34% for *BRCA1* and 0.11 to 0.27% for *BRCA2*) (12–15).

The prevalence of *BRCA1/2* in the general population varies by country and ethnicity (16, 17). It was 0.18% in a Malaysian group of 2,809 individuals, 0.26% among 22,731 Japanese, 0.38% in a Mexican population of 3,985 individuals, 0.53% in 50,726 US people and 2.17% in the Ashkenazi Jewish population, which is the highest (18–22). The prevalence of *BRCA1/2* mutation in the general Chinese population is intermediate.

The spectrum of *BRCA* variation in Chinese is rather different from those in non-Chinese populations (15, 17). It was reported that approximately 38-41.4% of *BRCA* variants were only present in the Chinese population (23, 24). Even when compared to neighboring India, only 4.1% and 0.4% of shared *BRCA1* and *BRCA2* variants were found in both populations (24).

In a large-scale cohort with 1,245 pathogenic variants identified, 48 most common pathogenic *BRCA1/2* variants (39.86% of total) were not reported as common variants in Caucasians (15). The pathogenic variant *BRCA1* c.5470_5477del was determined as a founder mutation in the Chinese Han population (25, 26). Interestingly, another systematic review with 2,128 *BRCA1/2* variants derived from 35,178 Chinese individuals from 23 provinces also reported that c.5470_5477del ranked as the highest frequency of all *BRCA1* variants identified while the c.3109C>T ranks highest in *BRCA2* (12). Further, *BRCA1* c.3770_3771delAG was the most common variant in Chinese ovarian cancer patients (27). The proportions of frameshift, nonsense, splice and missense mutations in Chinese ovarian cancer patients were determined as 51.2%, 39.3%, 7.1% and 2.4%, respectively (28). But the founder mutations in other ethnic populations, such as *BRCA1* c.66_67delAG, *BRCA2* c.5946delT in Ashkenazi Jewish, *BRCA* 1 c.303T>G, c.1623dupG in African, *BRCA1* c.390C>A in Japanese and Korean and many other founder mutations in different non-Chinese populations, were absent or at low prevalence in Chinese population (24).

Prevalence of *BRCA* mutations in different populations in China

We summarized the prevalence of *BRCA1/2* germline mutation in different populations from large-scale cohort studies published within five years (Table 1). A total of 41 studies were included for further analysis (13–15, 27, 29–35, 64).

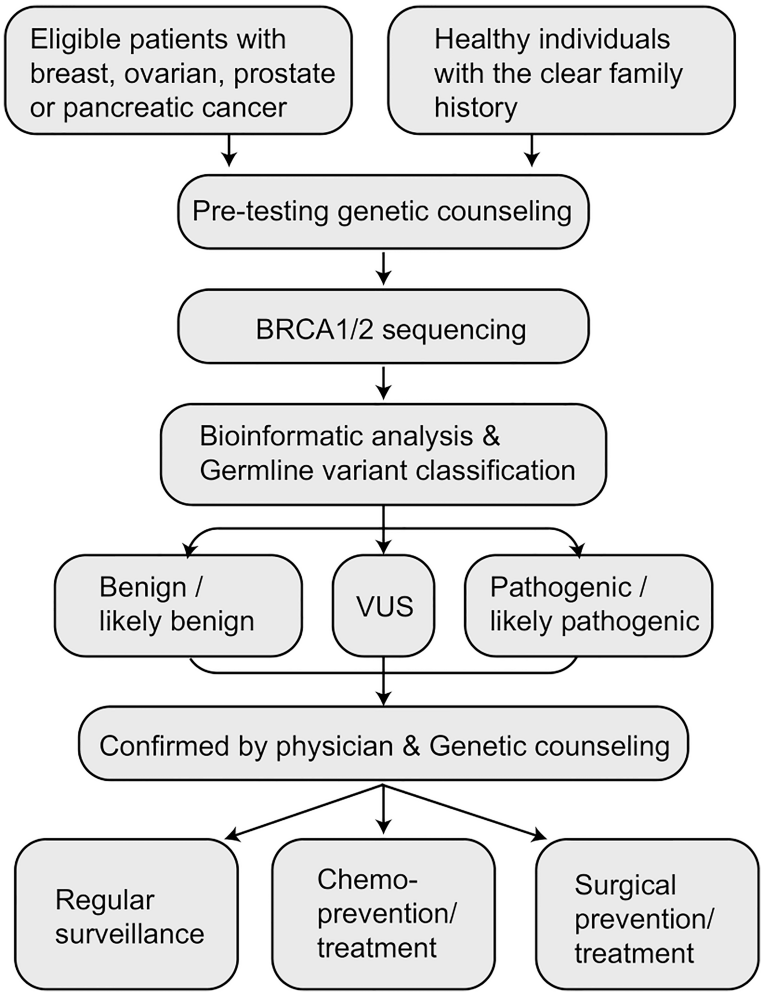


FIGURE 1
The procedure of population screening for BRCA germline mutation carriers in China (7–11).

In the Chinese cancer patients, a study showed that the prevalence rate was 5.53% for *BRCA1/2* (43.7% in *BRCA1* and 56.3% in *BRCA2*) in unselected breast cancer patients (15). In comparison, a higher prevalence of 9.06-19.54% for *BRCA1/2* mutation was observed in familial breast cancer patients (29, 31, 33). 60% of breast cancer patients carrying *BRCA1* deleterious mutation were classified as triple-negative breast cancer, while only 10 to 20% were triple-negative breast cancer in unselected cancer patients (33, 34). Patients with *BRCA1/2* mutated breast cancer generally show an earlier age of onset, on average 5 to 8 years earlier than patients with sporadic breast cancer (33, 35). *BRCA1/2* pathogenic variants are also enriched in bilateral breast cancer and patients with family history of breast or other cancers (33, 34). In unselected ovarian cancer patients, *BRCA1* pathogenic variants were more common compared to *BRCA2* (20.07% *vs.* 6.19%) (27). Pathogenic mutations in *BRCA1* genes were more related to a younger diagnosis age, serous ovarian carcinoma and hereditary breast and ovarian cancer syndrome (HBOC) (27). Among prostate cancer patients carrying germline mutations, *BRCA2* is the most common mutated gene among DNA damage repair pathway genes. The prevalence of *BRCA1* and *BRCA2* pathogenic variants was 0.38% and 4.30%, respectively, in prostate cancer patients (55). *BRCA2* was also reported as the most frequent gene in the germline in pancreatic cancer patients, with a prevalence rate of 1.9%; the frequency of *BRCA1* variants was 0.5% (57). However, there is a lack of multicenter studies on *BRCA* mutations in pancreatic cancer. It is worth noting that the actual prevalence may be higher than what is now predicted because the data for pathogenic variants interpretation are mainly from non-Chinese populations. In addition, most of the studies summarized in the Table 1 examined only single nucleotide variants and indels and did

not detect mutations of large genomic rearrangements (LGRs). It is possible that many unknown pathogenic variants have not been identified.

Despite increasing data from large-scale and multicenter *BRCA* studies having been reported, no *BRCA* data is reported for the Chinese living in many remote areas (12). Most *BRCA1/2* prevalence studies were from cities with relatively developed economies and medical care, such as Beijing, Shanghai, Hong Kong, Guangdong, Zhejiang and Sichuan. Possibly because genetic testing is not yet covered by basic medical insurance, patients in economically developed regions are more likely to afford expensive genetic testing. Meanwhile, economically and medically developed regions have more medical resources, such as genetic testing facilities and genetic counseling services (65). The bias is also because these regions have more investigators and research funds and are more likely to conduct clinical studies. However, considering the regional and ethnic specificity of *BRCA* gene variation, substantial efforts are needed to generate a comprehensive *BRCA* variation map for the Chinese population.

Methodologies for population *BRCA* screening in Chinese population

In the mid-1990s, the identification of the relationship between *BRCA1/2* mutation and cancer risk heralded the era of genetic testing for susceptibility to cancer. Subsequently, germline *BRCA1* and *BRCA2* mutations were extensively studied in the Caucasian populations, and associations with breast and ovarian cancers were established (66). Sanger sequencing has been widely used in *BRCA* variant identification since the 1990s, but the development of next-generation sequencing (NGS) revolutionized the detection strategy due to its affordability and efficiency.

NGS, including whole-genome sequencing (WGS), whole-exome sequencing (WES) and panel sequencing, have facilitated *BRCA* mutation research (67). Also because of the policy support in 2015, large-scale *BRCA* studies in China have increased rapidly since then (12, 42). Due to the lack of hotspot variation, NGS is currently the optimal option for *BRCA1/2* genetic testing in the Chinese population. NGS panel test is widely implemented for clinical *BRCA* test in China in recent years. The two-gene panel is a more preferred option for the general population, breast cancer and ovarian cancer patients, while pancreatic, prostate and other cancer patients tend to be suggested with the multi-gene panel in China (Tables 1, 2).

Because of its accuracy, Sanger sequencing remains to be a gold standard for detecting *BRCA* variants and validating NGS-detected *BRCA* variants and can be used in confirming the findings (67). Practical test- and laboratory-specific criteria have been proposed for confirmation strategy to facilitate timely delivery of clinical accuracy (73).

Many studies involving different populations have shown that LGRs in *BRCA1/2* can be identified in HBOC (74–76). Multiplex ligation-dependent probe amplification (MLPA) is a cheap, sensitive and reliable method for detecting gene rearrangements (77). In the eastern Chinese population, 2.9% of HBOC patients without detectable *BRCA1/2* small pathogenic variants were identified harboring LGRs in *BRCA* (78). The data are similar to those from the Myriad data set with high-risk patients, most of whom were diagnosed with early-onset ovary cancer or male breast cancer. The study reported an overall *BRCA1/2* mutation rate of 23.8%, of which 9.9% were LGRs. Thus, large genomic rearrangement testing is recommended if the NGS result is negative for high-risk populations to avoid the missed diagnosis of *BRCA1/2* mutation carriers (71).

Problems related to panel sequencing

NGS panel, the recommended method for *BRCA1/2* testing in clinical practice, still has some problems. First, variants of uncertain significance (VUS) increase with testing a larger panel or increasing genome sequencing length, making *BRCA1* and *BRCA2* interpretation more complex (79). For example, 24.7% of variants reported in the general population and 43.8% reported in breast cancer were identified as VUS, respectively (13, 31). Classification of VUS as pathogenic or benign variants has important clinical implications for cancer diagnosis and treatment (80). The methods to identify VUS as pathogenic or benign need to become more efficient and accurate, considering the huge abundance of VUS. *BRCA1/2* variants interpretation mainly follows the Chinese expert consensus on *BRCA1/2* variant interpretation (2021 version) (81) and the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guideline (82) in China.

Nevertheless, the population, disease-specific and sequence databases commonly used for interpretation contain few Chinese or Asian data. Lacking Chinese ethnic-specific data makes variant interpretation highly reliant on the peer-reviewed literature, which is also limited. This challenging context prevents many pathogenic variants from being identified and the VUS increases even more (83). Suggesting a new classification system for Chinese is needed, including but not limited to the databases based on Chinese populations and biological function identification of Chinese specific variants. Chinese Familial & Hereditary Cancer Susceptibility Gene Mutation Database (CFCSG-database) is one of the biggest cancer susceptibility gene mutation databases based on Chinese population. But the amount of *BRCA1* and *BRCA2*

TABLE 1 Summary of BRCA prevalence studies from China in recent 5 years.

Reference*	Year	BRCA1/2(+) number	BRCA1/2 (+) rate (%)	BRCA1 (+) N	BRCA1 (+) rate (%)	BRCA2 (+) N	BRCA2 (+) rate (%)	Sequencing methods	Singlecenter or multicenter	Regions	Study Population	Select criteria	Size	Age range	Median age	Average age
(13)	2021	43	0.38	13	0.11	30	0.26	NGS panel** +sanger	multicenter	nation-wide	general	healthy population	11386	>19	–	F34.8/ M43.0
(15)	2021	71	1.10	–	–	–	–	NGS panel +sanger	multicenter	–	general	healthy population	6434	–	–	34.8
(14)	2021	18	0.29	1	0.02	17	0.27	NGS panel +sanger	singlecenter	Macau	general	healthy population	6314	–	–	F42.0/ M41.0
(29)	2017	4	0.38	–	–	–	–	NGS panel +sanger	multicenter	Shanghai, Fujian	general	healthy population	1043	–	–	–
(27)	2018	8	0.45	6	0.34	2	0.11	NGS panel +sanger	multicenter	–	general	healthy population	1763	–	–	37.5
(30)	2019	138	15.65	89	10.09	49	5.56	NGS multi-gene panel (21***)+sanger	singlecenter	Guangdong	high risk population	high risk of HBOC	882	13-80	–	47.0
(31)	2021	23	19.83	11	9.48	12	10.34	NGS multi-gene panel (43) +sanger	singlecenter	Tianjin	breast cancer/ high risk population	familial patients and their direct relatives	116	26-76	51	50.0
(32)	2017	31	23.31	18	13.53	13	9.77	NGS panel +duplicate independent PCR	singlecenter	Zhejiang	breast/ovarian cancer	familial	133	22-74	–	43.0
(15)	2021	1174	5.53	–	2.3	–	3.1	NGS panel +sanger	multicenter	–	breast cancer	unselected	21216	–	–	49.7
(33)	2017	428	5.29	146	1.81	285	3.53	NGS multi-gene panel (62) +sanger	singlecenter	Beijing	breast cancer	unselected	8085	–	–	–
(34)	2019	148	5.34	74	2.67	76	2.74	NGS panel +sanger	singlecenter	Zhejiang	breast cancer	unselected	2769	–	–	49.4
(35)	2020	29	5.53	11	2.10	18	3.44	NGS multi-gene panel (62)	singlecenter	Guangdong	breast cancer	unselected	524	22-86	–	49.2
(36)	2021	13	3.82	5	1.47	8	2.35	NGS panel	singlecenter	Guangdong	breast cancer	unselected	340	–	–	49.9
(37)	2017	17	5.43	5	1.60	12	3.83	NGS panel +sanger	singlecenter	Hunan	breast cancer	unselected	313	21-84		51.2
(29)	2017	232	9.06	105	4.10	128	5.00	NGS panel +sanger	multicenter	Shanghai, Fujian	breast cancer	familial	2560	–	–	–
(33)	2017	146	18.14	59	7.33	87	10.81	NGS multi-gene panel (62) +sanger	singlecenter	Beijing	breast cancer	familial	805	–	–	–

(Continued)

10.3389/fonc.2022.1002360

TABLE 1 Continued

Reference*	Year	BRCA1/2(+) number	BRCA1/2 (+) rate (%)	BRCA1 (+) N	BRCA1 (+) rate (%)	BRCA2 (+) N	BRCA2 (+) rate (%)	Sequencing methods	Singlecenter or multicenter	Regions	Study Population	Select criteria	Size	Age range	Median age	Average age
(38)	2019	94	19.54	70	14.55	24	4.99	NGS multi-gene panel (22) +sanger	multicenter	nation-wide (28 centers)	breast cancer	familial	481	19-77	47	–
(33)	2017	198	3.32	56	0.94	142	2.38	NGS multi-gene panel (62) +sanger	singlecenter	Beijing	breast cancer	sporadic	5963	–	–	–
(39)	2019	159	16.97	82	8.75	81	8.64	NGS panel (40)	multicenter	nation-wide (26 centers)	breast cancer	high risk	937	8-77	–	37.5
(40)	2018	40	8.33	6	1.25	34	7.08	NGS multi-gene panel (20) +sanger	singlecenter	Guangdong	breast cancer	high risk	480	17-82	–	41.8
(41)	2017	35	7.94	9	2.04	26	5.90	SNaPshot/NGS/ MLPA+Sanger	multicenter	Hongkong	breast cancer	high risk	441	18-87	–	47.1
(42)	2018	76	17.39	–	–	–	–	NGS panel	multicenter	nation-wide (18 centers)	breast cancer	high risk	437	–	–	–
(29)	2017	15	3.48	–	–	–	–	NGS panel +sanger	multicenter	Shanghai, Fujian	breast cancer	high risk	431	–	–	–
(39)	2019	18	8.33	11	5.09	7	3.24	NGS panel +sanger	multicenter	Inner Mongolia, Jilin	breast cancer	high risk	216	21-67	42	–
(43)	2021	67	18.87	–	–	–	–	NGS panel +sanger	singlecenter	Shanghai	breast cancer	early-onset TNBC	355	24-40	34	–
(44)	2020	85	6.31	24	1.78	61	4.53	–	singlecenter	Fujian	breast cancer	early-onset	1347	<40	–	–
(45)	2019	4	14.81	2	7.41	2	7.41	NGS panel +sanger	singlecenter	Sichuan	breast cancer	early-onset	27	23-40	–	32.0
(46)	2020	35	10.77	24	7.38	9	2.77	–	singlecenter	Shanghai	breast cancer	TNBC	325	–	–	–
(47)	2021	26	20.97	20	16.13	6	4.84	NGS panel +MLPA	singlecenter	Shanghai	breast cancer	TNBC	124	24-55	46	–
(45)	2019	1	3.70	0	0.00	1	3.70	NGS panel +sanger	singlecenter	Sichuan	breast cancer	non-early-onset	27	41-68	–	52.0
(48)	2018	48	8.07	17	2.86	31	5.21	NGS panel +sanger	multicenter	Guangdong, Shandong, Chongqing	breast cancer	–	595	22-80	–	48.0
(28)	2019	129	23.58	84	15.36	45	8.23	NGS panel +sanger	multicenter	Shandong	ovarian/ fallopian tube/ peritoneal cancer	unselected	547	–	–	–

(Continued)

10.3389/fonc.2022.1002360

TABLE 1 Continued

Reference*	Year	BRCA1/2(+) number	BRCA1/2 (+) rate (%)	BRCA1 (+) N	BRCA1 (+) rate (%)	BRCA2 (+) N	BRCA2 (+) rate (%)	Sequencing methods	Singlecenter or multicenter	Regions	Study Population	Select criteria	Size	Age range	Median age	Average age
(49)	2021	14	22.58	12	19.35	2	3.23	NGS multi-gene panel +sanger/qPCR	singlecenter	Beijing	ovarian/ fallopian tube/ peritoneal cancer	unselected	62	34-82	56	–
(27)	2019	297	26.26	227	20.07	70	6.19	NGS panel +sanger	multicenter	nation-wide	ovarian cancer	unselected	1131	9-24	–	51.5
(50)	2017	235	28.45	172	20.82	63	7.63	NGS panel +sanger/qPCR	multicenter	Shanghai, Beijing, Shandong, Guangdong, Sichuan	ovarian cancer	unselected	826	–	52	–
(51)	2017	41	23.84	35	20.35	28	16.28	NGS panel	singlecenter	Beijing	ovarian cancer	unselected	172	18-81	–	52.5
(41)	2020	13	8.39	9	5.81	4	2.58	SNaPshot/NGS panel/MLPA +Sanger	multicenter	Hong Kong	ovarian cancer	unselected	155	9-85	-	44.7
(52)	2022	64	32.82	37	18.97	32	16.41	NGS panel	singlecenter	Guangdong	ovarian cancer	Hakka people	195	–	–	–
(53)	2017	30	26.09	24	20.87	6	5.22	NGS panel +MLPA	singlecenter	Shanghai	ovarian cancer	high grade serous ovarian cancer	115	38-79	51	–
(54)	2018	153	16.70	120	13.10	36	3.93	NGS panel	multicenter	nation-wide (25 centers)	ovarian cancer	epithelial ovarian cancer	916	20-81	–	54.2
(55)	2021	36	30.51	31	26.27	5	4.24	NGS multi-gene panel (18) +sanger/qPCR	singlecenter	Anhui	ovarian cancer	epithelial ovarian cancer	118	31-79	–	52.0
(56)	2017	9	18.00	3	6.00	6	12.00	WES+sanger	singlecenter	Beijing	ovarian cancer	epithelial ovarian cancer	50	25-79	53	–
(57)	2022	–	–	–	0.5	–	1.9	NGS multi-gene panel(381/733)	singlecenter	Shanghai	pancreatic cancer	unselected	1080	20-87	60	–
(58)	2021	–	–	–	0	–	0.33	NGS multi-gene panel(566/764)	singlecenter	Shanghai	pancreatic cancer	unselected	608	–	–	–
(59)	2021	10	5.13	1	0.51	9	4.62	NGS multi-gene panel(150/381/437)	singlecenter	Sichuan	pancreatic cancer	pancreatic ductal adenocarcinoma	195	27-79	59	–
(60)	2021	86	4.68	7	0.38	79	4.30	WES/NGS multi-gene panel(2~618)	multicenter	Shanghai, Hong Kong, Sichuan, Guangdong	prostate cancer	unselected	1836	61-73	–	67.0

(Continued)

10.3389/fonc.2022.1002360

TABLE 1 Continued

Reference*	Year	BRCA1/2(+) number	BRCA1/2 (+) rate (%)	BRCA1 (+) N	BRCA1 (+) rate (%)	BRCA2 (+) N	BRCA2 (+) rate (%)	Sequencing methods	Singlecenter or multicenter	Regions	Study Population	Select criteria	Size	Age range	Median age	Average age
(61)	2019	22	7.01	2	0.64	20	6.37	WES/NGS multi-gene panel(63/499/618)	singlecenter	Shanghai	prostate cancer	–	314	34-84	64	63.4
(62)	2021	–	–	–	0.4	–	5.3	WES	singlecenter	Shanghai	prostate cancer	unselected	246	57-69	65	–
(63)	2021	10	0.46	1	0.05	9	0.42	NGS panel (365genes+25 genes frequently re-arranged)	–	–	colorectal cancer	unselected	2160	–	–	–
(64)	2019	17	4.76	–	–	–	–	NGS panel (450genes+36 genes frequently re-arranged)	singlecenter	Beijing	liver cancer	unselected	357	16-88	–	56.0

*Only large-scale studies (n>50 in unselected cancer or n>300 in general population) are included.
**NGS panel mainly refers to the 2 gene panel (BRCA1 and BRCA2).
***The number of cancer susceptibility genes contained in NGS multi-gene panel.

10.3389/fonc.2022.1002360

TABLE 2 Summary of guidelines and consensus about BRCA1/2 genetic testing in recent 5 years in China.

Reference	Title	Organization	Year	Language	Target population	Recommended population for genetic testing
(10)	Guidelines of Chinese Society of Clinical Oncology (CSCO) - Pancreatic Cancer (2022 Edition)	Chinese Society of Clinical Oncology Guidelines Working Committee	2022	Chinese	pancreatic cancer patient	Germline genetic testing is recommended for all patients diagnosed with pancreatic cancer
(68)	Clinical Practice Guideline of BRCA1/2 Testing for Patients with Breast Cancer: Chinese Society of Breast Surgery (CSBrS) Practice Guideline 2021	Chinese Society of Breast Surgery (CSBrS)	2021	English	breast cancer patient	1.Breast cancer diagnosed ≤45 years old; 2.Breast cancer diagnosed 46 to 50 years old with one or more of the following: An additional breast cancer primary at any age; ≥1 close blood relative† with breast cancer at any age; An unknown or limited family history; 3.Diagnosed ≤60 years old with triple negative breast cancer; 4.Breast cancer diagnosed at any age with one or more of the following: ≥1 close blood relative† with breast cancer diagnosed 50 years old; ≥1 close blood relative† with ovarian carcinoma/metastatic prostate cancer/ pancreatic cancer/male breast cancer; ≥2 additional diagnoses of breast cancer at any age in patient and/or in close blood relatives; Personal history of ovarian carcinoma/pancreatic cancer; 5.Male breast cancer; 6.Patients with HER2negative recurrent metastatic breast cancer; 7.BRCA1/2 pathogenic/likely pathogenic variant were detected in tumor tissues; 8.Individual from a family with a known BRCA1/2 pathogenic/likely pathogenic variant; 9.Ovarian carcinoma; 10.High-grade prostate cancer with one or more of the following: ≥1 close blood relatives† with ovarian carcinoma/pancreatic cancer/ metastatic prostate cancer/breast cancer <50 years old; ≥2 close blood relatives† with breast/prostate cancer (any grade) at any age.
(9)	Expert Consensus on Clinical Treatment of Familial Hereditary Tumors in China (2021 Edition)- Familial Hereditary Breast Cancer	China Anti-Cancer Association, Familial Hereditary Cancer Committee	2021	Chinese	breast cancer patient	a. Individuals with a history of breast cancer with any of the following conditions: 1. Age at presentation ≤ 50 years. 2. Triple-negative breast cancer. 3. Male breast cancer. 4. Age at presentation >50 years and ≥1 other breast, ovarian, pancreatic or prostate cancer in the family. 5. Patients with operable primary HER-2 negative breast cancer with high risk of recurrence, regardless of family history of breast cancer or other tumors. 6. HER-2 negative metastatic breast cancer. b. Individuals with a history of breast cancer, regardless of whether they have any of the following conditions: 1. Immediate family members with known pathogenic or potentially pathogenic mutations in the BRCA1/2 gene. 2. A male breast cancer patient in the family. 3. Healthy individuals* may be tested if they have ≥2 cases of breast cancer in the family; or ≥2 tumor types including breast, ovarian, pancreatic, or prostate cancer with at least 1 breast cancer in the family.(*However, it is still recommended that individuals with cancer in the family be tested as a priority, especially those with early age of onset and multiple primary tumors; healthy individuals in the family should be considered for testing only when patients are not available.)
(69)	Consensus of Chinese Experts on Hot Isssues in GneticTesting of Advanced Breast Cancer (2021 edition)	International Medical society, Chinese Anti-cancer Association	2021	Chinese	advanced breast cancer patient	Patients with advanced breast cancer who are financially eligible and have accessible pathological specimens.
(8)	Expert Consensus on Clinical Treatment of Familial Hereditary	China Anti-Cancer Association, Familial	2021	Chinese	prostate cancer patient	Germline mutation testing for DNA damage repair genes, including BRCA2, BRCA1, ATM, PALB2, CHEK2, MLH1, MSH2, MSH6, and PMS2, is recommended for people at genetic risk for prostate cancer who meet any of the following criteria: 1.Known family members carry pathogenic mutations in the above genes.

(Continued)

TABLE 2 Continued

Reference	Title	Organization	Year	Language	Target population	Recommended population for genetic testing
	Tumors in China (2021 Edition)-Familial Hereditary Prostate Cancer	Hereditary Cancer Committee				2. Patients with a clear family history of tumors and multiple cases in the same family including bile duct cancer, breast cancer, pancreatic cancer, prostate cancer, ovarian cancer, colorectal cancer, endometrial cancer, gastric cancer, renal cancer, melanoma, small intestine cancer and uroepithelial cancer, especially if their age of diagnosis is ≤ 50 years; and patients with a brother, father or other family members diagnosed with prostate cancer or died of prostate cancer before the age of 60 years. 3.With a suspicious or unknown family history, recommended after adequate genetic counseling evaluation. 4. Tumor tissue testing reveals no germline verification of the above gene pathogenic mutation. 5.Intraductal carcinoma and ductal adenocarcinoma. 6.High risk and above, locally progressive and metastatic prostate cancer.
(7)	Expert Consensus on Clinical Treatment of Familial Hereditary Tumors in China (2021 Edition)-Familial Hereditary Ovarian Cancer	China Anti-Cancer Association, Familial Hereditary Cancer Committee	2021	Chinese	ovarian cancer patient	1.Patients with primary epithelial ovarian cancer; 2.Patients with recurrent epithelial ovarian cancer; 3.Individuals with germline mutations detected in ovarian cancer, further " cascade testing" of their family line is required
(70)	Chinese Expert Consensus on Genomic Testing of Prostate Cancer Patients (the 2020 edition)	China Anti-Cancer Association Genitourinary Cancer Committee	2020	Chinese	prostate cancer patient	a. To provide genetic counseling for the purpose of 1. Patients with a clear family history of prostate cancer who have not undergone risk assessment at first diagnosis or who are at very low to intermediate risk; patients with unknown or unclear family history need to be guided by oncologic genetic counseling to consider the need for testing 2. Patients with high-risk or very high-risk prostate cancer 3. Patients with locally progressive (N1) or metastatic (M1) prostate cancer, intraductal carcinoma of the prostate (IDC-P) or ductal adenocarcinoma of the prostate (DAP) pathology Prostate cancer patients 4. Patients with prostate cancer whose tumor tissue testing has identified mutations associated with risk of tumor development and who lack verification of germline variants will be considered for testing after genetic counseling recommendations. b. For the purpose of making treatment decisions 1. Patients with metastatic castration-resistant prostate cancer (mCRPC)
(11)	Guideline on Next-Generation Sequencing-Based BRCA1/2 Testing (2019)	Working Group of Guideline on Next-Generation Sequencing-Based BRCA1/2 Testing (2019)	2019	Chinese	not specific	a.To assess genetic risk, genetic counseling and germline BRCA1/2 gene testing are recommended for relevant high-risk populations, including (1) individuals from families with pathogenic/probably pathogenic mutations in the BRCA1/2 gene; (2) patients with pathogenic/probably pathogenic mutations in the BRCA1/2 gene identified by tumor testing but for whom it is not clear whether they are germline mutations; (3) all newly diagnosed patients with ovarian cancer, fallopian tube cancer and primary peritoneal cancer; (4) breast cancer patients with age of onset of 40 years or younger, triple negative breast cancer patients with age of onset of 60 years or younger, all male breast cancer patients; (5) all newly diagnosed pancreatic cancer patients; (6) patients with high risk and above, N1 and M1 prostate cancer, prostate intraductal cancer patients; (7) breast cancer and prostate cancer patients; (8) individuals with one or more 1st or 2nd degree blood relatives meeting the above testing criteria, etc. b.To guide the selection of subsequent treatment options, (1) germline and/or somatic BRCA1/2 gene testing is recommended for all newly diagnosed ovarian, fallopian tube, and primary peritoneal cancer patients, and BRCA1/2 gene testing using newly obtained tumor tissue is considered after recurrence); (2) germline BRCA1/2 gene testing is recommended for HER2-negative advanced breast cancer patients when considering chemotherapy gene testing; (3) germline and/or somatic cell BRCA1/2 gene testing is recommended for patients with locally advanced and metastatic pancreatic cancer at the time of diagnosis); (4) testing for

(Continued)

TABLE 2 Continued

Reference	Title	Organization	Year	Language	Target population	Recommended population for genetic testing
						germline and somatic cell variants containing at least DNA damage response genes such as BRCA1/2 is recommended for all patients with metastatic desmoplastic resistant prostate cancer
(71)	Expert Consensus on BRCA1/2 Gene Testing and Clinical Application in Chinese Breast Cancer Patients (2018 edition)	Chinese Medical Doctor Association, Chinese Society of Precision Medicine, Breast Cancer Committee	2018	Chinese	breast cancer patient	Breast cancer patients: ≤40 years of age onset ≤50 years of age with: (1) second primary breast cancer (2) ≥1 of the following family history criteria: ① ≥1 consanguineous relative with a history of breast cancer at any age; ② ≥1 consanguineous relative with a history of pancreatic cancer; ③ ≥1 relative with a history of prostate cancer (Gleason score ≥7); ④ Unknown or limited family history ≤60 years of age with (2) ≥1 consanguineous relative with a history of breast cancer at ≤50 years of age; (3) ≥1 consanguineous relative with a history of ovarian cancer; (4) ≥3rd degree relative with breast and/or ovarian cancer and ≥2 consanguineous relatives with breast cancer (at least 1 of whom is ≤50 years of age) and/or ① Family history of male breast cancer in a consanguineous relative; ② ≥2 consanguineous relatives with pancreatic and/or prostate cancer of any age (Gleason score ≥7); ③ Known familial pathogenic BRCA1/2 gene mutation
(72)	Guidelines for the Diagnosis and Treatment of Ovarian Malignancies (4th edition)	China Anti-Cancer Association Gynecology Cancer Committee	2018	Chinese	ovarian cancer patient	Genetic testing is recommended for individuals with one or more of the following: (1) Known BRCA1/2 mutation in the family. (2) Personal history of ovarian cancer or other HBOC-related tumors with age at diagnosis ≤50 years. (3) Have HBOC-associated tumor with age at diagnosis ≤ 60 years and a second primary tumor, or triple-negative breast cancer, or ≥ 1 close relative with HBOC-associated tumor (4) ≥2 close relatives with HBOC-associated tumors. (5) Male breast cancer patients, or male close relatives with breast cancer; BRCA1/2 mutation detected in tumor tissue, but germline analysis not performed.

variants is still limited in it. More large-scale population studies and function studies of *BRCA1/2* mutation in Chinese are needed to obtain more evidence to optimize the mutation interpretation.

As new shreds of evidence accumulate, the variant classification could be change over time. A study of 21,216 Breast cancer patients and 6,434 healthy controls performed VUS reclassification in the cohort. After the reclassification, 7 VUS were re-grouped into benign, which reduced the VUS ratio in both patient and healthy control (from 9.8 to 7.9% and from 6.9 to 5.3%) (15), indicating that the evidence should frequently be updated for VUS reclassification, and emphasizing the VUS carriers should be followed up.

Another notable issue concerns the price of BRCA mutation testing (84). Currently the price of BRCA mutation testing for a single sample in China is roughly 300 dollars ($), which is only paid by the patient side and not covered by the government side through basic medical insurance (85). Actually, the price of a single BRCA mutation testing is too high for the majority of ordinary Chinese. Therefore, financial investment from the Chinese government side is necessary to promote the widespread of BRCA mutation testing across China, e. g., Chinese government could offer reimbursement through Chinese basic medical insurance system for the high-risk population who took BRCA mutation testing. Additionally, evidence shows population-based BRCA mutation screening is also cost-effective for Chinese data with an incremental cost-effectiveness ratio of $18,066 from a societal perspective and $23,485 from a payer perspective per quality-adjusted life year (86).

In fact, while these issues are prominent in China, they also exist in many other countries and need to be addressed through collective efforts.

Genetic counseling for *BRCA* mutation carriers in China

Currently, the principles of *BRCA* mutation detection in China mainly refers to the guideline on next-generation sequencing-based *BRCA1/2* testing (2019) (11), the US National Comprehensive Cancer Network (NCCN) guidelines (87) and the European Society of Medical Oncology (ESMO) guidelines (88), as well as other Chinese expert consensus on specific cancers or genetic testing (summarized in Table 2). We summarized the criteria proposed in 10 different guidelines and consensuses for Chinese population *BRCA* screening in recent 5 years (7–11, 68–72).

Genetic counseling is essential in pre- and post-sequencing stage for the test individuals. The purpose is to accurately estimate the probability of cancer susceptibility gene mutations (89) and offer early prevention advice and medical management

such as regular surveillance, chemoprevention or surgical prevention for *BRCA* mutation carriers (27, 28). In a study with 839 breast cancer patients and 510 relatives, who are considered high-risk populations, 86.4% and 63.8% cases showed a strong willingness to accept genetic counseling and genetic testing, respectively (90). For those high-risk populations who are willing to do the genetic testing of *BRCA1/2*, the mutation rate was 19.9%. Despite the high willingness, most of the high-risk individuals lacked knowledge of cancer inheritance (90). We are glad to find out that another study exhibited that 79% of germline mutation carriers were aware of the risk and the importance of surveillance, while 56% accepted preventive interferences after genetic counseling on gynecologic tumors (91).

However, the development of cancer genetic counseling in China is in its beginning. Unlike some developed countries where specialized and certified genetics health professionals are available (92), cancer genetic counseling relies heavily on clinicians. Setting up standardized workflows and training eligible counselors is pivotal for promoting genetic counseling in China. Although the "oncologist-led *BRCA* consultation" mode has improved access to cancer genetic testing in developing countries (93), specialized cancer genetic counselors are urgently needed. Organizations like the Chinese Board of Genetic Counseling and others are now dedicated to training genetic counselors in more than 15 provinces across China (65). Meanwhile, the Chinese Anti-Cancer Association is urging hospitals nationwide to set up cancer genetic counseling clinics to accommodate the increased demand for counseling. Still, the training projects and qualified counselors are minimal and lack statistics.

Regular surveillance, prevention and treatment for *BRCA* mutation carriers in China

After genetic testing, the frequency of regular surveillance for female mutation carriers was significantly higher compared to non-carriers, according to the report on high-risk southern Chinese females (94).

Early-stage breast cancer lacks apparent signs and symptoms. Possible symptoms of breast cancer can be skin dimpling, red or thickening, nipple retraction and lymph nodes swelling. But a painless hard lump with irregular edges discovered accidentally by patients themselves is the most common early sign. Ninety-one percent of Chinese breast cancer patients had dense gland (95), which significantly affected the quality and effectiveness of palpation examination. For the surveillance of high-risk female carriers, in addition to regular breast self-examination and clinical breast examination, X-ray combined with ultrasound and magnetic resonance imaging (MRI) are usually selected as the methods recommended for women aged >40 years to detect early signs of breast cancer in China (96). Given that Chinese women have dense breasts and many younger patients with *BRCA1/2* mutated breast cancer, mammography screening has a lower sensitivity. A prospective study comparing different screening methods for patients with *BRCA1/2* mutations found the sensitivity of 77% with MRI compared with 36% with mammography and 33% with ultrasound (97–99).

Regular pelvic examination, tumor marker CA125 detection and transvaginal ultrasound are the methods recommended for detecting early signs of ovarian cancer (88). Annual prostate-specific antigen (PSA) testing and digital rectal examination are recommended for prostate cancer screening and surveillance, especially for *BRCA1* carriers (8). A study showed that multiparameter MRI has high diagnostic efficacy for *BRCA1* or *BRCA2* mutated prostate cancer patients. As soon as PSA elevation is detected, multiparameter MRI is recommended for *BRCA1* or *BRCA2* mutation carriers aged >55 years for further diagnosis (100). Besides, annual imaging examinations can be considered to prevent pancreatic cancer for *BRCA2* carriers, although the efficacy of this approach remains to be validated (88). The recommended starting age for monitoring breast cancer, ovarian cancer, prostate cancer and pancreatic cancer is 25, 30, 40 and 50 years, respectively, or ten years earlier than the earliest confirmed case in the family (81, 88, 94, 101).

Many studies confirmed that for *BRCA* mutation carriers, chemoprevention or surgical prevention play an important role in reducing the occurrence of HBOC (102–104). In high-risk women, prophylactic mastectomy can reduce the incidence of breast cancer by 90% and the mortality rate by 81% (103). A study showed that 23.8% and 32% of patients chose prophylactic mastectomy and prophylactic salpingo-oophorectomy; more than 17% of healthy carriers also had prophylactic surgery in Hongkong, China (102). In mainland China, however, healthy carriers and surgeons are more cautious about choosing prophylactic surgery. Only one study reported that three healthy carriers with deleterious *BRCA1/2* variant underwent prophylactic nipple-sparing mastectomy (105). Breast cancer patients carrying *BRCA1/2* deleterious variants had a 4.52-fold and 5.54-fold increased risk of contra-lateral breast cancer, respectively, compared to non-carriers (106). Preventive contra-lateral prophylactic mastectomy can be an optimal selection for *BRCA1/2* mutated breast cancer patients in China (9). Risk-reducing salpingo-oophorectomy, which can significantly reduce the risk of breast, ovarian, and fallopian tube cancers, is recommended for high-risk women after childbirth to prevent ovarian cancer (7, 107).

Studies found that *BRCA1/2*-mutated patients are more likely to benefit from platinum-based chemotherapy (108–110). Since DNA damage caused by platinum-based drugs

requires DNA homologous recombination for repair, the functional defects caused by mutations in the *BRCA1/2* gene make tumor cells more sensitive to platinum-based drugs. The TNT phase III trial compared the efficacy between carboplatin and docetaxel in unselected advanced TNBC. In the germline *BRCA1/2*-mutated subgroup, the objective response rate with carboplatin was 2-fold higher than it with docetaxel (68% *vs.* 33%) (110). Recently, cancer patients with *BRCA* mutation could be benefited from poly (ADP-ribose) polymerase (PARP) targeted therapy due to the increased sensitivity to PARP inhibitors (62, 111). PARP inhibitor specifically causes the death of cancer cells with *BRCA1/2* mutations through the "synthetic lethal effect" (112). The OlympiA trial has confirmed the efficacy of PARP inhibitors in the adjuvant treatment of early-stage *BRCA1/2*-mutated breast cancer (113), while the OlympiAD trial, as well as many other phase III clinical trials, have proved the role of PARP inhibitors in advanced *BRCA1/2*-mutated breast cancer (114, 115). PARP inhibitors are widely used for *BRCA1/2*-mutated ovarian cancer patients as maintenance therapy in China based on the results of several phase III trials, including SOLO-1, SOLO-2, PAOLA-1, PRIMA and NOVA (116–120). PARP inhibitor olaparib is recommended for metastatic castration-resistant prostate cancer patients based on the PROfound trial. The phase III PROfound study showed a more prolonged imaging-based progression-free survival in the olaparib group compared with the control group (median, 7.4 months *vs.* 3.6 months) (121). PARP inhibitors are increasingly used to treat *BRCA*-mutated patients, but whether they can be used for prevention needs further investigation.

Chemoprevention for cancer-free *BRCA1/2* carriers remains controversial. Only a small retrospective study has shown that tamoxifen, a selective estrogen receptor modulator, reduces the risk of breast cancer in healthy carriers of *BRCA2* mutations by 62%. But it is unclear whether it has a preventive effect in *BRCA1*-mutated healthy carriers (122). The evidence is not enough to support tamoxifen as a prevention strategy for healthy *BRCA1/2* mutated carriers (9). Oral contraceptives have proven preventive efficacy for ovarian cancer with a family history. However, it is controversial whether oral contraceptives increase the risk of breast cancer in *BRCA1/2* mutation carriers (123).

Conclusion and future perspective

Taken together, germline *BRCA1/2* mutations are common in Chinese patients with hereditary breast, ovarian, prostate and pancreatic cancers. Because of its ethnic specificity, the unique features in the spectrum of *BRCA* mutations have already been revealed but the extension of the sequencing efforts to the whole Chinese population remains yet to be achieved. Many Chinese consensuses today recommend *BRCA1/2* genetic testing for cancer patients only. Regarding the prevalence in healthy populations, approximately one in every 300 healthy Chinese is a *BRCA1/2* mutation carrier (12, 15). *BRCA* mutation-related cancer is one of the most preventable cancers. Whether or not to perform population screening should not solely be based on cost-effectiveness but should also consider more non-cost factors such as social, political, public interest and patients' benefits. Under the current political and economic conditions in China, to achieve early prevention of *BRCA* mutation carriers, we recommend that the criteria be relaxed and all Chinese diagnosed with breast, ovarian, pancreatic or prostate cancer, as well as healthy individuals with a clear family history, should undergo *BRCA1/2* genetic testing to provide a risk assessment. Subsequently, preventive measures such as regular surveillance, chemoprevention or surgical prevention for mutation carriers are recommended after authentic genetic counseling.

Evidence had shown that relying on personal and family history may not be sufficient to determine the risk for *BRCA1/2* variants (20). Population *BRCA* screening is considered the trend in the near future (124, 125). Thus, a growing number of healthy individuals harboring pathogenic mutations can be identified for cancer prevention. Population screening for carriers with *BRCA* germline mutations in the Chinese population is highly warranted to promote prevention, early detection, early diagnosis, and timely treatment of *BRCA* mutation-related cancers, which may increase 5-year survival for *BRCA* mutation-related cancer patients. Also, the ethical, psychological and legal issues cannot be ignored.

Author contributions

TC were responsible for the study concept and design. HL, MZ, LZ, KH, X-jW and TC drafted the manuscript, and all authors revised it for important intellectual content. The work reported in the paper has been performed by the authors, unless clearly specified in the text. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by grants from National Key Research-Development Program of China (2019YFE0198800), Key Research-Development Program of Zhejiang Province (2017C03013), Ten-Thousand Talents Plan of Zhejiang Province (2021R52020), and Start-up Funds for Recruited Talents in Zhejiang Cancer Hospital. The funding agencies had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data;

preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sun S, Brazhnik K, Lee M, Maslov AY, Zhang Y, Huang Z, et al. Single-cell analysis of somatic mutation burden in mammary epithelial cells of pathogenic Brca1/2 mutation carriers. *J Clin Invest* (2022) 132(5):e148113. doi: 10.1172/JCI148113

2. Kobayashi H, Ohno S, Sasaki Y, Matsuura M. Hereditary breast and ovarian cancer susceptibility genes (Review). *Oncol Rep* (2013) 30(3):1019–29. doi: 10.3892/or.2013.2541

3. Collins JM, Isaacs C. Management of breast cancer risk in Brca1/2 mutation carriers who are unaffected with cancer. *Breast J* (2020) 26(8):1520–7. doi: 10.1111/tbj.13970

4. Momozawa Y, Sasai R, Usui Y, Shiraishi K, Iwasaki Y, Taniyama Y, et al. Expansion of cancer risk profile for Brca1 and Brca2 pathogenic variants. *JAMA Oncol* (2022) 8(6):871–8. doi: 10.1001/jamaoncol.2022.0476

5. Li S, Silvestri V, Leslie G, Rebbeck TR, Neuhausen SL, Hopper JL, et al. Cancer risks associated with Brca1 and Brca2 pathogenic variants. *J Clin Oncol* (2022) 40(14):1529-41. doi: 10.1200/JCO.21.02112

6. Lubinski J. Breast cancer genetics: 20 years later. *Clin Genet* (2014) 85(1):5–6. doi: 10.1111/cge.12293

7. Familial Hereditary Cancer Committee CA-CA. Expert consensus on clinical treatment of familial hereditary tumors in China (2021 edition)-familial hereditary ovarian cancer. *Chin J Clin Oncol* (2022) 48(24):5. doi: 10.12354/j.issn.1000-8179.2021.20211800

8. Familial Hereditary Cancer Committee CA-CA. Expert consensus on clinical treatment of familial hereditary tumors in China (2021 edition)-familial hereditary prostate cancer. *Chin J Clin Oncol* (2022) 49(2):5. doi: 10.12354/j.issn.1000-8179.2022.20211805

9. Familial Hereditary Cancer Committee CA-CA. Expert consensus on clinical treatment of familial hereditary tumors in China (2021 Edition)(1) -familial hereditary breast cancer. *Chin J Clin Oncol* (2022) 48(23):1189–95. doi: 10.12354/j.issn.1000-8179.2021.20211553

10. Chinese Society of Clinical Oncology GWC. *Guidelines of Chinese society of clinical oncology (Csco) - pancreatic cancer (2022 edition)* Beijing: People's Medical Publishing House. (2022), 107.

11. Chen G, Chen G, Chen M, Cheng W, Cui W, Ding W, et al. Working group of guideline on Next-Generation Sequencing-Based BRCA1/2 testing. [Guideline on Next-Generation Sequencing-Based Brca1/2 testing (2019)]. *Chin J Pathol* (2019) 48(9):8. doi: 10.3760/cma.j.issn.0529-5807.2019.09.002

12. Gao X, Nan X, Liu Y, Liu R, Zang W, Shan G, et al. Comprehensive profiling of Brca1 and Brca2 variants in breast and ovarian cancer in Chinese patients. *Hum Mutat* (2020) 41(3):696–708. doi: 10.1002/humu.23965

13. Dong H, Chandratre K, Qin Y, Zhang J, Tian X, Rong C, et al. Prevalence of Brca1/Brca2 pathogenic variation in Chinese han population. *J Med Genet* (2021) 58(8):565–9. doi: 10.1136/jmedgenet-2020-106970

14. Qin Z, Kuok CN, Dong H, Jiang L, Zhang L, Guo M, et al. Can population brca screening be applied in non-ashkenazi Jewish populations? experience in Macau population. *J Med Genet* (2021) 58(9):587–91. doi: 10.1136/jmedgenet-2020-107181

15. Liu Y, Wang H, Wang X, Liu J, Li J, Wang X, et al. Prevalence and reclassification of Brca1 and Brca2 variants in a Large, unselected Chinese han breast cancer cohort. *J Hematol Oncol* (2021) 14(1):18. doi: 10.1186/s13045-020-01010-0

16. Sekine M, Nishino K, Enomoto T. Differences in ovarian and other cancers risks by population and brca mutation location. *Genes (Basel)* (2021) 12(7):1050. doi: 10.3390/genes12071050

17. Bhaskaran SP, Huang T, Rajendran BK, Guo M, Luo J, Qin Z, et al. Ethnic-specific Brca1/2 variation within Asia population: Evidence from over 78 000 cancer and 40 000 non-cancer cases of Indian, Chinese, Korean and Japanese populations. *J Med Genet* (2021) 58(11):752–9. doi: 10.1136/jmedgenet-2020-107299

18. Fernandez-Lopez JC, Romero-Cordoba S, Rebollar-Vega R, Alfaro-Ruiz LA, Jimenez-Morales S, Beltran-Anaya F, et al. Population and breast cancer patients' analysis reveals the diversity of genomic variation of the brca genes in the Mexican population. *Hum Genomics* (2019) 13(1):3. doi: 10.1186/s40246-018-0188-9

19. Gabai-Kapara E, Lahad A, Kaufman B, Friedman E, Segev S, Renbaum P, et al. Population-based screening for breast and ovarian cancer risk due to Brca1 and Brca2. *Proc Natl Acad Sci U.S.A.* (2014) 111(39):14205–10. doi: 10.1073/pnas.1415979111

20. Manickam K, Buchanan AH, Schwartz MLB, Hallquist MLG, Williams JL, Rahm AK, et al. Exome sequencing-based screening for Brca1/2 expected pathogenic variants among adult biobank participants. *JAMA Netw Open* (2018) 1(5):e182140. doi: 10.1001/jamanetworkopen.2018.2140

21. Momozawa Y, Iwasaki Y, Parsons MT, Kamatani Y, Takahashi A, Tamura C, et al. Germline pathogenic variants of 11 breast cancer genes in 7,051 Japanese patients and 11,241 controls. *Nat Commun* (2018) 9(1):4083. doi: 10.1038/s41467-018-06581-8

22. Maxwell KN, Domchek SM, Nathanson KL, Robson ME. Population frequency of germline Brca1/2 mutations. *J Clin Oncol* (2016) 34(34):4183–5. doi: 10.1200/JCO.2016.67.0554

23. Zhang J, Sun J, Chen J, Yao L, Ouyang T, Li J, et al. Comprehensive analysis of Brca1 and Brca2 germline mutations in a Large cohort of 5931 Chinese women with breast cancer. *Breast Cancer Res Treat* (2016) 158(3):455–62. doi: 10.1007/s10549-016-3902-0

24. Bhaskaran SP, Chandratre K, Gupta H, Zhang L, Wang X, Cui J, et al. Germline variation in Brca1/2 is highly ethnic-specific: Evidence from over 30,000 Chinese hereditary breast and ovarian cancer patients. *Int J Cancer* (2019) 145(4):962–73. doi: 10.1002/ijc.32176

25. Meng H, Yao L, Yuan H, Xu Y, Ouyang T, Li J, et al. Brca1 C.5470_5477del, a founder mutation in Chinese han breast cancer patients. *Int J Cancer* (2020) 146(11):3044–52. doi: 10.1002/ijc.32877

26. Li J, Han S, Zhang C, Luo Y, Wang L, Wang P, et al. Identification of Brca1: C.5470_5477del as a founder mutation in Chinese ovarian cancer patients. *Front Oncol* (2021) 11:655709. doi: 10.3389/fonc.2021.655709

27. Li A, Xie R, Zhi Q, Deng Y, Wu Y, Li W, et al. Brca germline mutations in an unselected nationwide cohort of Chinese patients with ovarian cancer and healthy controls. *Gynecol Oncol* (2018) 151(1):145–52. doi: 10.1016/j.ygyno.2018.07.024

28. Bu H, Chen J, Li Q, Hou J, Wei Y, Yang X, et al. Brca mutation frequency and clinical features of ovarian cancer patients: A report from a Chinese study group. *J Obstet Gynaecol Res* (2019) 45(11):2267–74. doi: 10.1111/jog.14090

29. Lang GT, Shi JX, Hu X, Zhang CH, Shan L, Song CG, et al. The spectrum of brca mutations and characteristics of brca-associated breast cancers in China: Screening of 2,991 patients and 1,043 controls by next-generation sequencing. *Int J Cancer* (2017) 141(1):129–42. doi: 10.1002/ijc.30692

30. Shao D, Cheng S, Guo F, Zhu C, Yuan Y, Hu K, et al. Prevalence of hereditary breast and ovarian cancer (Hboc) predisposition gene mutations among 882 hboc high-risk Chinese individuals. *Cancer Sci* (2020) 111(2):647–57. doi: 10.1111/cas.14242

31. Dong L, Zhang H, Zhang H, Ye Y, Cheng Y, Li L, et al. The mutation landscape of multiple cancer predisposition genes in Chinese Familial/Hereditary breast cancer families. *Cancer Biol Med* (2021) 19(6):850–70. doi: 10.20892/j.issn.2095-3941.2021.0011

32. Cao WM, Gao Y, Yang HJ, Xie SN, Ding XW, Pan ZW, et al. Novel germline mutations and unclassified variants of Brca1 and Brca2 genes in Chinese women with familial Breast/Ovarian cancer. *BMC Cancer* (2016) 16:64. doi: 10.1186/s12885-016-2107-6

33. Sun J, Meng H, Yao L, Lv M, Bai J, Zhang J, et al. Germline mutations in cancer susceptibility genes in a Large series of unselected breast cancer patients. *Clin Cancer Res* (2017) 23(20):6113–9. doi: 10.1158/1078-0432.CCR-16-3227

34. Deng M, Chen HH, Zhu X, Luo M, Zhang K, Xu CJ, et al. Prevalence and clinical outcomes of germline mutations in Brca1/2 and Palb2 genes in 2769 unselected breast cancer patients in China. *Int J Cancer* (2019) 145(6):1517–28. doi: 10.1002/ijc.32184

35. Chen B, Zhang G, Li X, Ren C, Wang Y, Li K, et al. Comparison of brca versus non-brca germline mutations and associated somatic mutation profiles in patients with unselected breast cancer. *Aging (Albany NY)* (2020) 12(4):3140–55. doi: 10.18632/aging.102783

36. Wang Q, Wu H, Lan Y, Zhang J, Wu J, Zhang Y, et al. Changing patterns in clinicopathological characteristics of breast cancer and prevalence of brca mutations: Analysis in a rural area of southern China. *Int J Gen Med* (2021) 14:7371–80. doi: 10.2147/IJGM.S333858

37. Li G, Guo X, Tang L, Chen M, Luo X, Peng L, et al. Analysis of Brca1/2 mutation spectrum and prevalence in unselected Chinese breast cancer patients by next-generation sequencing. *J Cancer Res Clin Oncol* (2017) 143(10):2011–24. doi: 10.1007/s00432-017-2465-8

38. Wang J, Li W, Shi Y, Huang Y, Sun T, Tang L, et al. Germline mutation landscape of Chinese patients with familial Breast/Ovarian cancer in a panel of 22 susceptibility genes. *Cancer Med* (2019) 8(5):2074–84. doi: 10.1002/cam4.2093

39. Li JY, Jing R, Wei H, Wang M, Xiaowei Q, Liu H, et al. Germline mutations in 40 cancer susceptibility genes among Chinese patients with high hereditary risk breast cancer. *Int J Cancer* (2019) 144(2):281–9. doi: 10.1002/ijc.31601

40. Wang YA, Jian JW, Hung CF, Peng HP, Yang CF, Cheng HS, et al. Germline breast cancer susceptibility gene mutations and breast cancer outcomes. *BMC Cancer* (2018) 18(1):315. doi: 10.1186/s12885-018-4229-5

41. Kwong A, Ho JCW, Shin VY, Kurian AW, Tai E, Esserman LJ, et al. Rapid detection of Brca1/2 recurrent mutations in Chinese breast and ovarian cancer patients with multiplex snapshot genotyping panels. *Oncotarget* (2018) 9(8):7832–43. doi: 10.18632/oncotarget.23471

42. Wei H, Wang M, Ou J, Jiang W, Tian F, Sheng Y, et al. Multicenter cross-sectional screening of the brca gene for Chinese high hereditary risk breast cancer populations. *Oncol Lett* (2018) 15(6):9420–8. doi: 10.3892/ol.2018.8538

43. Ye F, He M, Huang L, Lang G, Hu X, Shao Z, et al. Insights into the impacts of brca mutations on clinicopathology and management of early-onset triple-negative breast cancer. *Front Oncol* (2020) 10:574813. doi: 10.3389/fonc.2020.574813

44. Chen L, Fu F, Huang M, Lv J, Zhang W, Wang C. The spectrum of Brca1 and Brca2 mutations and clinicopathological characteristics in Chinese women with early-onset breast cancer. *Breast Cancer Res Treat* (2020) 180(3):759–66. doi: 10.1007/s10549-020-05573-x

45. Shen M, Yang L, Lei T, Xiao L, Li L, Zhang P, et al. Brca1/2 mutation spectrum in Chinese early-onset breast cancer. *Transl Cancer Res* (2019) 8(2):483–90. doi: 10.21037/tcr.2019.03.02

46. Ma D, Chen SY, Ren JX, Pei YC, Jiang CW, Zhao S, et al. Molecular features and functional implications of germline variants in triple-negative breast cancer. *J Natl Cancer Inst* (2021) 113(7):884–92. doi: 10.1093/jnci/djaa175

47. Ji G, Bao L, Yao Q, Zhang J, Zhu X, Bai Q, et al. Germline and tumor Brca1/2 pathogenic variants in Chinese triple-negative breast carcinomas. *J Cancer Res Clin Oncol* (2021) 147(10):2935–44. doi: 10.1007/s00432-021-03696-2

48. Liang Y, Yang X, Li H, Zhu A, Guo Z, Li M. Prevalence and spectrum of Brca1/2 germline mutations in women with breast cancer in China based on next-generation sequencing. *Med Sci Monit* (2018) 24:2465–75. doi: 10.12659/msm.905812

49. Li W, Shao D, Li L, Wu M, Ma S, Tan X, et al. Germline and somatic mutations of multi-gene panel in Chinese patients with epithelial ovarian cancer: A prospective cohort study. *J Ovarian Res* (2019) 12(1):80. doi: 10.1186/s13048-019-0560-y

50. Wu X, Wu L, Kong B, Liu J, Yin R, Wen H, et al. The first nationwide multicenter prevalence study of germline Brca1 and Brca2 mutations in Chinese ovarian cancer patients. *Int J Gynecol Cancer* (2017) 27(8):1650–7. doi: 10.1097/IGC.0000000000001065

51. You Y, Li L, Lu J, Wu H, Wang J, Gao J, et al. Germline and somatic Brca1/2 mutations in 172 Chinese women with epithelial ovarian cancer. *Front Oncol* (2020) 10:295. doi: 10.3389/fonc.2020.00295

52. Luo Y, Wu H, Huang Q, Rao H, Yu Z, Zhong Z. The features of Brca1 and Brca2 germline mutations in hakka ovarian cancer patients: Brca1 C.536 a>T maybe a founder mutation in this population. *Int J Gen Med* (2022) 15:2773–86. doi: 10.2147/IJGM.S355755

53. Ji G, Yao Q, Bao L, Zhang J, Bai Q, Zhu X, et al. Germline and tumor Brca1/2 mutations in Chinese high grade serous ovarian cancer patients. *Ann Transl Med* (2021) 9(6):453. doi: 10.21037/atm-20-6827

54. Shi T, Wang P, Xie C, Yin S, Shi D, Wei C, et al. Brca1 and Brca2 mutations in ovarian cancer patients from China: Ethnic-related mutations in Brca1 associated with an increased risk of ovarian cancer. *Int J Cancer* (2017) 140(9):2051–9. doi: 10.1002/ijc.30633

55. Wu X, Chen Z, Ren P, Zhao X, Tang D, Geng H, et al. Identifying sequence variants of 18 hereditary ovarian cancer-associated genes in Chinese epithelial ovarian cancer patients. *BioMed Res Int* (2021) 2021:5579543. doi: 10.1155/2021/5579543

56. Zhao Q, Yang J, Li L, Cao D, Yu M, Shen K, et al. Germline and somatic mutations in homologous recombination genes among Chinese ovarian cancer patients detected using next-generation sequencing. *J Gynecol Oncol* (2017) 28(4):e39. doi: 10.3802/jgo.2017.28.e39

57. Zhang X, Mao T, Zhang B, Xu H, Cui J, Jiao F, et al. Characterization of DNA damage response deficiency in pancreatic cancer patients from China. *Cancer Commun (Lond)* (2022) 42(1):70–4. doi: 10.1002/cac2.12238

58. Zhan Q, Wen C, Zhao Y, Fang L, Jin Y, Zhang Z, et al. Identification of copy number variation-driven molecular subtypes informative for prognosis and treatment in pancreatic adenocarcinoma of a Chinese cohort. *EBioMedicine* (2021) 74:103716. doi: 10.1016/j.ebiom.2021.103716

59. Shui L, Li X, Peng Y, Tian J, Li S, He D, et al. The Germline/Somatic DNA damage repair gene mutations modulate the therapeutic response in Chinese patients with advanced pancreatic ductal adenocarcinoma. *J Transl Med* (2021) 19(1):301. doi: 10.1186/s12967-021-02972-6

60. Zhu Y, Wei Y, Zeng H, Li Y, Ng CF, Zhou F, et al. Inherited mutations in Chinese men with prostate cancer. *J Natl Compr Canc Netw* (2021) 20(1):54–62. doi: 10.6004/jnccn.2021.7010

61. Wei Y, Wu J, Gu W, Qin X, Dai B, Lin G, et al. Germline DNA repair gene mutation landscape in Chinese prostate cancer patients. *Eur Urol* (2019) 76(3):280–3. doi: 10.1016/j.eururo.2019.06.004

62. Wu J, Wei Y, Pan J, Jin S, Gu W, Gan H, et al. Prevalence of comprehensive DNA damage repair gene germline mutations in Chinese prostate cancer patients. *Int J Cancer* (2021) 148(3):673–81. doi: 10.1002/ijc.33324

63. Liao H, Cai S, Bai Y, Zhang B, Sheng Y, Tong S, et al. Prevalence and spectrum of germline cancer susceptibility gene variants and somatic second hits in colorectal cancer. *Am J Cancer Res* (2021) 11(11):5571–80.

64. Lin J, Shi J, Guo H, Yang X, Jiang Y, Long J, et al. Alterations in DNA damage repair genes in primary liver cancer. *Clin Cancer Res* (2019) 25(15):4701–11. doi: 10.1158/1078-0432.CCR-19-0127

65. Sun L, Liang B, Zhu L, Shen Y, He L. The rise of the genetic counseling profession in China. *Am J Med Genet C Semin Med Genet* (2019) 181(2):170–6. doi: 10.1002/ajmg.c.31693

66. Mavaddat N, Peock S, Frost D, Ellis S, Platte R, Fineberg E, et al. Cancer risks for Brca1 and Brca2 mutation carriers: Results from prospective analysis of embrace. *J Natl Cancer Inst* (2013) 105(11):812–22. doi: 10.1093/jnci/djt095

67. Rehder C, Bean LJH, Bick D, Chao E, Chung W, Das S, et al. Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: A technical standard of the American college of medical genetics and genomics (Acmg). *Genet Med* (2021) 23(8):1399–415. doi: 10.1038/s41436-021-01139-4

68. Xie F, Wang S. Clinical practice guideline of Brca1/2 testing for patients with breast cancer: Chinese society of breast surgery (Csbrs) practice guideline 2021. *Chin Med J (Engl)* (2021) 134(13):1516–8. doi: 10.1097/CM9.0000000000001587

69. International Medical society CA-cA. [Consensus of Chinese experts on hot isssues in gnetictesting of advanced breast cancer (2021 edition)]. *Chin J Oncol* (2021) 44(1):8. doi: 10.3760/cma.j.cn112152-20211111-00837

70. Genitourinary Cancer Committee CA-CA. [Chinese expert consensus on genomic testing of prostate cancer patients (the 2020 edition)]. *China Oncol* (2020) 30(7):10. doi: 10.19401/j.cnki.1007-3639.2020.07.011

71. Cao X, Zeng X, Chen C, Chen J, Cheng J, Cui S, et al. Chinese Medical doctor association CSoPM, breast cancer committee. [Expert consensus on Brca1/2 gene testing and clinical application in Chinese breast cancer patients (2018

edition)]. *China Oncol* (2018) 28(10):14. doi: 10.19401/j.cnki.1007-3639.2018.10.011

72. China Anti-Cancer Association GCC. [Guidelines for the diagnosis and treatment of ovarian malignancies (4th edition)]. *ZHONGGUO SHIYONG FUKE YU CHANKE ZAZHI* (2018) 34(7):11. doi: 10.19538/j.fk2018070110

73. Lincoln SE, Truty R, Lin CF, Zook JM, Paul J, Ramey VH, et al. A rigorous interlaboratory examination of the need to confirm next-generation sequencing-detected variants with an orthogonal method in clinical genetic testing. *J Mol Diagn* (2019) 21(2):318–29. doi: 10.1016/j.jmoldx.2018.10.009

74. Kang P, Mariapun S, Phuah SY, Lim LS, Liu J, Yoon SY, et al. Large Brca1 and Brca2 genomic rearrangements in Malaysian high risk breast-ovarian cancer families. *Breast Cancer Res Treat* (2010) 124(2):579–84. doi: 10.1007/s10549-010-1018-5

75. Bozsik A, Pocza T, Papp J, Vaszko T, Butz H, Patocs A, et al. Complex characterization of germline Large genomic rearrangements of the Brca1 and Brca2 genes in high-risk breast cancer patients-novel variants from a Large national center. *Int J Mol Sci* (2020) 21(13):4650. doi: 10.3390/ijms21134650

76. van der Merwe NC, Oosthuizen J, Theron M, Chong G, Foulkes WD. The contribution of Large genomic rearrangements in Brca1 and Brca2 to south African familial breast cancer. *BMC Cancer* (2020) 20(1):391. doi: 10.1186/s12885-020-06917-y

77. Hogervorst FB, Nederlof PM, Gille JJ, McElgunn CJ, Grippeling M, Pruntel R, et al. Large Genomic deletions and duplications in the Brca1 gene identified by a novel quantitative method. *Cancer Res* (2003) 63(7):1449–53.

78. Cao WM, Zheng YB, Gao Y, Ding XW, Sun Y, Huang Y, et al. Comprehensive mutation detection of Brca1/2 genes reveals Large genomic rearrangements contribute to hereditary breast and ovarian cancer in Chinese women. *BMC Cancer* (2019) 19(1):551. doi: 10.1186/s12885-019-5765-3

79. Wong RSJ, Lee SC. Brca sequencing of tumors: Understanding its implications in the oncology community. *Chin Clin Oncol* (2020) 9(5):66. doi: 10.21037/cco-19-198

80. Li D, Shi Y, Li A, Cao D, Su H, Yang H, et al. Retrospective reinterpretation and reclassification of Brca1/2 variants from Chinese population. *Breast Cancer* (2020) 27(6):1158–67. doi: 10.1007/s12282-020-01119-7

81. Zhang B, Wang Z, Chen G, Zhou X, Wu H, Meng H, et al. Chinese Society of pathology CPQCC. [Chinese expert consensus on Brca1/2 variant Interpretation (2021 version)]. *Zhonghua Bing Li Xue Za Zhi* (2021) 50(6):565–71. doi: 10.3760/cma.j.cn112151-20201027-00809

82. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. *Genet Med* (2015) 17(5):405–24. doi: 10.1038/gim.2015.30

83. Qu S, Chen Q, Yi Y, Shao K, Zhang W, Wang Y, et al. A reference system for brca mutation detection based on next-generation sequencing in the Chinese population. *J Mol Diagn* (2019) 21(4):677–86. doi: 10.1016/j.jmoldx.2019.03.003

84. Hemminki K, Sundquist K, Sundquist J, Forsti A, Hemminki A, Li X. Familial risks and proportions describing population landscape of familial cancer. *Cancers (Basel)* (2021) 13(17):4385. doi: 10.3390/cancers13174385

85. Sun L, Cui B, Wei X, Sadique Z, Yang L, Manchanda R, et al. Cost-effectiveness of genetic testing for all women diagnosed with breast cancer in China. *Cancers (Basel)* (2022) 14(7):1839. doi: 10.3390/cancers14071839

86. Manchanda R, Sun L, Patel S, Evans O, Wilschut J, De Freitas Lopes AC, et al. Economic evaluation of population-based Brca1/Brca2 mutation testing across multiple countries and health systems. *Cancers (Basel)* (2020) 12(7):1929. doi: 10.3390/cancers12071929

87. Daly MB, Pal T, Berry MP, Buys SS, Dickson P, Domchek SM, et al. Genetic/Familial high-risk assessment: Breast, ovarian, and pancreatic, version 2.2021, nccn clinical practice guidelines in oncology. *J Natl Compr Canc Netw* (2021) 19(1):77–102. doi: 10.6004/jnccn.2021.0001

88. Paluch-Shimon S, Cardoso F, Sessa C, Balmana J, Cardoso MJ, Gilbert F, et al. Prevention and screening in brca mutation carriers and other Breast/Ovarian hereditary cancer syndromes: Esmo clinical practice guidelines for cancer prevention and screening. *Ann Oncol* (2016) 27(suppl 5):v103–v10. doi: 10.1093/annonc/mdw327

89. Hung FH, Wang YA, Jian JW, Peng HP, Hsieh LL, Hung CF, et al. Evaluating brca mutation risk predictive models in a Chinese cohort in Taiwan. *Sci Rep* (2019) 9(1):10229. doi: 10.1038/s41598-019-46707-6

90. Cheng X, Gu Z, Sun X, Zhuang Z. Study on the differences of opinions and choices of high-risk breast cancer populations in China before and after genetic testing. *Transl Cancer Res* (2019) 8(8):2893–905. doi: 10.21037/tcr.2019.11.43

91. Xue Y, Shi Y, Xu Y, Xu Z, Chen X, Wang C. Questionnaire survey on status quo of genetic counseling on gynecologic tumors. *Acad J Second Military Med Univ* (2021) 42(6):10. doi: 10.16781/j.0258-879x.2021.06.0641

92. Reid S, Spalluto LB, Lang K, Weidner A, Pal T. An overview of genetic services delivery for hereditary breast cancer. *Breast Cancer Res Treat* (2022) 191 (3):491–500. doi: 10.1007/s10549-021-06478-z

93. Yoon SY, Wong SW, Lim J, Ahmad S, Mariapun S, Padmanabhan H, et al. Oncologist-led brca counselling improves access to cancer genetic testing in middle-income Asian country, with no significant impact on psychosocial outcomes. *J Med Genet* (2022) 59(3):220–9. doi: 10.1136/jmedgenet-2020-107416

94. Kwong A, Chu AT, Wu CT, Tse DM. Attitudes and compliance of clinical management after genetic testing for hereditary breast and ovarian cancer among high-risk southern Chinese females with breast cancer history. *Fam Cancer* (2014) 13(3):423–30. doi: 10.1007/s10689-014-9706-7

95. Wang Y, Li Y, Song Y, Chen C, Wang Z, Li L, et al. Comparison of ultrasound and mammography for early diagnosis of breast cancer among Chinese women with suspected breast lesions: A prospective trial. *Thorac Cancer* (2022). doi: 10.1111/1759-7714.14666

96. Bao W, Chen W, Du L, Gu F, Guo L, Han J, et al. Consulting group of China guideline for the screening and early diagnosis and treatment of female breast cancer. [China guideline for the screening and early detection of female breast cancer (2021,Beijing)]. *China Cancer* (2021) 30(3):31. doi: 10.11735/j.issn.1004-0242.2021.03.A001

97. Sardanelli F, Podo F, D'Agnolo G, Verdecchia A, Santaquilani M, Musumeci R, et al. Multicenter comparative multimodality surveillance of women at genetic-familial high risk for breast cancer (Hibcrit study): Interim results. *Radiology* (2007) 242(3):698–715. doi: 10.1148/radiol.2423051965

98. Riedl CC, Ponhold L, Flory D, Weber M, Kroiss R, Wagner T, et al. Magnetic resonance imaging of the breast improves detection of invasive cancer, preinvasive cancer, and premalignant lesions during surveillance of women at high risk for breast cancer. *Clin Cancer Res* (2007) 13(20):6144–52. doi: 10.1158/1078-0432.CCR-07-1270

99. Kuhl CK, Schrading S, Leutner CC, Morakkabati-Spitz N, Wardelmann E, Fimmers R, et al. Mammography, breast ultrasound, and magnetic resonance imaging for surveillance of women at high familial risk for breast cancer. *J Clin Oncol* (2005) 23(33):8469–76. doi: 10.1200/JCO.2004.00.4960

100. Segal N, Ber Y, Benjaminov O, Tamir S, Yakimov M, Kedar I, et al. Imaging-based prostate cancer screening among brca mutation carriers-results from the first round of screening. *Ann Oncol* (2020) 31(11):1545–52. doi: 10.1016/j.annonc.2020.06.025

101. Kim YC, Zhao L, Zhang H, Huang Y, Cui J, Xiao F, et al. Prevalence and spectrum of brca germline variants in mainland Chinese familial breast and ovarian cancer patients. *Oncotarget* (2016) 7(8):9600–12. doi: 10.18632/oncotarget.7144

102. Kwong A, Wong CH, Shea C, Suen DT, Choi CL. Choice of management of southern Chinese brca mutation carriers. *World J Surg* (2010) 34(7):1416–26. doi: 10.1007/s00268-010-0477-5

103. Cao A, Huang L, Shao Z. The preventive intervention of hereditary breast cancer. *Adv Exp Med Biol* (2017) 1026:41–57. doi: 10.1007/978-981-10-6020-5_3

104. Cheng A, Li L, Wu M, Lang J. Pathological findings following risk-reducing salpingo-oophorectomy in brca mutation carriers: A systematic review and meta-analysis. *Eur J Surg Oncol* (2020) 46(1):139–47. doi: 10.1016/j.ejso.2019.09.002

105. Zhang D, Fu F, Xie L, Chu F, Wan Q, Xie Y. Clinical practice of prophylactic nipple- sparing mastectomy and immediate recon struction in Chinese healthy women with Brca1/Brca2 germline mutation. *Chin J Clin Oncol* (2020) 47(1):5. doi: 10.3969/j.issn.1000-8179.2020.01.429

106. Su L, Xu Y, Ouyang T, Li J, Wang T, Fan Z, et al. Contralateral breast cancer risk in Brca1 and Brca2 mutation carriers in a Large cohort of unselected Chinese breast cancer patients. *Int J Cancer* (2020) 146(12):3335–42. doi: 10.1002/ijc.32918

107. Rebbeck TR, Kauff ND, Domchek SM. Meta-analysis of risk reduction estimates associated with risk-reducing salpingo-oophorectomy in Brca1 or Brca2 mutation carriers. *J Natl Cancer Inst* (2009) 101(2):80–7. doi: 10.1093/jnci/djn442

108. Wattenberg MM, Asch D, Yu S, O'Dwyer PJ, Domchek SM, Nathanson KL, et al. Platinum response characteristics of patients with pancreatic ductal adenocarcinoma and a germline Brca1, Brca2 or Palb2 mutation. *Br J Cancer* (2020) 122(3):333–9. doi: 10.1038/s41416-019-0582-7

109. Byrski T, Dent R, Blecharz P, Foszczynska-Kloda M, Gronwald J, Huzarski T, et al. Results of a phase ii open-label, non-randomized trial of cisplatin chemotherapy in patients with Brca1-positive metastatic breast cancer. *Breast Cancer Res* (2012) 14(4):R110. doi: 10.1186/bcr3231

110. Tutt A, Tovey H, Cheang MCU, Kernaghan S, Kilburn L, Gazinska P, et al. Carboplatin in Brca1/2-mutated and triple-negative breast cancer brcaness subgroups: The tnt trial. *Nat Med* (2018) 24(5):628–37. doi: 10.1038/s41591-018-0009-7

111. Tung NM, Zakalik D, Somerfield MR. Hereditary breast cancer guideline expert p. adjuvant parp inhibitors in patients with high-risk early-stage Her2-negative breast cancer and germline brca mutations: Asco hereditary breast cancer

guideline rapid recommendation update. *J Clin Oncol* (2021) 39(26):2959–61. doi: 10.1200/JCO.21.01532

112. Curtin NJ, Szabo C. Poly(Adp-ribose) polymerase inhibition: Past, present and future. *Nat Rev Drug Discovery* (2020) 19(10):711–36. doi: 10.1038/s41573-020-0076-6

113. Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, et al. Adjuvant olaparib for patients with Brca1- or Brca2-mutated breast cancer. *N Engl J Med* (2021) 384(25):2394–405. doi: 10.1056/NEJMoa2105215

114. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline brca mutation. *N Engl J Med* (2017) 377(6):523–33. doi: 10.1056/NEJMoa1706450

115. Litton JK, Hurvitz SA, Mina LA, Rugo HS, Lee KH, Goncalves A, et al. Talazoparib versus chemotherapy in patients with germline Brca1/2-mutated Her2-negative advanced breast cancer: Final overall survival results from the embraca trial. *Ann Oncol* (2020) 31(11):1526–35. doi: 10.1016/j.annonc.2020.08.2098

116. Pujade-Lauraine E, Ledermann JA, Selle F, Gebski V, Penson RT, Oza AM, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a Brca1/2 mutation (Solo2/Engot-Ov21): A double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol* (2017) 18 (9):1274–84. doi: 10.1016/S1470-2045(17)30469-2

117. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med* (2018) 379(26):2495–505. doi: 10.1056/NEJMoa1810858

118. Ray-Coquard I, Pautier P, Pignata S, Perol D, Gonzalez-Martin A, Berger R, et al. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *N Engl J Med* (2019) 381(25):2416–28. doi: 10.1056/NEJMoa1911361

119. Gonzalez-Martin A, Pothuri B, Vergote I, DePont Christensen R, Graybill W, Mirza MR, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med* (2019) 381(25):2391–402. doi: 10.1056/NEJMoa1910962

120. Del Campo JM, Matulonis UA, Malander S, Provencher D, Mahner S, Follana P, et al. Niraparib maintenance therapy in patients with recurrent ovarian cancer after a partial response to the last platinum-based chemotherapy in the engot-Ov16/Nova trial. *J Clin Oncol* (2019) 37(32):2968–73. doi: 10.1200/JCO.18.02238

121. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for metastatic castration-resistant prostate cancer. *N Engl J Med* (2020) 382(22):2091–102. doi: 10.1056/NEJMoa1911440

122. Smith SG, Sestak I, Howell A, Forbes J. Cuzick j. participant-reported symptoms and their effect on long-term adherence in the international breast cancer intervention study I (Ibis I). *J Clin Oncol* (2017) 35(23):2666–73. doi: 10.1200/JCO.2016.71.7439

123. Park J, Huang D, Chang YJ, Lim MC, Myung SK. Oral contraceptives and risk of breast cancer and ovarian cancer in women with a Brca1 or Brca2 mutation: A meta-analysis of observational studies. *Carcinogenesis* (2022) 43(3):231–42. doi: 10.1093/carcin/bgab107

124. King MC, Levy-Lahad E, Lahad A. Population-based screening for Brca1 and Brca2: 2014 lasker award. *JAMA* (2014) 312(11):1091–2. doi: 10.1001/jama.2014.12483

125. Michaan N, Leshno M, Safra T, Sonnenblick A, Laskov I, Grisaru D. Cost effectiveness of whole population brca genetic screening for cancer prevention in Israel. *Cancer Prev Res (Phila)* (2021) 14(4):455–62. doi: 10.1158/1940-6207.CAPR-20-0411

frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 16 December 2022
DOI 10.3389/fonc.2022.1042789

OPEN ACCESS

EDITED BY
Maria Rosaria De Miglio,
University of Sassari, Italy

REVIEWED BY
Tibor Tot,
Falun Hospital, Sweden
Marco Fogante,
Marche Polytechnic University, Italy
Min Yan,
Henan Provincial Cancer Hospital, China

*CORRESPONDENCE
Ailin Song
songail@lzu.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 13 September 2022
ACCEPTED 28 November 2022
PUBLISHED 16 December 2022

CITATION
Zhang Y, Liu F, Gao Q, Chai Y, Ren Y, Tian H, Ma B and Song A (2022) Comparing the outcome between multicentric/multifocal breast cancer and unifocal breast cancer: A systematic review and meta-analysis. *Front. Oncol.* 12:1042789. doi: 10.3389/fonc.2022.1042789

Comparing the outcome between multicentric/multifocal breast cancer and unifocal breast cancer: A systematic review and meta-analysis

Yalan Zhang[1,2†], Fan Liu[1,2†], Qianqian Gao[1†], Yahui Chai[1,2], Yan Ren[1,2], Hongyou Tian[1,2], Bin Ma[3,4] and Ailin Song[1,2*]

[1]Department of General Surgery, Lanzhou University Second Hospital, Lanzhou University, Lanzhou, China, [2]Second Clinical Medical College, Lanzhou University, Lanzhou, China, [3]Evidence-Based Medicine Center, School of Basic Medical Sciences, Lanzhou University, Lanzhou, China, [4]Key Laboratory of Evidence-Based Medicine and Knowledge Translation of Gansu Province, Lanzhou University, Lanzhou, China

Objective: This systematic review and meta-analysis compares the outcome between MMBC and unifocal breast cancer (UFBC), in order to provide a theoretical basis for the design of an appropriate clinical therapeutic strategy of MMBC patients.

Methods: PubMed, Embase, The Cochrane Library, Web of science, CNKI, WanFang Data, CBM and VIP database were searched from inception to July 2021, and observational studies reporting the outcome of patients with MMBC and UFBC were included. We extracted or calculated the mortality rates of MMBC and UFBC patients; and obtained the hazard ratios; odds ratios; relative risks; and the corresponding 95% confidence intervals from the eligible studies. All the meta-analyses were conducted by using the Stata 15.0 software.

Results: 31 eligible studies comprising a total of 15,703 individuals were included. The meta-analysis revealed that MMBC did not have a significant association with poor overall survival (*HR*=1.04, 95% *CI*=0.96-1.12), disease-free survival (*HR*= 1.07, 95% *CI*= 0.84-1.36), breast cancer-specific survival (*HR*=1.42, 95% *CI*= 0.89-2.27), recurrence-free survival (*HR*= 0.878, 95% *CI*= 0.652-1.182), local recurrence-free survival (*HR*= 0.90, 95% *CI*= 0.57-1.42), and contralateral breast cancer risk (*RR*= 0.908, 95% *CI*= 0.667-1.234). However, MMBC appeared to have a correlation with a slightly higher risk of death (*OR*=1.31, 95% *CI*=1.18-1.45).

Conclusion: Patients with MMBC appeared to have a higher risk of death, however, it may not be independently associated with poorer outcomes. Considering the inter-study heterogeneity and other limitations, our results need to be validated by further multicenter prospective studies with a large sample size in the future.

KEYWORDS

Multicentric/multifocal breast cancer (MMBC), unifocal breast cancer (UFBC), prognosis, systematic review, meta-analysis

1 Introduction

In 2020, the estimated number of new breast cancer cases was about 2.26 million and cancer deaths were projected to be around 0.68 million worldwide (1). Globally, breast cancer is one of the most common cancers and the most frequent cause of cancer death among women.

Breast cancer usually presents as a single lesion, but in unilateral breast cancer, multiple lesions may appear simultaneously or sequentially. To enable further study and differentiate it from the subtypes with only one separate lesion - unifocal breast cancer (UFBC), researchers have subdivided such cases into two categories. The first one is multicentric breast cancer (MCBC), wherein two or more tumors are present in more than one quadrant of the same breast, but some researchers suggest that regardless of whether the different lesions are present in multiple quadrants of the same breast, those separated by >4-5 cm from each other should be called MCBC (2, 3). The second one is multifocal breast cancer (MFBC), wherein two or more tumors are found in the same quadrant of the breast (4, 5). Regarding the minimum distance between the MFBC lesions, Lüttges et al. (6) suggested that it should be at least 2 cm, while Ustaalioglu et al. (7) suggested that the spacing distance over 1 mm was enough. However, other investigators suggested that independent lesions in the specimen needed to be observed by the naked eye (disregarding microscopic lesions) (3, 8).Moreover, others indicated that multiple lesions should be clearly separated by non-cancerous tissue or carcinoma *in situ* (5, 8–11). Considering the difficulties with measurement and precision, these two categories are often studied together, and called multicentric/multifocal breast cancer (MMBC) (9).

At present, the prevalence of MMBC ranges between 6% - 77% (5, 12–14). Although MMBC is a common occurrence, its clinicopathological characteristics, precise therapeutic strategies, and prognosis and survival are not well characterized. Past studies have shown that MMBC was correlated with an increase in the lymph-node involvement, less differentiation, *HER*-2 positivity and lymphovascular invasion as compared to UFBC (4, 13, 15). In terms of the prognosis, many studies have explored the differences between MMBC and UFBC, but the findings have been largely inconclusive. Some studies have shown that MMBC patients had a higher mortality rate and shorter survival than the UFBC patients, and suggested that MMBC as an independent prognostic risk factor (4, 16, 17). However, others reported that MMBC patients had a similar prognosis as the UFBC patients (5, 10, 18), in terms of the OS and the DFS. The eighth edition of the AJCC TNM staging system of breast cancer indicates that the overall prognostic impact of smaller lesions on MMBC is not considered. However, the guidelines also emphasize the importance of a comprehensive judgment in the real clinical practice, especially when synchronous invasive tumors are identified (19). Therefore, there are conflicting reports regarding the prognosis of MMBC and UFBC patients, and whether MMBC is associated with a poorer prognosis is controversial (4, 5, 9, 16, 17, 20–24).

Thus, the current study summarizes the studies related to the comparison of prognosis between MMBC and UFBC patients, and synthesizes a systematic review and meta-analysis to evaluate the differences in the prognosis, in order to provide a theoretical basis for the design of an appropriate therapeutic strategy for treating MMBC patients.

2 Methods

The study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (25).

2.1 Eligibility criteria

The inclusion criteria for the study were as follows: (1) Participants: female patients with pathologically proven stages I-III of unilateral invasive breast cancer, aged ≥18 years, without contralateral breast cancer, without distant metastases, without any previous or concomitant malignant disease, without any limitation due to race or nationality; (2) Exposure: patients with clinically or image-based or pathologically diagnosed MMBC or UFBC; (3) Outcomes: mortality rates of MMBC and UFBC, overall survival (OS), disease-free survival (DFS), breast cancer-specific survival (BCSS), recurrence-free survival (RFS), local recurrence-free survival (LRFS), risk of contralateral breast cancer (CBC); (5) Type of study: case-control and cohort studies.

The exclusion criteria were as follows: (1) articles without a clear definition of MMBC; (2) MFBC or MCBC only; (3) duplicate articles; (4) articles published in languages other than English or Chinese.

2.2 Information sources and search strategy

The following eight electronic databases were independently searched by two researchers (YLZ and FL) and the timeline was set at July 2021: PubMed; Embase; the Cochrane Library; Web of Science; CNKI; WanFang Data; CBM; and the VIP database. The references of the included studies and previous MMBC related systematic reviews were also checked, and the relevant literature was manually added if available. Before the final analyses, we re-searched the literature to ensure that any study meeting the inclusion criteria was included as far as possible. The detailed search strategies are showed in Appendix Table 1.

2.3 Study selection

Two researchers (YLZ and FL) checked all the collected studies independently and if there was any disagreement between them, a discussion or the third reviewer (QQG)'s decision was taken into account. All the retrieved literature were imported into Endnote X9 software. After removing the duplicates, we firstly screened the articles by the title and abstract and then identified the final included studies through the full-text reading of previously screened literature. Then, we recorded the reasons for excluding the literature in the last two steps.

2.4 Data collection

Three researchers were involved in the data collection task. Two of them (YLZ and FL) independently collected the data from the included studies and recorded them in a pre-defined spreadsheet by using the Microsoft Excel 2021 software. The differences of opinion were discussed, and if they were still unresolved, a third Reviewer (QQG)'s opinion was taken into account. We extracted the following information from the included studies: (1) the first author's name and the publication year, region where the study was conducted, study design, and recruitment period; (2) the sample size and age; (3) follow-up time; (4) definition of MMBC; (5) the AJCC edition used for the T-staging; (7) mortality rates of MMBC and UFBC patients. If the data needed further confirmation, the corresponding author of the article was contacted by email.

2.5 Quality assessment

The risk of bias was assessed by using the Newcastle-Ottawa Scale (NOS) (26), whose full mark was 9, a score of 8 to 9 was considered as low risk of bias (high quality literature), a score of 5~7 was considered as moderate risk (moderate quality literature), and a score of 0~4 was considered as high risk (low quality literature). The risk of bias was independently assessed by two researchers (YLZ and FL) and the discrepancies were resolved by discussion or a third reviewer's (QQG) decision was taken into account.

2.6 Statistical analysis

In this study, we extracted or calculated the mortality rates for MMBC and UFBC patients, and the *HRs*, *RRs* and *ORs* with the corresponding 95% *CIs* were obtained from the multivariate analyses of the included studies. If two or more studies reported the data of an outcome, a meta-analysis was performed, otherwise, only a descriptive analysis was performed. All the meta-analyses were completed by using the Stata 15.0 software. The Cochrane's Q-test was applied to evaluate the inter-study heterogeneity and the I^2 statistic was used to quantify the degree of heterogeneity. If the studies were without statistical heterogeneity, the meta-analysis was conducted using the fixed-effects model. If $I^2 \geq 50\%$ and $P<0.10$, it indicated that there was a significant and substantial heterogeneity (27) among the studies, and hence a random-effects model was employed after excluding the significant clinical heterogeneity. When there was a significant clinical heterogeneity, sensitivity and subgroup analyses were used, or only a descriptive analysis was performed. $P<0.05$ was considered as statistically significant. The publication bias was evaluated using funnel plots or the Egger's test when over 10 studies were included. When $P > 0.05$, it suggested the absence of publication bias. And if there was a publication bias, the trim-and-fill method was used to assess the further effect of publication bias on the results.

3 Results

3.1 Search results

The eight databases were searched and a total of 15,703 articles were retrieved. After removing the duplicate articles, 10,027 were available for further screening. 9933 articles were excluded after browsing the titles and abstracts, and the full-text was examined for 94 studies. Lastly, 31 articles (2–5, 7–11, 13, 14, 16–18, 20–24, 28–39) met the eligibility criteria mentioned previously. The detailed selection procedures and statistics are shown in Figure 1.

3.2 Study characteristics

Among the included studies, four (5, 14, 18, 39) were prospective cohort studies, twenty-seven (2–4, 7–11, 13, 16, 17, 20–24, 28–38) were retrospective cohort studies, and one (23) was a retrospective age-matched cohort study. The total number of participants was 88,147 and the sample sizes ranged from 118 (29) to 25,320 (14). The follow-up for fifteen studies was over 60 months (4, 8–11, 14, 17, 20, 23, 29, 31, 33, 34, 36, 39), and seven studies were published within 5 years (5, 9, 21–23, 33, 35). The detailed information is presented in Appendix Table 2.

3.3 Assessment of the quality of the included articles

The quality of the included studies was evaluated using the NOS scale. Seventeen studies (3, 4, 7, 9–11, 14, 16, 17, 23, 28, 29, 33–35, 38, 39) were of high quality, while fourteen (2, 5, 8, 13, 18, 20–22, 24, 30–32, 36, 37) were of moderate quality and none of the study was of low quality. The details are listed in Appendix Figure 1.

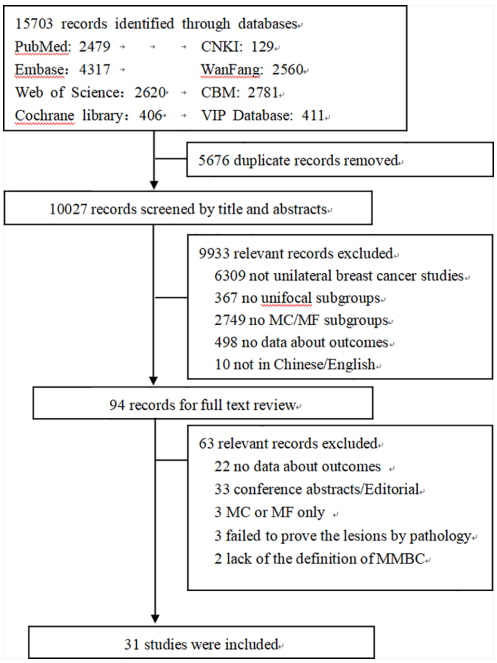


FIGURE 1
Flowchart of literature selection.

3.4 Outcomes

3.4.1 Overall survival

9 studies (7, 10, 13, 16, 20, 21, 30, 32, 39) were enrolled in the analysis of OS, 8 (7, 10, 13, 16, 20, 30, 32, 39) of them reported *HRs* and 1 (21) reported *OR*. The heterogeneity test was not statistically significant (I^2 =45.1%, *P*=0.059), and 8 *HRs* were selected for the meta-analysis using the fixed-effects model. The analysis revealed no statistically significant relationship between MMBC and poor OS in the multivariate analysis (*HR*=1.04, 95% *CI*= 0.96-1.12, I^2 =45.1%, *P*=0.059, 8 studies) (Figure 2). Moreover, Djordjevic-Jovanovic et al. (21) reported no remarkable difference in the 5-year OS between UFBC and MMBC patients in a multivariate analysis (*OR*=0.91, 95% *CI*=0.65-1.21, *P*=0.51).

3.4.2 Disease-free survival

In total, four studies (7, 16, 24, 30) were integrated into the *HRs* analysis of DFS. Heterogeneity tests showed statistical significance and therefore a random effects model was applied. The results indicated that compared to UFBC, MMBC was not associated with poorer DFS by multivariate analysis (*HR*=1.07, 95% *CI*=0.84-1.36, I^2 =76.6%, *P*=0.001, 4 studies) (Figure 3).

3.4.3 Breast cancer specific survival

Four studies (4, 13, 17, 39) were included for the analysis of BCSS, 3 studies (13, 17, 39) reported *HRs*, and 1 study (4) reported *RR*. The 3 *HRs* were selected for the meta-analysis, and the heterogeneity was noticeable (I^2 =65.0%, *P*=0.022), thus we chose a random-effects model to perform the analysis. In the multivariate analysis, the meta-analysis showed that in comparison with UFBC, MMBC had no clear correlation with poorer BCSS (*HR*=1.42, 95% *CI*=0.89-2.27, 3 studies) (Figure 4). Moreover, Boyages et al. (4) reported four *RR* values for BCSS from multivariate analysis. They used aggregate tumor size of each foci in MMBC or the dominant tumor size of MMBC to determine the "T-stage" and set a 2 cm tumor diameter boundary. The results showed that when the tumor diameter was less than 2 cm, there was no statistical difference in the 10-year BCSS between the UFBC and MMBC patients (Dominant: *RR* (95% *CI*) =0.86 (0.39-1.87), *P*=0.695; Aggregate: *RR* (95% *CI*) =1.00 (0.36-2.76), *P*=1.00). When the tumor diameter was greater than 2 cm, the results of the aggregate tumor size staging method also indicated no significant difference between the two groups, but the largest or the dominant tumor size staging system showed a different result (Dominant: *RR* (95% *CI*) =1.91(1.15-3.16), *P*=0.012; Aggregate: *RR* (95% *CI*) =1.13(0.82-2.09), *P*=0.267).

3.4.4 Recurrence-free survival

The analysis of *HRs* for the RFS was comprised of two studies (13, 32). A fixed-effect model meta-analysis demonstrated that in the multivariate analysis, compared with UFBC, MMBC was not significantly associated with poorer RFS (*HR*= 0.878, 95% *CI*=0.652-1.182, I^2 =0.00%, *P*=0.977, 2 studies) (Figure 5).

3.4.5 Local recurrence-free survival

For LRFS, three studies (5, 24, 39) were included in the *HRs* meta-analysis. The results were analyzed using a fixed-effects model, and suggested that MMBC was not significantly associated with poorer LRFS by multivariate analysis (*HR*=0.90, 95% *CI*=0.57-1.42, I^2 =48.2%, *P*=0.145, 3 studies) (Figure 6).

3.4.6 Mortality rates

10 studies (4, 9, 13, 14, 16, 17, 20, 23, 24, 32) described the mortality rates of MMBC and UFBC patients. Meta-analysis by fixed-effects model showed that in comparison with UFBC, MMBC was associated with a higher mortality (*OR*=1.31, 95% *CI*=1.18-1.45, I^2 =36.0%, *P*=0.12, 10 studies) (Figure 7).

3.4.7 Contralateral breast cancer

Only one study (14) among the included literature reported the multivariate analysis results of CBC risk. Yerushalmi et al. (14) concluded that MMBC was not significantly associated with higher risk of CBC (*RR*=0.908, 95% *CI*=0.667-1.234, *P* = 0.537).

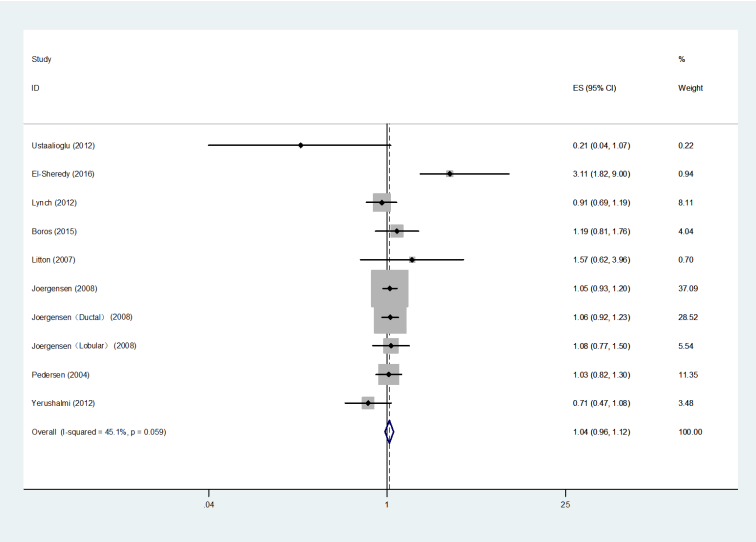


FIGURE 2
Forest plot of OS comparing MMBC and UFBC.

4 Discussion

In this review, we pooled the data for MMBC and UFBC with regards to the OS, DFS, RFS, BCSS, LRFS, mortality, and CBC aspects and performed a meta-analysis. The final results showed that MMBC patients had a similar prognosis as the UFBC patients, except for a slightly higher mortality rate.

4.1 MMBC patients may have a slightly higher mortality rate

In this paper, we found that MMBC patients had a slightly higher mortality rate than the UFBC patients (*OR*=1.31, 95% *CI*=1.18-1.45), which could mainly be because MMBC patients had a relatively high total tumor load and more aggressive biological behavior. In a previously published review,

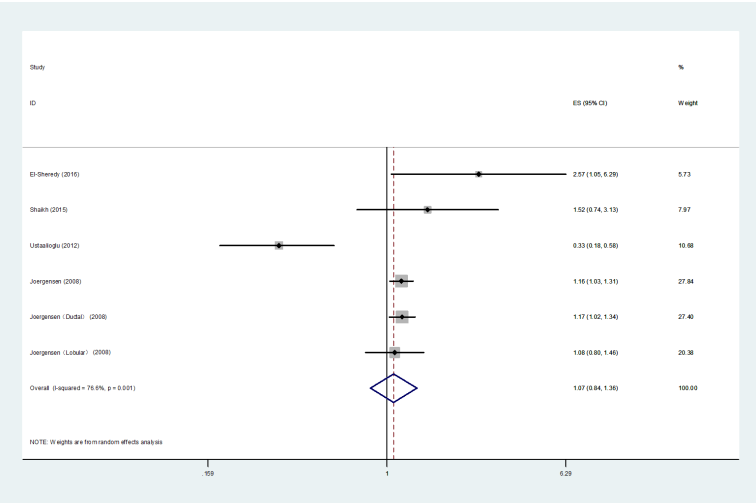


FIGURE 3
Forest plot of DFS comparing MMBC and UFBC.

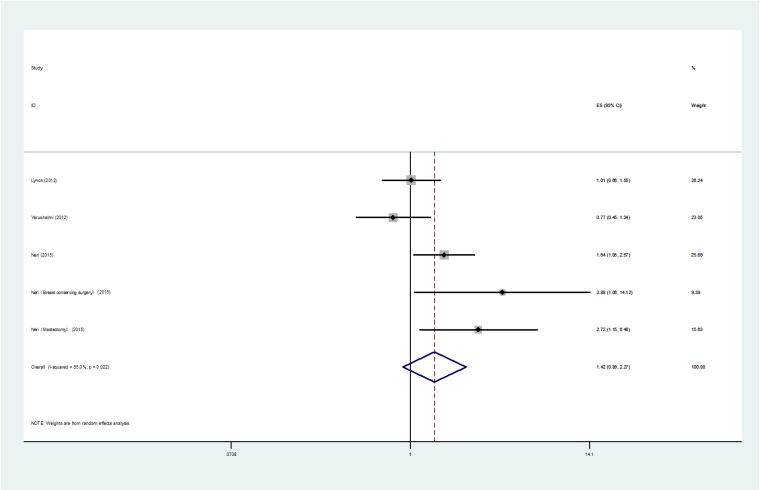


FIGURE 4
Forest plot of BCSS comparing MMBC and UFBC.

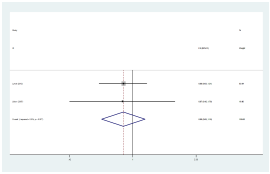


FIGURE 5
Forest plot of RFS comparing MMBC and UFBC.

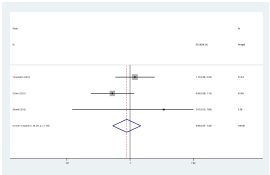


FIGURE 6
Forest plot of LRFS comparing MMBC and UFBC.

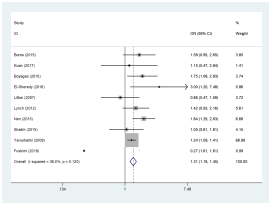


FIGURE 7
Forest plot of the mortality rates of MMBC versus UFBC patients.

compared to UFBC, MMBC was shown to have a higher proportion of poorly differentiated tumors and a greater risk of vascular invasion (2), which suggested that MMBC was associated with extensive intra-ductal lesions and an invasive lobular carcinoma component, which might increase the risk of positive surgical margins (40–42). At the same time, MMBC patients were more likely to develop tumor recurrence and metastases. And Neri et al. (17) found that MMBC was associated with the absence of ER and Her2-neu positive status which may reduce the possibility of MMBC patients benefiting from endocrine therapy and targeted therapy. Moreover, Lang et al. (43) reported a higher rate of axillary lymph node metastasis and a higher Ki67 proliferation index in MMBC patients compared to the UFBC patients, which to some extent suggested that MMBC patients might have a poorer outcome. However, previous studies (23, 44) reported that when controlling for the age, there was no significant difference in mortality between UFBC and MMBC patients (5.3% versus 7%, $P = 0.89$ with a median follow-up period of 3 years and 13% versus 14.7%, $P = 0.89$ with a median follow-up period of 7 years). And it is worth noting that more MMBC patients in this cohort opted for total mastectomy, which could have provided a survival benefit for patients these patients. Additionally, Yerushalmi et al. (39) reported that patients who underwent breast-conserving surgery in stages I-II also showed a similar mortality as compared to MMBC and UFBC patients, but the MMBC group had less severe disease compared to the UFBC

patients. The results for the comparison of mortality rates between the two groups in our study showed that MMBC patients had a higher risk of death, but in the early stage breast cancer, appropriate surgery and adjuvant treatment may also offer survival benefits for MMBC patients (4, 5, 39). Therefore, early screening for breast cancer is important, and timely diagnosis and early intervention may not only provide a survival benefit, but also allow some patients to be suitable for and benefit from less invasive surgical modalities.

4.2 MMBC per se may not represent a poorer prognosis

The findings from the current study showed that there were no significant differences between UFBC and MMBC patients in terms of the OS, DFS, RFS, BCSS, and LRFS in multivariate analysis, which may be due to the fact that MMBC per se is not associated with a worse prognosis. It was reported that the MMBC patients were younger, had larger tumors, had greater involvement of lymph nodes, and many of them were in pre-menopausal stage in comparison to the UFBC patients (11, 13, 28, 45, 46). The above risk factors made MMBC patients more likely to undergo mastectomy as well as receive more adjuvant therapy to some extent. When these factors were controlled in the multivariate analysis, most of the studies showed that MMBC no longer had an independent effect on the OS and DFS. However, some studies still found that MMBC was an independent prognostic factor for the OS and DFS in multivariate analysis (11, 16, 47). Meanwhile, the results from a previous systematic review (48) showed that MMBC was associated with poorer OS, but after excluding one study (49) with significant heterogeneity, the results no longer showed that MMBC was associated with poorer OS. Upon reviewing recent studies (5, 9, 21–23), we found that MMBC may be associated with some worse prognosis factors, but MMBC patients often had a similar prognosis as UFBC, which could be due to the advances in imaging technologies and pathological diagnostic techniques and the continuous optimization of the therapeutic options. Pre-operative breast MRI shows good utility in determining tumor boundaries and detection of additional tumor foci, and is not influenced by different histotypes, which helps to provide the best local treatment for MMBC patients (50). In the past, the majority of MMBC patients underwent mastectomy for the discerned higher risk for in-breast recurrence and less good cosmetic outcome. But in recent publications, breast conserving surgery can be performed in selected MMBC patients (51) and the use of daVinci Robot can improve cosmetic results (52).

For the multivariate analysis of RFS, LRFS and BCSS, the general trend supported the finding that MMBC patients had a similar prognosis to that of UFBC patients. However, only few studies were included in these outcome indicators, which may affects the results reliability. A study on outcomes in 1163 MFBC/UFBC patients reported that MFBC was independently significantly associated with LRFS, DFS, and OS, but this study did not adjust for pathologic stage, T stage and nodal status (49). Thus, further prospective studies with larger samples are needed to confirm the above findings.

Our meta-analysis results on DFS and BCSS indicated a significant and substantial heterogeneity among the included studies. And we think clinical factors cause heterogeneity mainly. With fewer studies included under each outcome indicators, the subgroup analysis may not produce meaningful results. But a decreased heterogeneity was also seen when we attempted to perform subgroup analysis based on some clinical factors (Appendix Figure 2).

4.3 MMBC may not increase the risk of CBC

Among the included studies, only one study (14) reported the results related to the development of contralateral breast cancer in multivariate analysis. And the results supported the opinion that MMBC was only a representative of intra-mammary spreading, whereas CBC was an independent event. This finding may help to alleviate anxiety and panic among the patients with MMBC, as some patients may receive excessive treatment or even make a hasty decision to undergo prophylactic surgery after the diagnosis owing to their fear of developing CBC. Moreover, the study by Kurtz et al. (31) also showed a similar probability of CBC in MMBC and UFBC (3% versus 4%) patients. However, some of the current tools to assess the risk of CBC also incorporate MMBC as a risk term and have shown a better predictive power (53). There isn't enough evidence regarding the association between MMBC and CBC, and it is hoped that more original studies will report relevant data to support CBC-related analysis.

4.4 The prognostic role of the remaining lesions in MMBC needs further investigation

Breast cancer is a heterogeneous group of diseases with regard to clinical manifestation, tumor morphology, and immunohistochemical differences within tumors (54). And a recent publication emphasized that MMBC has a higher risk of metastasis, recurrence, and a worse prognosis, compared to UFBC with similar staging (TNM), and sometimes the largest one is not always the most aggressive one, and more than one tumor should be evaluated (55). Data from Boyages et al. (4) on BCSS showed that the use of different criteria for assessing the tumor T-staging could influence the final results, which showed that for tumors >20 mm in diameter, MMBC was associated with poorer BCSS after using the largest or dominant tumor size of MMBC to assess the T-staging. On the other hand, when the aggregated diameter of the lesions were used to assess the tumor staging, MMBC and UFBC patients

were found to have a similar prognosis. However, Duraker et al. (2) reported that MMBC and UFBC patients had similar prognosis regardless of whether the T-staging was assessed by using the largest tumor diameter or the aggregated diameter of all the lesions. Also, several studies (13, 28) have concluded that the current TNM staging could be a good assessment of MMBC tumor load, and also showed that the difference in the overall prognosis between MMBC and UFBC patients was not statistically significant. However, it is worth noting that Fushimi et al. (9) reported that MMBC was not associated with a worse prognosis, but at the same time showed that MMBC was a major prognostic factor for DFS after assessing the T-staging using the aggregated diameter of the lesions (*HR*= 2.710, 95% *CI*= 1.011-7.264, *P*= 0.048). Therefore, the method for assessing the T-stage of MMBC may influence the results for prognosis in multivariate analyses, and the prognostic impact of the remaining lesions in MMBC requires further investigation.

4.5 Strengths and limitations

In this systematic review and meta-analysis, several studies were included to assess the difference in the prognosis between MMBC and UFBC patients. Here, we searched eight databases using a relatively broad terminology and our search strategy ensured as far as possible, that none of the potentially relevant studies were excluded. However, the current study suffers from some limitations. First, most of the included studies were retrospective cohort studies which may existed selection bias and data analysis bias. Second, the definition and diagnostic criteria of MMBC was not completely consistent across all the included studies. These discrepancies affected the detection of MMBC, and it could have affected the reliability of the meta-analysis results. Third, as the heterogeneity among the included studies were significant and fewer studies were included under some of the outcome indicators, the source of heterogeneity was difficult to determine and limited the accuracy of our findings further. Finally, the limitation of the choice of language could have increased the publication or language bias.

5 Conclusions

In summary, patients with MMBC appeared to have a higher risk of death, however, it may be not independently associated with poor OS, DFS, RFS, BCSS, LRFS, and CBC risk. With appropriate surgical interventions and adjuvant therapies, the prognosis of patients with MMBC and UFBC was similar, but the prognostic impact of every lesion in MMBC still needs further investigation. Further multicenter prospective studies with larger sample size are needed for validating the findings from the current study.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

All authors contributed to the study conception and design. Study selection, data extraction and analysis were performed by YZ, FL and QG. The first draft of the manuscript was written by YZ and all authors commented on previous versions of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by Natural Science Foundation of Gansu Province, China (grant number: 20JR5RA342).

Acknowledgments

The authors would like to thank all the reviewers who participated in the review, as well as the Science and Technology Department of Gansu Province, the Center for Evidence-Based Medicine of Lanzhou University and the Second Hospital of Lanzhou University for their support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1042789/full#supplementary-material

References

1. *Latest global cancer data: cancer burden rises to 19.3 million new cases and 10.0 million cancer deaths in 2020* . Available at: https://www.iarc.who.int/faq/latest-global-cancer- data-2020-qa/.

2. Duraker N, Caynak ZC. Axillary lymph node status and prognosis in multifocal and multicentric breast carcinoma. *Breast J* (2014) 20(1):61–8. doi: 10.1111/tbj.12205

3. Katz A, Strom EA, Buchholz TA, Theriault R, Singletary SE, McNeese MD. The influence of pathologic tumor characteristics on locoregional recurrence rates following mastectomy. *Int J Radiat Oncol Biol Physics* (2001) 50(3):735–42. doi: 10.1016/S0360-3016(01)01500-0

4. Boyages J, Jayasinghe UW, Coombs N. Multifocal breast cancer and survival: Each focus does matter particularly for larger tumours. *Eur J Cancer* (2010) 46 (11):1990–6. doi: 10.1016/j.ejca.2010.03.003

5. Ozturk A, Ilgun S, Ucuncu M, Gachayev F, Ordu C, Alco G, et al. The effect of multifocal and multicentric tumours on local recurrence and survival outcomes in breast cancer. *J BUON* (2021) 26(1):196–203.

6. Lüttges J, Kalbfleisch H, Prinz P. Nipple involvement and multicentricity in breast cancer. a study on whole organ sections. *J Cancer Res Clin Oncol* (1987) 113 (5):481–7. doi: 10.1007/BF00390043

7. Ustaalioglu BO, Bilici A, Kefeli U, Seker M, Oncel M, Gezen C, et al. The importance of Multifocal/Multicentric tumor on the disease-free survival of breast cancer patients single center experience. *Am J Clin Oncol Cancer Clin Trials* (2012) 35(6):580–6. doi: 10.1097/COC.0b013e31822d9cd6

8. Wilson LD, Beinfield M, Mckhann CF, Haffty BG. Conservative surgery and radiation in the treatment of synchronous ipsilateral breast cancers. *Cancer* (1993) 72(1):137–42. doi: 10.1002/1097-0142(19930701)72:1<137::AID-CNCR2820720126>3.0.CO;2-E

9. Fushimi A, Yoshida A, Yagata H, Takahashi O, Hayashi N, Suzuki K, et al. Prognostic impact of multifocal and multicentric breast cancer versus unifocal breast cancer. *Surg Today* (2019) 49(3):224–30. doi: 10.1007/s00595-018-1725-9

10. Pedersen L, Gunnarsdottir KA, Rasmussen BB, Moeller S, Lanng C. The prognostic influence of multifocality in breast cancer patients. *Breast* (2004) 13 (3):188–93. doi: 10.1016/j.breast.2003.11.004

11. Tot T, Gere M, Pekár G, Tarján M, Hofmeyer S, Hellberg D, et al. Breast cancer multifocality, disease extent, and survival. *Hum Pathol* (2011) 42(11):1761–9. doi: 10.1016/j.humpath.2011.02.002

12. Jain S, Rezo A, Shadbolt B, Dahlstrom JE. Synchronous multiple ipsilateral breast cancers: implications for patient management. *Pathology* (2009) 41(1):57–67. doi: 10.1080/00313020802563502

13. Lynch SP, Lei X, Chavez-Macgregor M, Hsu L, Meric-Bernstam F, Buchholz TA, et al. Multifocality and multicentricity in breast cancer and survival outcomes. *Ann Oncol* (2012) 23(12):3063–9. doi: 10.1093/annonc/mds136

14. Yerushalmi R, Kennecke H, Woods R, Olivotto IA, Speers C, Gelmon KA. Does multicentric/multifocal breast cancer differ from unifocal breast cancer? an analysis of survival and contralateral breast cancer incidence. *Breast Cancer Res Treat* (2009) 117(2):365–70. doi: 10.1007/s10549-008-0265-1

15. Aalders KC, Kuijer A, Straver ME, Slaets L, Litiere S, Viale G, et al. Characterisation of multifocal breast cancer using the 70-gene signature in clinical low-risk patients enrolled in the EORTC 10041/BIG 03-04 MINDACT trial. *Eur J Cancer* (2017) 79:98–105. doi: 10.1016/j.ejca.2017.03.034

16. El-Sheredy HG, El-Benhawy SA, Matrawy K, Ramadan R, Hamed Y. Multifocal/multicentric versus unifocal breast cancer: What is the difference? *Middle East J Cancer* (2016) 7(2):69–78.

17. Neri A, Marrelli D, Megha T, Bettarini F, Tacchini D, De Franco L, et al. "Clinical significance of multifocal and multicentric breast cancers and choice of surgical treatment: a retrospective study on a series of 1158 cases". *BMC Surg* (2015) 15:1. doi: 10.1186/1471-2482-15-1

18. Fowble B, Yeh IT, Schultz DJ, Solin LJ, Rosato EF, Jardines L, et al. The role of mastectomy in patients with stage I-II breast cancer presenting with gross multifocal or multicentric disease or diffuse microcalcifications. *Int J Radiat Oncol Biol Physics* (1993) 27(3):567–73. doi: 10.1016/0360-3016(93)90381-5

19. Giuliano AE, Connolly JL, Edge SB, Mittendorf EA, Rugo HS, Solin LJ, et al. Breast cancer-major changes in the American joint committee on cancer eighth edition cancer staging manual. *CA Cancer J Clin* (2017) 67(4):290–303. doi: 10.3322/caac.21393

20. Boros M, Voidazan S, Moldovan C, Georgescu R, Toganel C, Moncea D, et al. Clinical implications of multifocality as a prognostic factor in breast carcinoma - a multivariate analysis study comprising 460 cases. *Int J Clin Exp Med* (2015) 8(6):9839–46.

21. Djordjevic-Jovanovic L, Karanikolic A, Bojic T, Pesic I, Djordjevic M, Marinkovic M. Characteristics and outcomes of patients with multifocal/multicentric and unifocal breast cancer. *J BUON* (2017) 22(3):652–7.

22. Karakas Y, Dizdar O, Aksoy S, Hayran M, Altundag K. The effect of total size of lesions in Multifocal/Multicentric breast cancer on survival. *Clin Breast Cancer Karakas* (2018) 18(4):320–7. doi: 10.1016/j.clbc.2017.11.002

23. Kuan LL, Tiong LU, Parkyn R, Walters D, Lai C, Walsh D. Disease recurrence and survival in patients with multifocal breast cancer: a follow-up study with 7-year results. *ANZ J Surg* (2017) 87(10):E125–E8. doi: 10.1111/ans.13193

24. Shaikh T, Tam TY, Li T, Hayes SB, Goldstein L, Bleicher R, et al. Multifocal and multicentric breast cancer is associated with increased local recurrence regardless of surgery type. *Breast J* (2015) 21(2):121–6. doi: 10.1111/tbj.12366

25. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* (2021) 372:n71. doi: 10.1136/bmj.n71

26. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* (2010) 25(9):603–5. doi: 10.1007/s10654-010-9491-z

27. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *Bmj* (2003) 327(7414):557–60. doi: 10.1136/bmj.327.7414.557

28. Cabioglu N, Ozmen V, Kaya H, Tuzlali S, Igci A, Muslumanoglu M, et al. Increased lymph node positivity in multifocal and multicentric breast cancer. *J Am Coll Surg* (2009) 208(1):67–74. doi: 10.1016/j.jamcollsurg.2008.09.001

29. Egan RL. Multicentric breast carcinomas: Clinical-radiographic-pathologic whole organ studies and 10-year survival. *Cancer* (1982) 49(6):1123–30. doi: 10.1002/1097-0142(19820315)49:6<1123::AID-CNCR2820490610>3.0.CO;2-R

30. Joergensen LE, Gunnarsdottir KA, Lanng C, Moeller S, Rasmussen BB. Multifocality as a prognostic factor in breast cancer patients registered in Danish breast cancer cooperative group (DBCG) 1996-2001. *Breast* (2008) 17(6):587–91. doi: 10.1016/j.breast.2008.06.004

31. Kurtz JM, Jacquemier J, Amalric R, Brandone H, Ayme Y, Hans D, et al. Breast-conserving therapy for macroscopically multiple cancers. *Ann Surg* (1990) 212(1):38–44. doi: 10.1097/00000658-199007000-00006

32. Litton JK, Eralp Y, Gonzalez-Angulo AM, Broglio K, Uyei A, Hortobagyi GN, et al. Multifocal breast cancer in women < or =35 years old. *Cancer* (2007) 110 (7):1445–50. doi: 10.1002/cncr.22928

33. Mastropasqua MG, Addante F, Pirola S, Ingravallo G, Viale G. Correlation of size and focality with prognosis in small breast carcinoma: a single institution case series. *Breast* (2020) 54:164–9. doi: 10.1016/j.breast.2020.10.006

34. Middleton LP, Vlastos G, Mirza NQ, Eva S, Sahin AA. Multicentric mammary carcinoma: evidence of monoclonal proliferation. *Cancer* (2002) 94 (7):1910–6. doi: 10.1002/cncr.10452

35. Milulescu A, Ilie S, Toesca A, Filipescu A, Clim N, Mitran M, et al. A retrospective study on multifocal and multicentric vs. unifocal breast cancer. preliminary results. *Ginecoeu* (2017) 13(2):59–64. doi: 10.18643/gieu.2017.59

36. Pekar G, Hofmeyer S, Tabár L, Tarján M, Chen TH, Yen AM, et al. Multifocal breast cancer documented in large-format histology sections: long-term follow-up results by molecular phenotypes. *Cancer* (2013) 119(6):1132–9. doi: 10.1002/cncr.27877

37. Tan MP, Sitoh NY, Sitoh YY. Optimising breast conservation treatment for multifocal and multicentric breast cancer: A worthwhile endeavour? *World J Surg* (2016) 40(2):315–22. doi: 10.1007/s00268-015-3336-6

38. Wolters R, Wöckel A, Janni W, Novopashenny I, Ebner F, Kreienberg R, et al. Comparing the outcome between multicentric and multifocal breast cancer: What is the impact on survival, and is there a role for guideline-adherent adjuvant therapy? a retrospective multicenter cohort study of 8,935 patients. *Breast Cancer Res Treat* (2013) 142(3):579–90. doi: 10.1007/s10549-013-2772-y

39. Yerushalmi R, Tyldesley S, Woods R, Kennecke HF, Speers C, Gelmon KA. Is breast-conserving therapy a safe option for patients with tumor multicentricity and multifocality? *Ann Oncol* (2012) 23(4):876–81. doi: 10.1093/annonc/mdr326

40. Bani MR, Lux MP, Heusinger K, Wenkel E, Magener A, Schulz-Wendtland R, et al. Factors correlating with reexcision after breast-conserving therapy. *Eur J Surg Oncol* (2009) 35(1):32–7. doi: 10.1016/j.ejso.2008.04.008

41. Lagios MD. Pathologic features related to local recurrence following lumpectomy and irradiation. *Semin Surg Oncol* (1992) 8(3):122–8.

42. Sabel MS, Rogers K, Griffith K, Jagsi R, Kleer CG, Diehl KA, et al. Residual disease after re-excision lumpectomy for close margins. *J Surg Oncol* (2009) 99 (2):99–103. doi: 10.1002/jso.21215

43. Lang Z, Wu Y, Li C, Li X, Wang X, Qu G. Multifocal and multicentric breast carcinoma: A significantly more aggressive tumor than unifocal breast cancer. *Anticancer Res* (2017) 37(8):4593–8. doi: 10.18643/gieu.2017.59

44. Tiong LU, Parkyn R, Walters D, Field J, Lai C, Walsh DC. Dilemma in multifocal breast cancer – largest versus aggregate diameter. *ANZ J Surg* (2011) 81 (9):614–8. doi: 10.1111/j.1445-2197.2010.05569.x

45. Coombs NJ, Boyages J. Multifocal and multicentric breast cancer: does each focus matter? *J Clin Oncol Off J Am Soc Clin Oncol* (2005) 23(30):7497–502. doi: 10.1200/JCO.2005.02.1147

46. Rezo A, Dahlstrom J, Shadbolt B, Rodins K, Zhang Y, Davis AJ. Tumor size and survival in multicentric and multifocal breast cancer. *Breast* (2011) 20(3):259–63. doi: 10.1016/j.breast.2011.01.005

47. Weissenbacher TM, Zschage M, Janni W, Jeschke U, Dimpfl T, Mayr D, et al. Multicentric and multifocal versus unifocal breast cancer: Is the tumor-node-metastasis classification justified? *Breast Cancer Res Treat* (2010) 122(1):27–34. doi: 10.1007/s10549-010-0917-9

48. Vera-Badillo FE, Napoleone M, Ocana A, Templeton AJ, Seruga B, Al-Mubarak M, et al. Effect of multifocality and multicentricity on outcome in early stage breast cancer: a systematic review and meta-analysis. *Breast Cancer Res Treat* (2014) 146(2):235–44. doi: 10.1007/s10549-014-3018-3

49. Chung AP, Huynh K, Kidner T, Mirzadehgan P, Sim MS, Giuliano AE. Comparison of outcomes of breast conserving therapy in multifocal and unifocal invasive breast cancer. *J Am Coll Surg* (2012) 215(1):137–46. doi: 10.1016/j.jamcollsurg.2012.05.006

50. Prochowski Iamurri A, Ponziani M, Macchini M, Fogante M, Pistelli M, De Lisa M, et al. Evaluation of multifocality and multicentricity with breast magnetic resonance imaging in each breast cancer subtype. *Clin Breast Cancer* (2018) 18(2): e231–e5. doi: 10.1016/j.clbc.2017.10.012

51. Fang M, Zhang X, Zhang H, Wu K, Yu Y, Sheng Y. Local control of breast conservation therapy versus mastectomy in multifocal or multicentric breast cancer: A systematic review and meta-analysis. *Breast Care (Basel Switzerland)* (2019) 14(4):188–93. doi: 10.1159/000499439

52. Toesca A, Peradze N, Manconi A, Galimberti V, Intra M, Colleoni M, et al. Robotic nipple-sparing mastectomy for the treatment of breast cancer: Feasibility and safety study. *Breast* (2017) 31:51–6. doi: 10.1016/j.breast.2016.10.009

53. Völkel V, Hueting TA, Draeger T, van Maaren MC, de Munck L, Strobbe LJA, et al. Improved risk estimation of locoregional recurrence, secondary contralateral tumors and distant metastases in early breast cancer: the INFLUENCE 2.0 model. *Breast Cancer Res Treat* (2021) 189(3):817–26. doi: 10.1007/s10549-021-06335-z

54. Polyak K. Heterogeneity in breast cancer. *J Clin Invest* (2011) 121(10):3786–8. doi: 10.1172/JCI60534

55. Onisâi M, Dumitru A, Iordan I, Aliuş C, Teodor O, Alexandru A, et al. Synchronous multiple breast cancers-do we need to reshape staging? *Medicina (Kaunas Lithuania)* (2020) 56(5). doi: 10.3390/medicina56050230

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 04 January 2023
DOI 10.3389/fonc.2022.1023714

OPEN ACCESS

EDITED BY
Hussain Gadelkarim Ahmed,
University of Khartoum, Sudan

REVIEWED BY
Abdelbaset Mohamed Elasbali,
jouf university college of applied medical science qurayyat, Saudi Arabia
Aliete Cunha-Oliveira,
Coimbra Nursing School, Portugal

*CORRESPONDENCE
Phuong Dung (Yun) Trieu
phuong.trieu@sydney.edu.au

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 20 August 2022
ACCEPTED 05 December 2022
PUBLISHED 04 January 2023

CITATION
Trieu PD(Y), Mello-Thoms CR, Barron ML and Lewis SJ (2023) Look how far we have come: BREAST cancer detection education on the international stage.
Front. Oncol. 12:1023714.
doi: 10.3389/fonc.2022.1023714

Look how far we have come: BREAST cancer detection education on the international stage

Phuong Dung (Yun) Trieu[1]*, Claudia R. Mello-Thoms[1,2], Melissa L. Barron[1] and Sarah J. Lewis[1]

[1]Discipline of Medical Imaging Sciences, School of Health Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia, [2]Department of Radiology, Carver College of Medicine, University of Iowa, Iowa City, IA, United States

The development of screening mammography over 30 years has remarkedly reduced breast cancer–associated mortality by 20%-30% through detection of small cancer lesions at early stages. Yet breast screening programmes may function differently in each nation depending on the incidence rate, national legislation, local health infrastructure and training opportunities including feedback on performance. Mammography has been the frontline breast cancer screening tool for several decades; however, it is estimated that there are 15% to 35% of cancers missed on screening which are owing to perceptual and decision-making errors by radiologists and other readers. Furthermore, mammography screening is not available in all countries and the increased speed in the number of new breast cancer cases among less developed countries exceeds that of the developed world in recent decades. Studies conducted through the BreastScreen Reader Assessment Strategy (BREAST) training tools for breast screening readers have documented benchmarking and significant variation in diagnostic performances in screening mammogram test sets in different countries. The performance of the radiologists from less well-established breast screening countries such as China, Mongolia and Vietnam were significant lower in detecting early-stage cancers than radiologists from developed countries such as Australia, USA, Singapore, Italy. Differences in breast features and cancer presentations, discrepancies in the level of experiences in reading screening mammograms, the availability of high-quality national breast screening program and breast image interpretation training courses between developed and less developed countries are likely to have impact on the variation of readers' performances. Hence dedicated education training programs with the ability to tailor to different reader cohorts and different population presentations are suggested to ameliorate challenges in exposure to a range of cancer cases and improve the interpretation skills of local radiologists. Findings from this review provide a good understanding of the radiologist' performances and their improvement using the education interventions, primarily the BREAST program, which has

been deployed in a large range of developing and developed countries in the last decade. Self-testing and immediate feedback loops have been shown to have important implications for benchmarking and improving the diagnostic accuracy in radiology worldwide for better breast cancer control.

KEYWORDS

breast cancer, screening mammography, diagnostic accuracy, training & development, early detection

Introduction

Breast cancer is classified as the most common malignancy and the leading cause of cancer-related morbidity and mortality for women over the world. It has become a severe health problem as accounting for a third of all new cancer cases diagnosed among females (1). With advances in technology attributing to earlier diagnoses, as well as changes in environmental and lifestyle factors, an increasing trend of breast cancer incidence has been observed from developed countries in North America, Europe and Australia, as well as in developing countries across the Pacific region and towards Asia and Africa (2). Although there has been an increase in the number of new breast cancer cases detected worldwide annually, the prevalence of this disease is relatively low in both high income and middle/low-income countries. The average risk of a Caucasian woman in the United States or Australia developing breast cancer in her lifetime is approximately 13%. This means there is a 1 in 8 chance a women will develop breast cancer (3). With Mongoloid and Negroid women originally from Asia and Africa, this rate is estimated lower at 10%-11%. Therefore, only a small number of cancer cases are detected regardless of the large number of breast screening cases performed each year.

Examining mammograms in a screening environment requires expertise in image interpretation as detecting small and early signs of cancer lesions is more complex than diagnosing cancer in patients presenting with advanced stages. Developed countries that have an established nationwide/population-based breast screening program include Australia (4), the Netherlands (5), the United Kingdom (6) and the United States of America (7). These countries have regulations that require radiologists and other reader types to participate in continuing medical education (CME) and training to maintain a high level of performance. For example, Australian radiologists who interpret screening mammograms are obliged to complete a 5-year registrar program that includes breast imaging interpretation curricula. Once registered, screening radiologists must read a minimum of 2000 screening mammograms per year, obtain at least 4 CME hours annually and participate in an audit once every 3 years (4). BREAST (Breastscreen REader Assessment Strategy) have been developed to help radiologists at all stages of their expertise development, from those who have minimal experience and less time dedicated to screening mammograms through to those who wish to continuously update and test their knowledge (8–10). BREAST provides radiologists and screen readers with the opportunity to self-assess and improve their diagnostic performance in a simulated but highly authentic environment. This article aims to review international trends in current breast cancer status and a review of published educational tools that specifically related to breast cancer detection *via* mammograms that are available across a range of countries. Through this, an assessment of the effectiveness of the BREAST interactive training programs to improve radiologists' diagnostic efficacy for early breast cancer detection is undertaken.

Breast cancer: Incidence and mortality rates

Breast cancer is the most common solid organ oncology presentation for women, with over 2.2 million new cancer cases worldwide in 2020, contributing to 24.5% of all cancer cases and almost 685,000 deaths, a 30% increase compared with the WHO statistics in 2012 (1, 11). Asian countries, representing 59.5% of the world's population, make up the largest component, with 45.4% of new cases and 50.5% of deaths related to breast cancer. European countries, with 11% of the population, stand second with 23.5% of new cases and 20.7% of deaths. Although North America and Oceania represent only 8% of the global population, they account for 13.6% of new breast cancer cases and 8% of patients who die from this disease (1) (Figure 1).

Data from GLOBOCAN (WHO) in 2020 show that age-standardized breast cancer incidence rate was highest in high income countries (HIC) in Europe (Belgium, Netherlands, Luxembourg, France, Denmark, Finland, UK, Italy), Northern America (US, Canada), Oceania (Australia, New Zealand) and Asia (Singapore, Japan), ranging from 75 to 113 cases per

100,000 women. Mortality rates peak in low-middle-income (LMICs) and low-income countries (LICs) in Latin America and Caribbean (Barbados, Bahamas), Africa (Jamaica, Nigeria, Namibia, Ethiopia), and Asian Oceania (Fiji, Papua New Guinea, Malaysia, Thailand, Vietnam) with the range from 20 to 42 deaths per 100,000 women (age-standardized) (1). The high incidence of breast cancer in high-income countries has been described as reflecting the increase in the accessibility of mammography screening programs and the prevalence of well-known breast cancer risk factors (e.g sedentary lifestyles, late reproductive records and being overweight after menopause) (12–17), while high mortality rates in LMICs and LICs were found to be associated with lack of access to quality health care and treatment (18–22).

The incidence of breast cancer has steadily increased by an average of 1.4% per year for all age groups since 1990, based on the published report by the World Bank involving 185 countries across seven regions (23). This increase took place in more than 60% of nations experiencing socio-economic turmoil (24), whilst the data indicated that incidence rates had stabilized in HICs such as Canada, Europe, Australia and New Zealand (25) whereas in the US, stabilisation has been shown for white women but the incidence rate continues to increase for black and Hispanic women (26). The growth in the incidence rate among LICs and MICs are primarily due to an increase in risk factors associated with urbanization, including adopting western diets, obesity, lack of physical activity, early menarche (before age 12 years), late menopause (after 55 years old), delayed childbirth (after 30 years old) and a decrease in the number of children and shorter breastfeeding periods (27–30). For example, the obesity ratio in Australia and New Zealand in 2016 was approximately one to three adults, whilst obesity prevalence in Bangladesh, India and Vietnam was recorded as below 4%. However, there was a surge of 28% in the obesity rate in LICs and MLICs in Asia Pacific region from 2010 to 2016, with the increase particularly high at 50% among adults from 1.4% to 2.1% in Vietnam and 3.5% to 5.3% in Laos (31). Improved access to family planning initiatives in conjunction with socioeconomic growth between 1990s and 2000s has also led to a significant drop in fertility rates in Latin America, Africa and Asia from 5 – 7 births (1970s-1980s) to 1.5 - 3 births per woman (32).

Mortality rates from breast cancer have reduced over time ranging from 0.55% to 1.75% (from 20-26 per 100,000 women in 1990 to 17 per 100,000 women in 2017) in most HICs in Europe, Central Asia and North America, however it is consistently high and rising in many LMICs and LICs (23). The morality reduction in HICs is likely due to increasing early cancer detection by screening mammography programs and modern treatment methods, although the impacts of treatment on each individual may differ as well as the participation rate for routine screening alongside the accessibility of effective treatment programs. Contrary to the downtrend recorded in HICs, the uptrend in breast cancer mortality has been reported in Asia, Latin America, and Africa (25) plus within population subgroups in some countries such as for black and Hispanic women in the US where the mortality rates are 28.4 per 100,000 women (26). A study comparing the data between 1990 and 2017 showed that the breast cancer mortality rate went up annually ranging from 0.36% per year in Middle East, North Africa to 0.56% in East Asia Pacific, Latin American Caribbean and Sub-Saharan Africa (23). It is described that the surge in breast cancer mortality in Japan that arose since the 1960s, is linked to the country undergoing a transition from a traditional Asian diet based on plant to a Western diet based on meat (this transition had occurred a decade earlier), which has been linked to the increase in obesity and overweight prevalence (33). Furthermore, even in some high-middle-income countries such as Malaysia and China, mammography screening has not yet been widely adopted at the population stage for various reasons such as sociocultural barriers, lack of equipment and clinician expertise and availability (34, 35).

The survival rate, which compares breast cancer mortality rates to incidence rates, was found to be lowest in less developed countries in Africa and South-Central Asia and highest in developed countries in North America, Europe and Oceania with the 5-year survival rate ranged from 53% in South Africa to 85% in Australia and 82% (Black women) and 92% white women in the US (26, 36). The low survival rate in low and middle-income countries highlights the fact that a large number of women were likely diagnosed in the late stages due to restricted or lack of screening programs and limited access to high-quality cancer treatment, in addition to insufficient staff and medical infrastructure including pathology services, radiotherapy units, and cancer treatment drugs (37). For example, in the period 2009 to 2010, over 75% of Nigerian breast cancer patients were detected with stage III or IV cancers (38), similarly to Vietnam, where 75% cancer cases were found to have local or distant metastasis (39). In contrast, high survival rates were observed in Northern America, Australia/New Zealand, Western and Northern Europe indicating low death rates in spite of high incidence rates as a consequence of early diagnosis and the availability of modern treatment methods (27). In HICs such as USA, Canada, UK, Australia and New Zealand, national breast screening programs are available, and women aged 50—75 are actively invited to have a free mammogram at set intervals, usually 1-3 years apart (40–42). Recalled women are frequently assessed with ultrasound, digital breast tomosynthesis or magnetic resonance imaging. However, optimal diagnostic and treatment methods for breast cancer are not commonly accessible in low-income populations. Efficient treatment is constrained by inadequate medical imaging equipment, including pathology and radiation therapy units and expensive cancer drugs (43). A systematic review highlighting radiotherapy capacity showed that there were more than 25 countries, mainly in Africa and Asia, that did not even have radiotherapy services

(44). The International Atomic Energy Agency (IAEA) has anticipated a shortage of at least 5000 radiotherapy machines in developing nations (45). There are other barriers such as religious beliefs, cultural beliefs, and shame associated with breast cancer and undertaking treatment (46).

Breast screening programs

An effective mammography screening program is a primary health service for detecting early abnormal lesions which will help to diminish the mortality risk from breast cancer for patients (40, 47). Digital mammography, with a considerably high specificity and sensitivity (over 90%) (48), is the main imaging tool used for breast cancer diagnosis and screening programs worldwide. Breast screening programs have been implementing for a number of decades in HICs with strong rates of successes. For instance, the UK has the National Health Service Breast Screening Programme which screened approximately 1.88 million women aged from 50 to 70 years old (73.4% participation rate from invitation) in 2010-2011, reported a cancer detection rate at 7.8 per 1000 women and 5-year survival for cancer patients of 85% (49). A similar result of the effectiveness of breast screening programs was found in Europe with a decrease of 25–30% breast cancer mortality for women between 50 and 74 years old. In Australia, the mortality rate has also decreased significantly since BreastScreen program began—from 74 deaths per 100,000 women in 1991 to less than 50 deaths per 100,000 since 2010 (40, 50). Overall, breast cancer screening recommendations are relatively similar across the HICs, with the most common age group targeted to be 50 to 70 years old for biannually screening. The American College of Radiology has the longest screening range, ranging from 45 to 75 years old with a suggestion for annual screening, whilst the UK has the longest screening interval time of 3 years (47).

The World Health Organization (WHO) estimates that up to 11 million cancer cases will be diagnosed in low- and middle-income countries by 2030, which is an 80% increase compared with 2008. By extrapolation, cancer will be the leading cause of death by the end of the 21st century and is predicted to be the greatest obstacle for advancing human life expectancy (51). Early detection of cancer is one way to prevent death. However, screening for early signs of illness in asymptomatic patients is performed much less frequently in LICs and MICs than in HICs. Apart from lack of infrastructure as mentioned above, differences in breast characteristics among women in various populations can also influence the effectiveness of breast screening programs. Compared to Caucasian women (American, European, or Oceanian), Asian women have low breast cancer rates despite generally having small, dense breasts, and the mean onset age of breast cancer for Asian women is around 40–50 years old, which is 10 years younger than that for Caucasian women (46, 52). It is possible that some of the differences in risk profiles between Western and Asian women is related to the structure and gene expression profile of the normal breast. For example, normal breast epithelium is much more likely to be ER-positive in Caucasian women than in Japanese women (53). In addition, breast size is a highly heritable trait, with a twin study estimating the heritability of bra cup size to be 56% (54). Several genome-wide association studies have also identified common genetic variants associated with breast size (55, 56). Asian women typically have smaller breasts than women of Caucasian ancestry. A large cohort of 24,353 Singaporean women showed that the average bust line and total breast area was 91.2 cm and 102.3 cm^2 (57) while the UK and Australian women were found with breast volumes calculated using the photograph-contours ranged from 90 to 1544 cm^3 (58). Several demographics, reproductive and lifestyle factors have been suggested to influence breast size, but most of these links are anecdotal in nature. Variables found to be significantly associated with bust line and total breast area included Body Mass Index (BMI), marital status, and working status. Age, ethnicity, and number of children were significant predictors of breast area, but not bust line (57).

Additionally, Asian women have comparatively denser parenchyma when compared to Caucasian women, which in turn is related to a reduce efficacy with mammography screening for early cancer detection (46, 59). For example, Maskarinec et al. investigated variations in mammography densities between Japanese, Chinese and Caucasian (US) women and found that both Japanese and Chinese women had an average of 15% smaller unadjusted dense area, yet the proportion of breast density tissues was 20% higher than in Caucasian women (60). However, many of these studies were conducted in the early days of mammography when radiographers had limited experience in mammographic positioning and the equipment such as the compression paddles were not as developed or of high quality compared to present day equipment and techniques. In a recent study of 28231 Singaporean women undergoing screening mammography, the authors reported that the range of mammographic abnormalities was similar to the findings in the Caucasian population (61).

In many Asian countries, especially LICs, ultrasound has been considered as a good alternative for mammography in breast cancer screening, because of its advantage in women with high dense breasts, wide accessible and low operating costs (62). Nevertheless, there are also drawbacks related to ultrasound such as its accuracy dependent on the skills of the probe operator, it is less adept at detecting calcifications and can produce a higher rate of false positives than mammography (62, 63). Whether mammography screening programs should be implemented more widely in certain populations or LIC/MIC countries is a challenging concept. With resource-restricted healthcare systems, most LIC/MIC nations consider that “awareness of breast disease” may be a priority before conducting extensive population-based screening (64). However, significant economic growth and social development

that has taken place in recent years, along with infrastructure and lifestyle changes, have led to many LICs and MICs to consider the introduction for formal mammography screening programs more widely.

There were six MIC countries (Russia, Brazil, Mexico, Uruguay, Hungary, Macedonia) which have nationwide or regional mammography screening programs with various recommendation for screening women from age 40 or 50 to 69 biennially. Screening participation rates in these countries, however, fluctuate considerably and is well below 70%, with modelling showing that a participation rate of 70% is optimal for breast cancer mortality reduction (65). Eight countries including South Africa, China, India, Indonesia, Colombia, Ukraine, Bulgaria, and Egypt conducted pilot studies to evaluate the diagnostic accuracy or cost effectiveness of a mammography screening program, which were aimed to notify policy makers practicability of a nationwide screening program. Yet the effectiveness of these pilots was not clear, so the implementation of national screening program is still on hold. One example was the trial from India which found that breast self-exam performed annually from age 40 to 60 had been almost as effective as biennial mammography screening in terms of reducing breast cancer mortality, while incurring only half of the total cost for a mammography screening program (66) suggesting that western mammography screening programs may not be cost-effective, especially given competing medical priorities and economic conditions. This may also be a feature of the population where higher breast density is documented for southern Asian/Indian women (Figure 2).

Other countries that have not published research on mammography screening occasionally provide population-based surveys for public awareness of breast cancer. In general, these surveys show that women in LICs are less aware of breast cancer and have been shown to have very low mammography utilization. For example, less than 20% of Iranian women have undertaken mammography (67). One survey in a less developed area of South Africa reported that no women at all have been screened using mammography (68). Although some countries have indicated their intention to introduce mammography screening programs, they are often referred to as "diagnostic mammography programs" after the mammogram has been identified as abnormal or after women who have experienced suspicious symptoms of breast cancer.

Breast screening reader training programs and BREAST

For a mammography screening program to succeed, diagnostic accuracy plays a vital role. In HICs, diagnostic efficacy of breast screening readers (radiologists, breast physicians or reporting radiographers) who interpret the mammograms is regularly monitored through clinical audit programs (69), so that readers with low performance levels can be identified and obtain further training. Nevertheless, most screen readers are exposed to low number of cancer cases in a clinical practice because of breast cancer's low incidence (approximately 8-15 cancers per 1000 screening women in HICs and even lower in MICs and LICs). Sensitivity and specificity are two of the most important parameters to assess the correct diagnosis of cancer and non-cancer in the population, however these metrics take time to collect due to screening intervals which range from 1-3 years in many established programs, and for *ad-hoc* screening, the interval period can be hugely variable. Realistically, clinical audit programs can take several years to collect sufficient data to classify reader performances against national standards. Once training programs are established, it may again take years for any progress in diagnostic performances to be identified.

In countries without a breast screening program, the radiologists are even less likely to be exposure to early breast cancer cases on mammograms and thus be unaware about their diagnostic performance due to the lack of clinical audit data. Fortunately, there is a high demand for assessment and training programs with immediate feedback to identify and improve low performance readers, and this leads to the introduction and implementation of mammogram test set innovations such as the Breastscreen REader Assessment STrategy (BREAST) (8–10), PERFORMS (70) and Detected-X (71). These are novel web-based training solutions which present radiologists, breast physicians and radiology trainees (also known as registrars) with high-quality test sets of challenging mammographic examinations (Full Field Digital Mammography (FFDM) or Digital Breast Tomosynthesis (DBT) to interpret, and then provide scores and instant feedback on their diagnostic performances at the end of the test set where overall metrics such as sensitivity, specificity and ROC AUC (Area Under the Receiver Operating Characteristic Curve) can be calculated (Figures 3, 4). Applying test sets to training platforms offers many benefits. Test sets could be arranged in such a way that the intervention is likely to explain for measured changes in diagnostic performances of radiologists. In addition, training sets are typically heavily enriched with pathology-proven cancer cases so have a much higher prevalence and results are almost immediately available to users.

Among a range of established education programs, BREAST has confirmed its usefulness and effectiveness through the largest number of publications in peer-reviewed journals with high impact factors. Between 2011 and 2021, BREAST has investigated diagnostic performances of radiologists, breast physicians and reporting radiographers in a variety of HIC, MIC and LIC countries with and without national breast screening programs including Australia, UK, Italy, Singapore, China, Mongolia, Iran and Vietnam *via* their mammogram-based test sets. The number of participants in the published studies have ranged from 10 to 117 and the number of mammographic cases included in test sets range from 35 to 60. Findings from studies show that radiologists from LIC countries with lack of national breast screening programs such as China, Mongolia, and Vietnam (72–74) displayed a significantly lower diagnostic accuracy in detecting cancer lesions on mammograms than radiologists from developed countries with

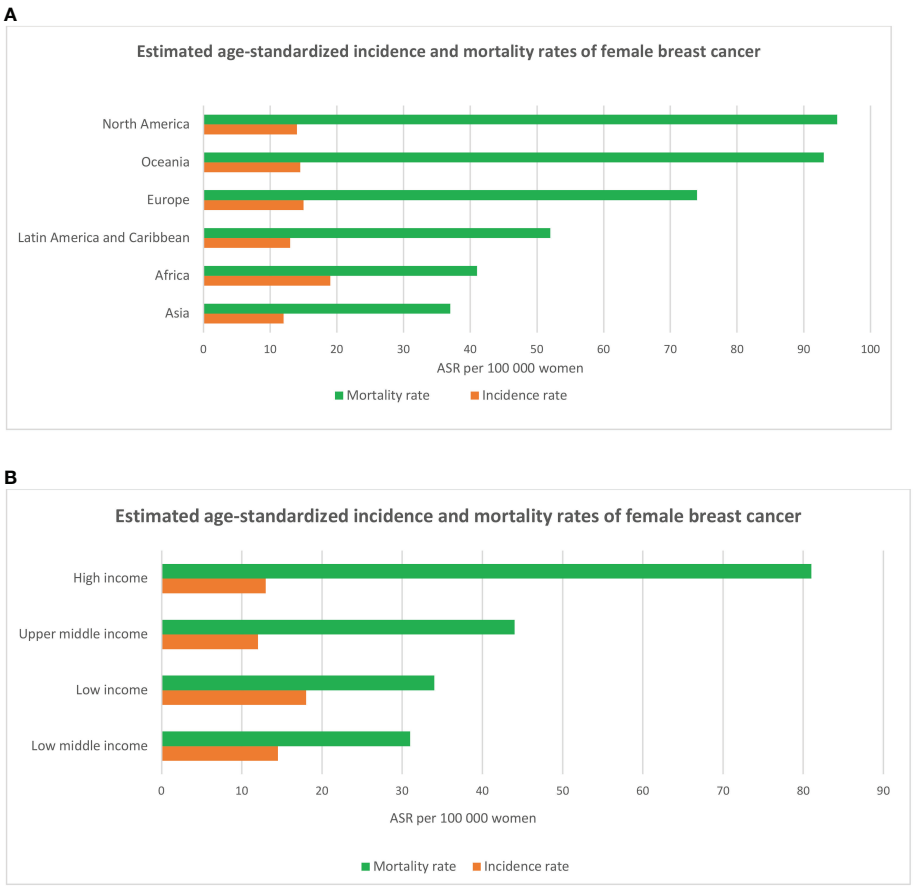


FIGURE 1
Estimated breast cancer age-standardized incidence and mortality rates across six continents **(A)** and in regions with different levels of income **(B)** according to the statistics of the International Agency for Research on Cancer (IARC - WHO) in 2020.

well-established breast screening programs such as Australia, UK, Italy, Singapore (73, 75, 76) (Figure 5). The average differences in the performances between the two groups of countries (LIC versus HIC) were 8% in specificity (0.78 vs 0.70), 12% in sensitivity (0.85 vs 0.73), 29% in cancer location sensitivity (0.76 vs 0.47), 11% in ROC AUC (Area under the ROC Curve) (0.87 vs 0.76) and 24% in JAFROC FOM (Jackknife free-response receiver operating characteristic – figure of merit) (0.75 vs 0.51). This difference was not only reported in digital mammogram test sets but also found in DBT test sets. For example, in a recent study, it was found that the false positive and false negative rates of Chinese radiologists reading the DBT test set *via* the BEAST platform was 52% and 69% compared with 36% and 35% in Australian radiologists (77). This large difference in cancer detection accuracy might imply that a great number of cancer cases could be missed or incorrectly reported in the clinical practices among MIC and LIC countries, which could have harmful implications for treatment outcomes of patients.

In addition, BREAST studies reported findings based on performances of radiologists from different countries in reading mammograms with different level of breast density and the ability to detect various types of cancer appearances. The most challenging type of cancer lesions to detect on mammograms for LIC radiologists were small lesions such as stellate/spiculated masses along with architectural distortions (the missed rate was 55%-75%) (77, 78), while discrete masses and asymmetric density (or non-specific density) were more likely to be missed (31%-37%) or rated as equivocal (47%-50%) by HIC radiologists (77, 79). This is in line with findings from the PERFORMS program where well-defined masses and asymmetric density accounted for the highest percentage of incorrectly diagnosed cases (25%) among UK radiologists (70). This difference could be related to a large proportion of breast cancer patients in LICs in Asia that present with advanced stages compared with women in HICs. Studies in China, Taiwan, India, Vietnam (LICs and MLICs) demonstrated that the proportion of breast cancer patients with local and distant metastasis were 55% to 85% while this rate in Japan and South Korea were 40-45%, and 28%-35% in Australia, Europe, Canada and USA (59) (39, 46). Hence, radiologists in LICs and MICs with very limited breast screening

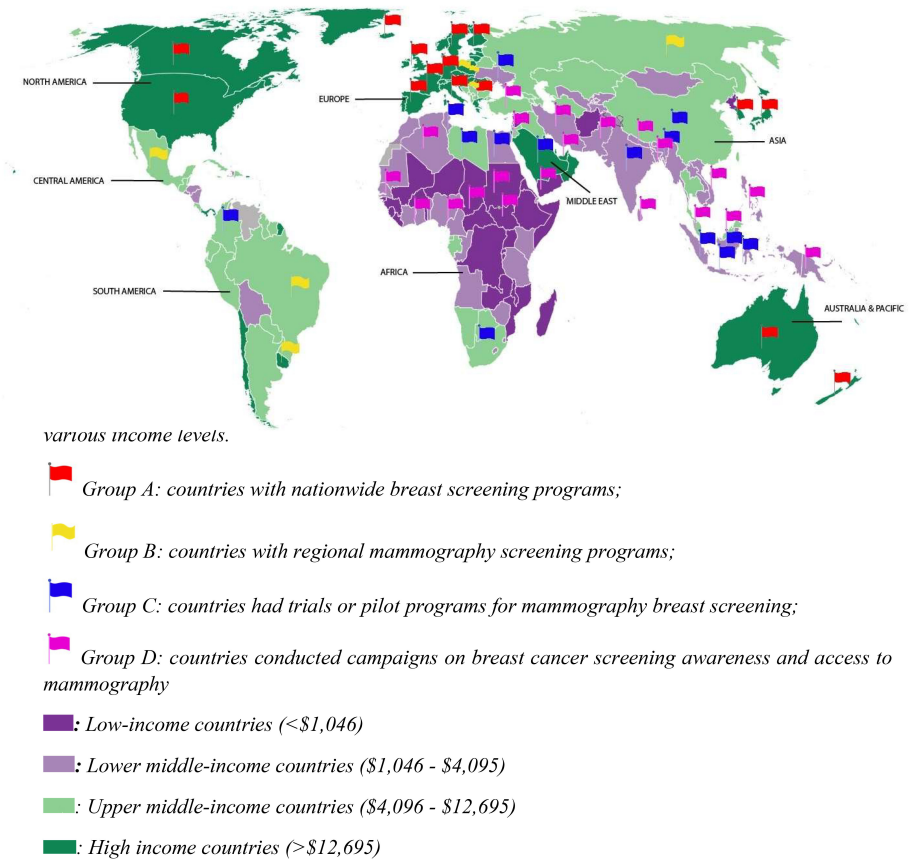


FIGURE 2
Distribution of mammography screening programs across continents and countries with various income levels.

abilities may not be accustomed to recalling women with small lesions or detect early cancers such as stellate lesions.

Furthermore, BREAST data has shown that Asian radiologists were more likely to achieve higher diagnostic accuracy when reading high density mammograms than mammograms with low breast density compared with their counterparts in Westernized countries. This discrepancy could be explained by the fact that Asian women tend to have smaller and higher dense breasts when viewed on mammography than Western/Caucasian women with the odds ratio for women with dense breasts versus fatty breasts increasing from 1.2 for women aged less than 45 to 1.6 for women over 65 years old according to a study of over 28,000 women of different races in the United States (80). Similarly, studies in Asian populations also support this finding with approximately 70% of mammograms in Vietnam demonstrating high breast density (81) and Chinese women had 10% higher breast density rates than Australian women (82). Thus, Asian radiologists are more likely to encounter high breast density mammographic cases compared to radiologists in Western countries where more women with low dense breasts reside.

The low level of diagnostic accuracy of the radiologists from countries with lack of breast screening programs compared with those that interpret screening cases regularly can be explained partly by the difference in expertise levels. In BREAST studies, the majority of radiologists (56%-82%) from MICs and LICs (China, Mongolia and Vietnam) reported that they read equal to or less than 20 mammograms per week whilst more than 65% of radiologists in HICs (Australia, Singapore) stated they read more than 20 cases per week. When breast screening experts interpret a mammogram, they will firstly extract information from an initial global impression, which requires a solid knowledge (also known as a memory schema) of what is normal anatomical breast features in order to differentiate abnormalities. This type of skill requires breast image readers to have considerable experience that can be achieved through conducting minimum annual readings facilitated by an active screening program (83, 84).

The number of cases read per year has been shown to be an essential component of high diagnostic performances (85). National accreditation standards in HICs such as Australia and the UK require between 2000 and 5000 reads per year (84) whereas it is lower at 960 cases every 2 years in the US. While effective training and ongoing clinical practice can develop mammographic interpretation skills, it is difficult for radiologists from LICs and

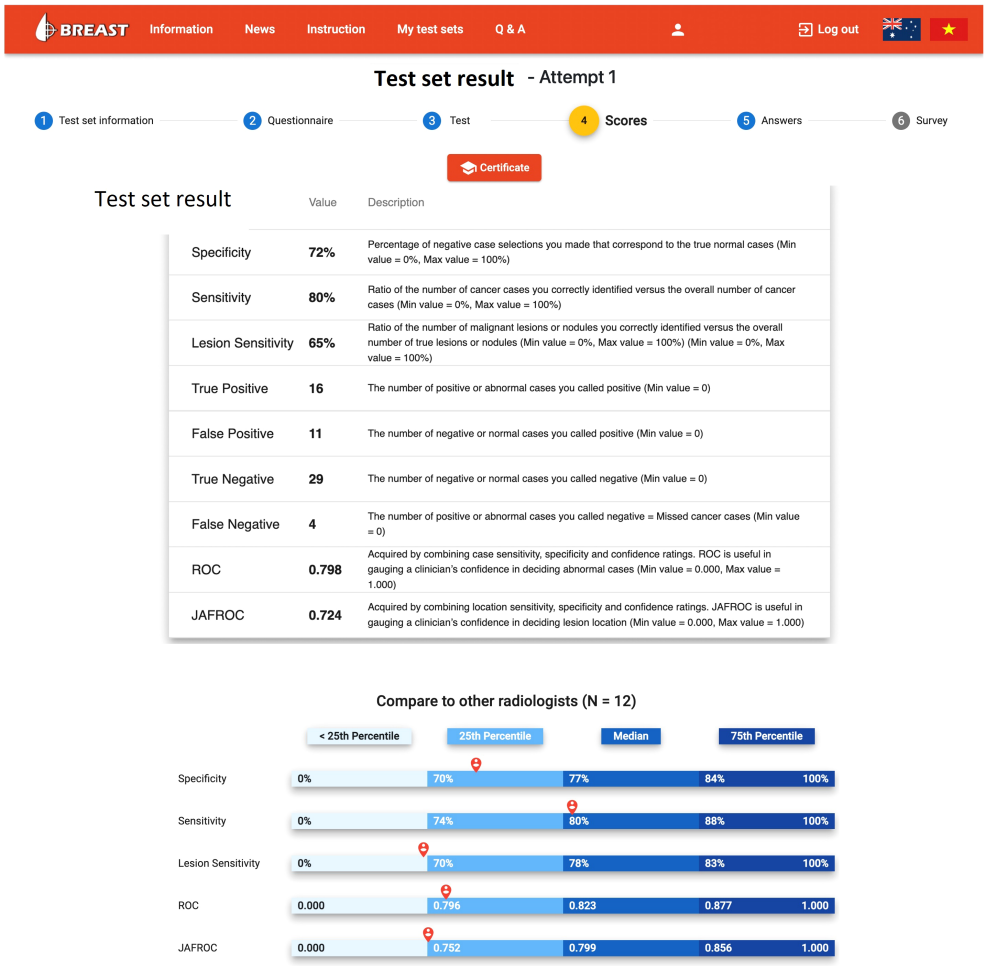


FIGURE 3
The diagnostic report on the BREAST platform to a radiologist when a mammogram test set is completed (www.breastaustralia.com).

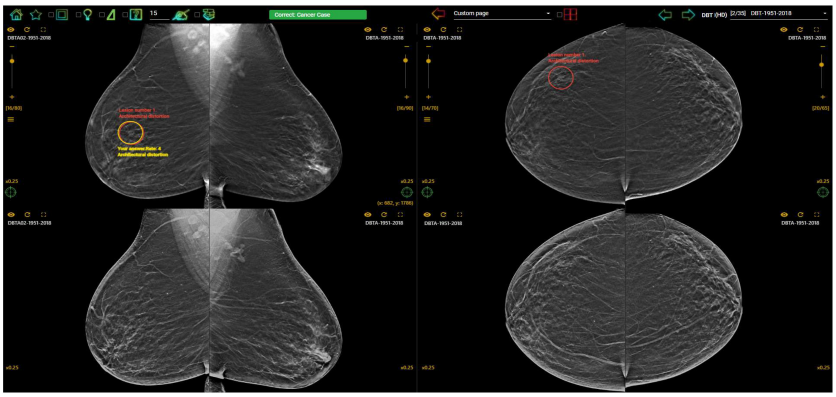


FIGURE 4
Feedback on the BREAST platform for a DBT case interpreted by a radiologist with a correct cancer location detection on the DBT slice (red circle (truth) and yellow circle (user's marking) were overlapped) on RMLO (Right Mediolateral Oblique) view and a missed cancer location (red circle) on RCC (Right Craniocaudal) view. The first row displayed DBT images and synthesized views were shown on the second row (www.breastaustralia.com).

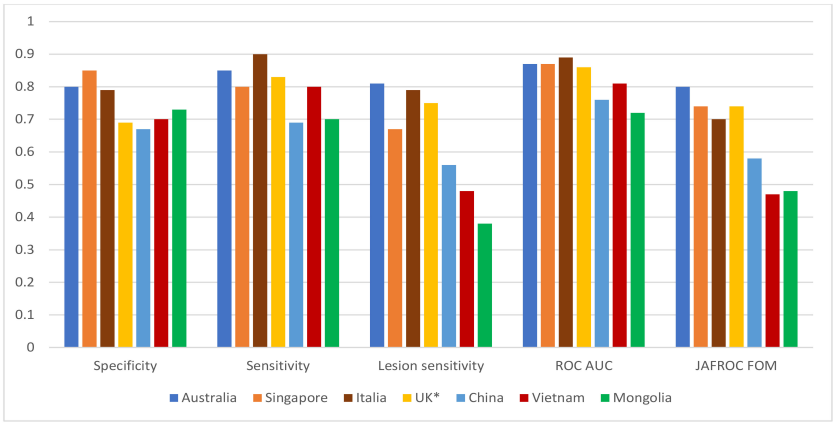


FIGURE 5
Diagnostic performances of radiologists in different countries in full-field digital mammogram BREAST test sets. *: Reporting radiographers.

MICs to achieve adequate experience in interpreting mammograms without an effective feedback loop that shows errors, successes and can offer both immediate and comprehensive feedback so that learning takes place at the point of self-testing of performance. Furthermore MIC and LIC countries tend to have shorter radiology training periods when compared to HIC such as Australia which has a 5 year radiology training period (74) (86, 87). The variation of diagnostic errors among radiologists between HICs and MICs/LICs highlights the need for effective education and training strategies tailored to better suit with local clinicians to enhance breast cancer diagnostic efficiency. One approach that could help radiologists with low levels of experience or less access to mammographic caseloads is building online interactive training platforms similar to BREAST as a continuing professional development activity. The BREAST platform currently provides the users with access to the mammogram test sets (both FFDM and DBT) at the same quality as DICOM images directly from the BREAST platform or through the PACS (Picture Archiving and Communication System). Studies have shown reasonable levels of agreement between diagnostic performances of radiologists in clinical reporting and their performance in test set environments in mammogram interpretation (69) and the use of training test sets is likely to improve diagnostic skills of radiologists in identifying abnormal lesions on screening mammograms and consequently improve patient health outcome.

The BREAST test sets, which can be made available through *via* the online platform or through workshops appended to scientific meetings/conferences has been used as an official training tool for BreastScreen Australia and BreastScreen New Zealand readers for more than a decade. Previous studies provide evidence that BREAST test sets have a positive effect on diagnostic efficacy of radiology fellows as a part of the quality assurance module of the national breast cancer screening program in Australia. Results show that the lesion sensitivity of readers recorded an increase from 20% to 31% among radiologists who read BREAST test sets regularly and this improvement was recorded in 83% of radiologists and an extraordinary 100% in radiology trainees (9). Recently, Qenam et al. (2022) reported a positive association of the improvement in positive predictive value and specificity of Australian radiologists through BREAST test sets with their diagnostic enhancement in clinical audits, further supporting the need for online educational tools like BREAST to exist (88).

Training test sets, *via* the online BREAST platform, have also been used to improve the diagnostic accuracy of radiologists from LICs. As a national example, a number of studies have been undertaken to map radiologists' performances in reading mammograms in Vietnam (73, 89), where initial benchmarking reported that the detection of spiculated masses and stellate lesions by Vietnamese radiologists was significantly lower than calcification, discrete mass or asymmetric density (90). Therefore, Vietnamese radiologists were provided tailored BREAST training sets designed to focus on the type of lesions that they missed, with a similar level of difficulty as the pre-test set. Results showed significant improvement in diagnostic accuracy of radiologists in Vietnam, with an increase of 20.6% in the detection of stellate/spiculated mass after dedicated the training test set (90). This indicates that the cancer detection of radiologists on mammograms from less developed countries can be improved with an appropriate training intervention after areas for improvement have been mapped.

Limitations and future opportunities

The results discussed here in relation to the BREAST program have some limitations. The majority of the test set images come from the BreastScreen Australia digital library and hence represent

women that attend screening in Australia. The population with the highest participation in BreastScreen Australia is White women although Australia is a very multi-cultural country with a large migrant population from Europe and Asia, and with one in four women attending being born overseas. A small number of test sets available through the BREAST program do include images from local populations where agreements have been secured with other national institutions (such as images from Vietnam for Vietnamese test results and from Iran for Iranian results). Furthermore, a number of test sets are engineered to simulate diversified populations, such as the high-density test sets which are curated using BSA images but include women who have greater than 50% mammographic breast density. In this case, BREAST has used this collection of cases to represent an Asian population and it should be acknowledged that there are a number of limitations with this approach. The greatest authenticity comes from case collection from local populations and tested with local clinicians and education enterprises such as BREAST and others need to strive to work collaboratively with different countries and organisations to create an international radiology education community that works together yet is culturally and diversely appropriate.

Although there are obvious advantages to this online training method *via* the use of test sets, several limitations must be taken into account to consider future development. Firstly, the performances of readers were evaluated based on the gold standard set by a panel of clinical experts who curated the test sets and this is further correlated by histopathology results. However, within test sets that are designed to be completed within a reasonable timeframe for concentration, completion and feedback, there is naturally a limited number and variety of cases in the test sets which may not represent all scenarios in the screening environment. Furthermore, using the test set method might have a psychological or social desirability effect as participants are aware of being tested, and they might increase their recall rate in an attempt to maximize sensitivity. In addition, although performance within BREAST test sets do show good correlation to clinical performance, there remains the scenario that client/patient care is not affected by the choices they make within self-assessment modules or tests. Thus, the purposes of BREAST test sets are to increase diagnostic efficacy though practice, targeted learning objectives, feedback and reflection. Therefore test sets need to be incorporated into a holistic and multidisciplinary educational regime to improve expertise that also includes other documented links to improved performance, such as building a broad social learning network and participation in multidisciplinary team activities (91, 92).

Further work is needed to fully understand the precise mechanism behind the findings of why radiologists in different countries have varying performance in detecting specific types of abnormal lesions on mammograms and how clinical variability can be reduced. The power of a global breast cancer detection community that builds expertise by sharing resources from one country to another needs to continue and BREAST has achieved strong results leveraging from investment by the Australian government and assisting with mapping and improving cancer detection *via* test sets in other HIC, MIC and LIC countries. Future research and shared education might involve multi-ethnicity mammograms and eye-tracking or brain tracking technology to trace how radiologists detect lesions on mammograms so that an understanding of decision-making errors can be included. Additionally, BREAST focuses on the use of screening cases as it is intrinsically linked to the national program BreastScreen Australia, and extension of interactive learning environments is very feasible to include other imaging modalities and scenarios, such as ultrasound, MRI and contrast enhanced mammography as well as the use of artificial intelligence to predict reader error and provide personalised test sets.

In conclusion, this review showed that there was significant variation in diagnostic performances in screening mammogram test sets in different countries. Difference in breast features, discrepancies in mammogram reading experiences, the availability of high-quality national breast screening program and breast image interpretation training courses between developed and less developed countries are likely to have an impact on the variation of readers' performances. The online educational and training methods using real-life clinical cases *via* test sets like BREAST which were shown to improve the diagnostic performances of radiologists and radiology trainees are significantly helpful to radiologists and breast image readers in different countries with and without breast screening programs in improving their diagnostic accuracy in mammogram interpretation, especially when cancer incidence rates and population demand for advanced medical imaging methods continues to rise.

Author contributions

All authors contributed to the review conception and design. Material preparation and the first draft was performed and prepared by PT and SL. The final manuscript was written and reviewed by PT, SL, MB and CM-T. All authors read and approved the manuscript.

Funding

This work has been supported through the BREAST research grant funded by Australia Department of Health.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Youlden DR, Cramb SM, Yip CH, Baade PD. Incidence and mortality of female breast cancer in the Asia-pacific region. *Cancer Biol Med* (2014) 11(2):101–15. doi: 10.7497/j.issn.2095-3941.2014.02.005

3. Howlader NN. *Breast: cancer risk from birth over time, 2016-2018, by risk type, female, all races (includes Hispanic).* (Tennessee: Institute NC) (2022). AM; Krapcho, M. SEER*Explorer.

4. Breast Screen Australia National Accreditation Standards *BreastScreen Australia quality improvement program.* (Tasmania, Australia) (2008).

5. Dutch-Expert-Centre-for-Screening *Kwaliteitsregister voor screeningsradiologen in het bevolkingsonderzoek op borstkanker in nederland.* (2018), Quality register for screening radiologists of the breast cancer screening in the Netherlands:Bilthoven, The Netherlands.

6. NHSBSP *Quality assurance guidelines for breast cancer screening radiology.* UK: NHS Cancer Screening Programmes (2011).

7. Destouet JM, Bassett LW, Yaffe MJ, Butler PF, Wilcox PA. The ACR's mammography accreditation program: ten years of experience since MQSA. *J Am Coll Radiol JACR* (2005) 2(7):585–94. doi: 10.1016/j.jacr.2004.12.005

8. Brennan PC, Trieu PD, Tapia K, Ryan J, Mello-Thoms C, Lee W. BREAST: A novel strategy to improve the detection of breast cancer. In: Hiroshi Fujita TH, Muramatsu C, editors. *Lecture notes in computer science - the 12th international workshop on breast imaging*, (Gifu, Japan: Springer) (2014). 5839 :438–43.

9. Trieu PDY, Tapia K, Frazer H, Lee W, Brennan P. Improvement of cancer detection on mammograms *via* BREAST test sets. *Acad radiol* (2019) 26(12):e341–e7. doi: 10.1016/j.acra.2018.12.017

10. Lewis SJ, Borecky N, Li T, Barron ML, Brennan P, Trieu PD. Radiologist self-training: a study of cancer detection when reading mammograms at work clinics or workshops. *J Cancer Educ* (2022) 5:1–7. doi: 10.1007/s13187-022-02156-w

11. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* (2015) 136(5):E359–86. doi: 10.1002/ijc.29210

12. Hirose K, Hamajima N, Takezaki T, Inoue M, Kuroishi T. Association of family history and other risk factors with breast cancer risk among Japanese pre-menopausal and post-menopausal women. *Cancer Causes Control* (2001) 12:349–58. doi: 10.1023/A:1011232602348

13. Tung HT, Tsukuma H, Tanaka H, Kinoshita N, Koyama Y, Ajiki W, et al. Risk factors for breast cancer in Japan, with special attention to anthropometric measurements and reproductive history. *Japanese J Clin Oncol* (1999) 29(3):137–46. doi: 10.1093/jjco/29.3.137

14. McCredie M, Paul C, Skegg DC, Williams S. Reproductive factors and breast cancer in new Zealand. *Int J Cancer* (1998) 76(2):182–8. doi: 10.1002/(SICI)1097-0215(19980413)76:2<182::AID-IJC3>3.0.CO;2-T

15. Mizota Y, Yamamoto S. Prevalence of breast cancer risk factors in Japan. *Japanese J Clin Oncol* (2012) 42(11):1008–12. doi: 10.1093/jjco/hys144

16. Yoo KY, Kim Y, Park SK, Kang D. Lifestyle, genetic susceptibility and future trends of breast cancer in Korea. *Asian Pac J Cancer Prev* (2006) 7(4):679–82.

17. Protani M, Page A, Taylor R, Glazebrook R, Lahmann PH, Branch E, et al. Breast cancer risk factors in Queensland women attending population-based mammography screening. *Maturitas* (2012) 71(3):279–86. doi: 10.1016/j.maturitas.2011.12.008

18. Trieu PD, Brennan P, Giuffre B, Mello-Thoms C, Tapia K, Santangelo N, et al. Evaluation of the effect of zoom function on lesion detection by soft-copy reading of screening mammograms. *J Med Imaging Radiat Oncol* (2015) 59(3):292–9. doi: 10.1111/1754-9485.12298

19. Xu YL, Sun Q, Shan GL, Zhang J, Liao HB, Li SY, et al. A case-control study on risk factors of breast cancer in China. *Arch Med Sci AMS.* (2012) 8(2):303–9. doi: 10.5114/aoms.2012.28558

20. Gao YT, Shu XO, Dai Q, Potter JD, Brinton LA, Wen W, et al. Association of menstrual and reproductive factors with breast cancer risk: results from the shanghai breast cancer study. *Int J Cancer* (2000) 87(2):295–300. doi: 10.1002/1097-0215(20000715)87:2<295::AID-IJC23>3.0.CO;2-7

21. Pathy NB, Yip CH, Taib NA, Hartman M, Saxena N, Iau P, et al. Breast cancer in a multi-ethnic Asian setting: results from the Singapore-Malaysia hospital-based breast cancer registry. *Breast* (2011) 20 Suppl 2:S75–80. doi: 10.1016/j.breast.2013.02.011

22. Sighoko D, Kamate B, Traore C, Malle B, Coulibaly B, Karidiatou A, et al. Breast cancer in pre-menopausal women in West Africa: analysis of temporal trends and evaluation of risk factors associated with reproductive life. *Breast* (2013) 22(5):828–35. doi: 10.1016/j.breast.2013.02.011

23. Lima SM, Kehm RD, Terry MB. Global breast cancer incidence and mortality trends by region, age-groups, and fertility patterns. *EClinicalMedicine* (2021) 38:100985. doi: 10.1016/j.eclinm.2021.100985

24. Heer E, Harper A, Escandor N, Sung H, McCormack V, Fidler-Benaoudia MM. Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study. *Lancet Glob Health* (2020) 8(8):e1027–e37. doi: 10.1016/S2214-109X(20)30215-1

25. DeSantis CE, Bray F, Ferlay J, Lortet-Tieulent J, Anderson BO, Jemal A. International variation in female breast cancer incidence and mortality rates. *Cancer epidemiol Biomarkers Prev Publ Am Assoc Cancer Research cosponsored by Am Soc Prev Oncol* (2015) 24(10):1495–506. doi: 10.1158/1055-9965.EPI-15-0535

26. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA: Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708

27. Youlden DR, Cramb SM, Dunn NA, Muller JM, Pyke CM, Baade PD. The descriptive epidemiology of female breast cancer: an international comparison of screening, incidence, survival and mortality. *Cancer Epidemiol* (2012) 36(3):237–48. doi: 10.1016/j.canep.2012.02.007

28. Boyle P, Howell A. The globalisation of breast cancer. *Breast Cancer Res BCR.* (2010) 12 Suppl 4:S7. doi: 10.1186/bcr2736

29. Porter P. "Westernizing" women's risks? breast cancer in lower-income countries. *New Engl J Med* (2008) 358(3):213–6. doi: 10.1056/NEJMp0708307

30. Shin HR, Joubert C, Boniol M, Hery C, Ahn SH, Won YJ, et al. Recent trends and patterns in breast cancer incidence among Eastern and southeastern Asian women. *Cancer Causes Control* (2010) 21(11):1777–85. doi: 10.1007/s10552-010-9604-8

31. WHO (World-Health-Organization). (2020). Obesity and overweight. Retrieved from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

32. Bongaarts J, Sinding S. Population policy in transition in the developing world. *Science* (2011) 333(6042):574–6. doi: 10.1126/science.1207558

33. Zhang J, Dhakal IB, Zhao Z, Li L. Trends in mortality from cancers of the breast, colon, prostate, esophagus, and stomach in East Asia: role of nutrition transition. *Eur J Cancer Prev Off J Eur Cancer Prev Organisation* (2012) 21(5):480–9. doi: 10.1097/CEJ.0b013e328351c732

34. Dahlui M, Gan DE, Taib NA, Lim JN. Breast screening and health issues among rural females in Malaysia: how much do they know and practice? *Prev Med* (2013) 57 Suppl:S18–20. doi: 10.1016/j.ypmed.2012.12.010

35. Satoh M, Sato N. Relationship of attitudes toward uncertainty and preventive health behaviors with breast cancer screening participation. *BMC women's Health* (2021) 21(1):171. doi: 10.1186/s12905-021-01317-1

36. Allemani C, Weir HK, Carreira H, Harewood R, Spika D, Wang XS, et al. Global surveillance of cancer survival 1995-2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). *Lancet* (2015) 385(9972):977–1010. doi: 10.1016/S0140-6736(14)62038-9

37. Anderson BO, Yip CH, Smith RA, Shyyan R, Sener SF, Eniu A, et al. Guideline implementation for breast healthcare in low-income and middle-income countries: overview of the breast health global initiative global summit 2007. *Cancer* (2008) 113(8 Suppl):2221–43. doi: 10.1002/cncr.23844

38. Unger-Saldana K. Challenges to the early diagnosis and treatment of breast cancer in developing countries. *World J Clin Oncol* (2014) 5(3):465–77. doi: 10.5306/wjco.v5.i3.465

39. Trieu PD, Mello-Thoms C, Brennan PC. Female breast cancer in Vietnam: a comparison across Asian specific regions. *Cancer Biol Med* (2015) 12(3):238–45. doi: 10.7497/j.issn.2095-3941.2015.0034

40. Peintinger F. National breast screening programs across Europe. *Breast Care (Basel)* (2019) 14(6):354–8. doi: 10.1159/000503715

41. Domingo L, Hofvind S, Hubbard RA, Roman M, Benkeser D, Sala M, et al. Cross-national comparison of screening mammography accuracy measures in U.S., Norway, and Spain. *Eur Radiol* (2016) 26(8):2520–8. doi: 10.1007/s00330-015-4074-8

42. Taylor R, Morrell S, Estoesta J, Brassil A. Mammography screening and breast cancer mortality in new south Wales, Australia. *Cancer Causes Control* (2004) 15(6):543–50. doi: 10.1023/B:CACO.0000036153.95908.f2

43. El Saghir NS, Adebamowo CA, Anderson BO, Carlson RW, Bird PA, Corbex M, et al. Breast cancer management in low resource countries (LRCs): consensus statement from the breast health global initiative. *Breast* (2011) 20 Suppl 2:S3–11. doi: 10.1016/j.breast.2011.02.006

44. Grover S, Xu MJ, Yeager A, Rosman L, Groen RS, Chackungal S, et al. A systematic review of radiotherapy capacity in low- and middle-income countries. *Front Oncol* (2014) 4:380. doi: 10.3389/fonc.2014.00380

45. IAEA (International Atomic Energy Agency). (2017). Radiotherapy in Cancer Care: Facing The Global Challenge. Retrieved from Vienna: https://www-pub.iaea.org/MTCD/Publications/PDF/P1638_web.pdf

46. Leong SPL, Shen ZZ, Liu TJ, Agarwal G, Tajima T, Paik NS, et al. Is breast cancer the same disease in Asian and Western countries? *World J Surg* (2010) 34 (10):2308–24. doi: 10.1007/s00268-010-0683-1

47. Ebell MH, Thai TN, Royalty KJ. Cancer screening recommendations: an international comparison of high income countries. *Public Health Rev* (2018) 39:7. doi: 10.1186/s40985-018-0080-0

48. Kemp Jacobsen K, O'Meara ES, Key D, SMB D, Kerlikowske K, Vejborg I, et al. Comparing sensitivity and specificity of screening mammography in the united states and Denmark. *Int J Cancer* (2015) 137(9):2198–207. doi: 10.1002/ijc.29593

49. NHSBSP *Breast disease management: A multidisciplinary manual*. (Oxford: Oxford University Press) (2014).

50. AIHW BreastScreen Australia monitoring report 2021. In: *Welfare AIoHa*. Canberra: Australian Government (2021).

51. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin* (2018) 68(6):394–424. doi: 10.3322/caac.21492

52. Rajaram N, Mariapun S, Eriksson M, Tapia J, Kwan PY, Ho WK, et al. Differences in mammographic density between Asian and Caucasian populations: a comparative analysis. *Breast Cancer Res Treat* (2017) 161(2):353–62. doi: 10.1007/s10549-016-4054-y

53. Lawson JS, Field AS, Champion S, Tran D, Ishikura H, Trichopoulos D. Low oestrogen receptor alpha expression in normal breast tissue underlies low breast cancer incidence in Japan. *Lancet* (1999) 354(9192):1787–8. doi: 10.1016/S0140-6736(99)04936-3

54. Wade TD, Zhu G, Martin NG. Body mass index and breast size in women: same or different genes? *Twin Res Hum Genet* (2010) 13(5):450–4. doi: 10.1375/twin.13.5.450

55. Eriksson N, Benton GM, Do CB, Kiefer AK, Mountain JL, Hinds DA, et al. Genetic variants associated with breast size also influence breast cancer risk. *BMC Med Genet* (2012) 13:53. doi: 10.1186/1471-2350-13-53

56. Li J, Foo JN, Schoof N, Varghese JS, Fernandez-Navarro P, Gierach GL, et al. Large-Scale genotyping identifies a new locus at 22q13.2 associated with female breast size. *J Med Genet* (2013) 50(10):666–73. doi: 10.1136/jmedgenet-2013-101708

57. Lim LY, Ho PJ, Liu J, Chay WY, Tan MH, Hartman M, et al. Determinants of breast size in Asian women. *Sci Rep* (2018) 8(1):1201. doi: 10.1038/s41598-018-19437-4

58. Dimmock M, McKinley J, Massey A, Hausermann D, Tam N, Stewart E, et al. Designing a breast support device for phase contrast tomographic imaging: getting ready for a clinical trial. *Br J Radiol* (2022) 95(1138):20211243. doi: 10.1259/bjr.20211243

59. Bhoo-Pathy N, Yip CH, Hartman M, Uiterwaal CS, Devi BC, Peeters PH, et al. Breast cancer research in Asia: adopt or adapt Western knowledge? *Eur J Cancer* (2013) 49(3):703–9. doi: 10.1016/j.ejca.2012.09.014

60. Maskarinec G, Meng L, Ursin G. Ethnic differences in mammographic densities. *Int J Epidemiol* (2001) 30(5):959–65. doi: 10.1093/ije/30.5.959

61. Sng KW, Ng EH, Ng FC, Tan PH, Low SC, Chiang G, et al. Spectrum of abnormal mammographic findings and their predictive value for malignancy in Singaporean women from a population screening trial. *Ann Acad Med Singap* (2000) 29(4):457–62.

62. Nothacker M, Duda V, Hahn M, Warm M, Degenhardt F, Madjar H, et al. Early detection of breast cancer: benefits and risks of supplemental breast ultrasound in asymptomatic women with mammographically dense breast tissue. *A systematic review BMC cancer* (2009) 9:335. doi: 10.1186/1471-2407-9-335

63. Sohn YM, Kim MJ, Kwak JY, Moon HJ, Kim SJ, Kim EK. Breast ultrasonography in young Asian women: analyses of BI-RADS final assessment category according to symptoms. *Acta Radiol* (2011) 52(1):35–40. doi: 10.1258/ar.2010.100250

64. Harford JB. Breast-cancer early detection in low-income and middle-income countries: do what you can versus one size fits all. *Lancet Oncol* (2011) 12(3):306–12. doi: 10.1016/S1470-2045(10)70273-4

65. WHO *Guide for effective programmes; cancer control: knowledge into action; module 4: palliative care*. Geneva: Organization WH (2008).

66. Okonkwo QL, Draisma G, der Kinderen A, Brown ML, de Koning HJ. Breast cancer screening policies in developing countries: a cost-effectiveness analysis for India. *J Natl Cancer Institute* (2008) 100(18):1290–300. doi: 10.1093/jnci/djn292

67. Kadivar M, Joolaee S, Joulaee A, Bahrani N, Hosseini N. Breast cancer knowledge, attitudes and screening behaviors in two groups of Iranian women: physicians and non-health care personnel. *J Cancer Educ* (2012) 27(4):770–3. doi: 10.1007/s13187-012-0386-4

68. Maree J, Wright S, Lu X. Breast cancer risks and screening practices among women living in a resource poor community in tshwane, south Africa. *Breast J* (2013) 19(4):453–4. doi: 10.1111/tbj.12143

69. Soh BP, Lee W, McEntee MF, Kench PL, Reed WM, Heard R, et al. Screening mammography: test set data can reasonably describe actual clinical reporting. *Radiology* (2013) 268(1):46–53. doi: 10.1148/radiol.13122399

70. Scott HJ, Gale AG. Breast screening: PERFORMS identifies key mammographic training needs. *Br J radiol* (2006) 79:S127–33. doi: 10.1259/bjr/25049149

71. Suleiman ME, Rickard M, Brennan PC. Perfecting detection through education. *Radiog (Lond)* (2020) 26(2):S49–53. doi: 10.1016/j.radi.2020.06.006

72. Li T, Taba ST, Khong PL, Tan TX, Trieu PDY, Chan E, et al. Reading high breast density mammograms: Differences in diagnostic performance between radiologists from Hong Kong SAR/Guangdong province in China and Australia. *Asian Pac J Cancer Prev* (2020) 21(9):2623–9. doi: 10.31557/APJCP.2020.21.9.2623

73. Jackson RL, Double CR, Munro HJ, Lynch J, Tapia KA, Trieu PD, et al. Breast cancer diagnostic efficacy in a developing south-East Asian country. *Asian Pac J Cancer Prev* (2019) 20(3):727–31. doi: 10.31557/APJCP.2019.20.3.727

74. Demchig D, Mello-Thoms C, Lee WB, Khurelsukh K, Ramish A, Brennan PC. Mammographic detection of breast cancer in a non-screening country. *Br J Radiol* (2018) 91(1091):20180071. doi: 10.1259/bjr.20180071

75. Brennan ACR V, Cook N, Dean K, Dryburgh S, Lowe H, Mahon C, et al. A cohort of Italian radiologists match international counterparts in breast cancer detection efficacy. *J Radiol Review*. (2020) 7:21–5. doi: 10.23736/S2723-9284.20.00254-5

76. Williams S, Aksoy U, Reed W, Cielecki L, Woznitza N. Digital mammographic interpretation by UK radiographer mammographers: A JAFROC analysis of observer performance. *Radiog (Lond)*. (2021) 27(3):915–9. doi: 10.1016/j.radi.2021.02.015

77. Li T, Gandomkar Z, Trieu PDY, Lewis SJ, Brennan PC. Differences in lesion interpretation between radiologists in two countries: Lessons from a digital breast tomosynthesis training test set. *Asia Pac J Clin Oncol* (2022) 18(4):441–7. doi: 10.1111/ajco.13686

78. Trieu PDY, Puslednik L, Colley B, Brennan A, Rodriguez VC, Cook N, et al. Interpretative characteristics and case features associated with the performances of radiologists in reading mammograms: A study from a non-screening population in Asia. *Asia Pac J Clin Oncol* (2021) 17(1):139–48. doi: 10.1111/ajco.13429

79. Trieu PDY, Lewis SJ, Li T, Ho K, Tapia KA, Brennan PC. Reader characteristics and mammogram features associated with breast imaging reporting scores. *Br J radiol* (2020) 93(1114):20200363. doi: 10.1259/bjr.20200363

80. El-Bastawissi AY, White E, Mandelson MT, Taplin S. Variation in mammographic breast density by race. *Ann Epidemiol* (2001) 11(4):257–63. doi: 10.1016/s1047-2797(00)00225-8

81. Trieu PD, Mello-Thoms C, Peat JK, Do TD, Brennan PC. Risk factors of female breast cancer in Vietnam: A case-control study. *Cancer Res Treat Off J Korean Cancer Assoc* (2017) 49(4):990–1000. doi: 10.4143/crt.2016.488

82. Li T, Li J, Heard R, Gandomkar Z, Ren J, Dai M, et al. Understanding mammographic breast density profile in China: A sino-Australian comparative study of breast density using real-world data from cancer screening programs. *Asia Pac J Clin Oncol* (2022) 18(6):696–705. doi: 10.1111/ajco.13763

83. Kan L, Olivotto IA, Warren Burhenne LJ, Sickles EA, Coldman AJ. Standardized abnormal interpretation and cancer detection ratios to assess reading volume and reader performance in a breast screening program. *Radiology* (2000) 215(2):563–7. doi: 10.1148/radiology.215.2.r00ma42563

84. Rawashdeh MA, Lee WB, Bourne RM, Ryan EA, Pietrzyk MW, Reed WM, et al. Markers of good performance in mammography depend on number of annual readings. *Radiology* (2013) 269(1):61–7. doi: 10.1148/radiol.13122581

85. Sickles EA, Wolverton DE, Dee KE. Performance parameters for screening and diagnostic mammography: specialist and general radiologists. *Radiology* (2002) 224(3):861–9. doi: 10.1148/radiol.2243011482

86. Rehani B, Zhang YC, Rehani MM, Palko A, Lau L, Lette MN, et al. Radiology education in Europe: Analysis of results from 22 European countries. *World J Radiol* (2017) 9(2):55–62. doi: 10.4329/wjr.v9.i2.55

87. Kyaw MM, Dedova I, Young N, Moscova M. Quality of radiology training and role of royal Australian and new Zealand college of radiology in supporting radiology trainees in NSW: Results of the first radiology trainee survey. *J Med Imaging Radiat Oncol* (2021) 65(3):261–71. doi: 10.1111/1754-9485.13148

88. Qenam BA, Li T, Frazer H, Brennan PC. Clinical performance progress of BREAST participants: the impact of test-set participation. *Clin radiol* (2022) 77(2): e130–e7. doi: 10.1016/j.crad.2021.10.008

89. Caspar F, Copps E, Diplas A, Hackney L, Jackson K, Kearins I, et al. Mammographic interpretation in Vietnam: Tailored educational strategies are needed to increase clinicians' expertise. *Asia Pac J Clin Oncol* (2021) 17(5):e212–e6. doi: 10.1111/ajco.13436

90. Trieu PDY, Lewis SJ, Li T, Ho K, Wong DJ, Tran OTM, et al. Improving radiologist's ability in identifying particular abnormal lesions on mammograms through training test set with immediate feedback. *Sci Rep* (2021) 11(1):9899. doi: 10.1038/s41598-021-89214-3

91. Tavakoli Taba S, Hossain L, Heard R, Brennan P, Lee W, Lewis S. Personal and network dynamics in performance of knowledge workers: A study of Australian breast radiologists. *PloS One* (2016) 11(2):e0150186. doi: 10.1371/journal.pone.0150186

92. Taba ST, Hossain L, Willis K, Lewis S. Social networks and expertise development for Australian breast radiologists. *BMC Health Serv Res* (2017) 17 (1):131. doi: 10.1186/s12913-016-1938-9

frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 06 January 2023
DOI 10.3389/fonc.2022.1043185

OPEN ACCESS

EDITED BY
Maria Rosaria De Miglio,
University of Sassari, Italy

REVIEWED BY
Mingxing Xie,
Huazhong University of Science and Technology, China
Haining Zheng,
Peking University, China
Enrico Cassano,
European Institute of Oncology (IEO), Italy

*CORRESPONDENCE
Jun Li
1287424798@qq.com
Xin-Wu Cui
cuixinwu@live.cn

†These authors have contributed equally to this article

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 13 September 2022
ACCEPTED 25 November 2022
PUBLISHED 06 January 2023

CITATION
Li J, Wang S-R, Li Q-L, Zhu T, Zhu P-S, Chen M and Cui X-W (2023) Diagnostic value of multiple ultrasound diagnostic techniques for axillary lymph node metastases in breast cancer: A systematic analysis and network meta-analysis. *Front. Oncol.* 12:1043185. doi: 10.3389/fonc.2022.1043185

Diagnostic value of multiple ultrasound diagnostic techniques for axillary lymph node metastases in breast cancer: A systematic analysis and network meta-analysis

Jun Li[1,2*†], Si-Rui Wang[1,2†], Qiao-Li Li[1,2], Tong Zhu[3], Pei-Shan Zhu[1,2], Ming Chen[1] and Xin-Wu Cui[4*]

[1]Department of Medical Ultrasound, the First Affiliated Hospital of Medical College, Shihezi University, Xinjiang, China, [2]NHC Key Laboratory of Prevention and Treatment of Central Asia High Incidence Diseases (First Affiliated Hospital, School of Medicine, Shihezi University), Shihezi, Xinjiang, China, [3]School of Medicine, Shihezi University, Shihezi, China, [4]Department of Medical Ultrasound, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: Early diagnosis of axillary lymph node metastasis is very important for the recurrence and prognosis of breast cancer. Currently, Lymph node biopsy is one of the important methods to detect lymph node metastasis in breast cancer, however, its invasiveness might bring complications to patients. Therefore, this study investigated the diagnostic performance of multiple ultrasound diagnostic methods for axillary lymph node metastasis of breast cancer.

Materials and methods: In this study, we searched PubMed, Web of Science, CNKI and Wan Fang databases, conducted Bayesian network meta-analysis (NMA) on the studies that met the inclusion criteria, and evaluated the consistency of five different ultrasound imaging techniques in axillary lymph node metastasis of breast cancer. Funnel graph was used to evaluate whether it had publication bias. The diagnostic performance of each ultrasound imaging method was ranked using SUCRA

Results: A total of 22 papers were included, US+CEUS showed the highest SUCRA values in terms of sensitivity (SEN) (0.874), specificity (SPE) (0.911), positive predictive value (PPV) (0.972), negative predictive value (NPV) (0.872) and accuracy (ACC) (0.990).

Conclusion: In axillary lymph node metastasis of breast cancer, the US+CEUS combined diagnostic method showed the highest SUCRA value among the five ultrasound diagnostic methods. This study provides a theoretical basis for

preoperative noninvasive evaluation of axillary lymph node metastases in breast cancer patients and clinical treatment decisions.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42022351977.

KEYWORDS

ultrasound, ultrasound elastography, contrast-enhanced ultrasound, breast cancer, lymph nodes metastasis, network meta-analysis

1 Introduction

Breast Cancer is the most common malignancy tumor in women worldwide, and its incidence is much higher than other cancers (1), it ranks first in incidence and second in mortality among female malignant tumors (2). The occurrence of axillary lymph nodes metastasis is a key factor affecting the recurrence and prognosis of breast cancer. In order to avoid the spread of cancer cells through lymph nodes, axillary lymph node dissection is often performed in breast cancer patients. Although this method can effectively inhibit the recurrence of breast cancer and improve the prognosis, it may cause a series of complications, such as lymph node edema, Cellulitis, etc. Currently, the axillary staging and treatment of early breast cancer has changed from complete axillary lymph node dissection to sentinel lymph node biopsy (SLNB), which has a higher accuracy rate and a lower rate of postoperative complications (3). However, as an invasive procedure, SLNB may still lead to postoperative complications such as subcutaneous effusion, nerve injury, and restriction of shoulder joint movement, and the incidence of SLNB is 7.1% (4). Therefore, an accurate assessment of the extent of axillary lymph node involvement by non-invasive methods before surgery can minimize the incidence of postoperative complications caused by invasive methods. In non-invasive diagnosis, the sensitivity (SEN) of axillary lymph node palpation is only 33% to 68% (5), computer tomography (CT), positron emission tomography (PET) and other diagnosis methods (6) have the disadvantages of high price, radiation, etc., and do not show the obvious correlation in the evaluation of axillary lymph node metastasis in breast cancer (7, 8).

As one of the main detection methods of non-invasive imaging, ultrasound (US) has the advantages of no radiation, economy, convenience, and real-time imaging, and has become a common imaging method for the diagnosis of axillary lymph node metastasis in breast cancer. However, some studies have shown that 2D ultrasound has low SEN and specificity(SPE) in detecting benign and malignant lymph nodes due to its poor imaging of deep axillary lymph nodes and inability to show typical morphological changes (9). Ultrasound elastography (UE), contrast-enhanced ultrasound (CEUS), and other techniques may allow better differentiation between benign and malignant masses (10, 11). Studies have shown that elastography has high diagnostic performance in distinguishing benign from metastatic LNs, however, Park et al (12) showed that elastography did not have a significant advantage in evaluating metastatic lymph nodes. Tsai et al (13) found that US+UE showed higher SEN and SPE than US and UE alone. With the continuous progress of ultrasound technology, CEUS is widely used in clinical practice, and has higher SEN and SPE for lymph node metastasis, so that accuracy of diagnosing axillary lymph node metastasis in breast cancer is better improved.

The diagnostic performance of ultrasound diagnostic techniques for breast Cancer axillary lymph nodes is still controversial, and the results obtained by different clinical trials are also different. Therefore, we comprehensively analyze the diagnostic performance of US, UE, CEUS, US+UE, and US+CEUS.

This study conducted an NMA of the diagnostic performance of US, UE, CEUS, US+UE, and US+CEUS using two or more published studies of ultrasound imaging methods, comparison of different ultrasound imaging techniques for detection of SEN, SPE, positive predictive value (PPV), negative predictive value (NPV), accuracy (ACC) in axillary lymph node metastases. Helping clinicians find more accurate methods for diagnosing axillary lymph node metastases in breast cancer thereby improving patient outcomes.

2 Method

2.1 Retrieval strategy

We searched for relevant studies published in Chinese National Knowledge Infrastructure, PubMed, Web of Science, and Wan Fang before July 2022. Using “lymph node”, “Lymphatic Metastasis”, Elasticity imaging Techniques”, “Ultrasonography”, “Breast” cancer”, “ Contrast Ultrasound “ and other keywords were searched (Table 1). The included

TABLE 1 Search strategy.

No.	Retrieval type
#1	lymph node【Mesh】
#2	Neoplasm Staging【Mesh】
#3	Staging, Neoplasm【Title/Abstract】
#4	Tumor Staging【Title/Abstract】
#5	TNM Staging System【Title/Abstract】
#6	TNM Classifications【Title/Abstract】
#7	Preoperative Staging【Title/Abstract】
#8	Lymphatic Metastasis【Mesh】
#9	Lymphatic Metastases【Title/Abstract】
#10	Lymph Node Metastasis【Title/Abstract】
#11	Lymph Nodes Metastasis【Title/Abstract】
#12	Metastasis, Lymph Node【Title/Abstract】
#13	Axilla 【Title/Abstract】
#14	#1OR #2OR #3OR #4OR #5OR #6OR #7OR #8OR #9OR #10OR #11OR #12OR #13
#15	Ultrasound Contrast【Title/Abstract】
#16	Elasticity Imaging Techniques【Mesh】
#17	Elastography【Title/Abstract】
#18	Elastogram【Title/Abstract】
#19	B-mode【Title/Abstract】
#20	Ultrasonography【Mesh】
#21	Diagnostic Ultrasound【Title/Abstract】
#22	Ultrasound Imaging【Title/Abstract】
#23	Ultrasonic Imaging【Title/Abstract】
#24	Ultrasonic Diagnosis【Title/Abstract】
#25	Ultrasound Diagnosis【Title/Abstract】
#26	#15OR #16OR #17OR #18OR #19OR #20OR #21OR#22 OR#23 OR #24OR #25
#27	Breast Neoplasms【Mesh】
#28	Breast Tumors【Title/Abstract】
#29	Mammary Cancer【Title/Abstract】
#30	Breast Malignant Neoplasm【Title/Abstract】
#31	Breast Malignant Tumors【Title/Abstract】
#32	Human Mammary Carcinoma【Title/Abstract】
#33	Breast Carcinoma【Title/Abstract】
#34	Breast Cancer【Title/Abstract】
#35	#27 OR#28 OR#29OR #30 OR#31OR#32#33 OR#34
#36	#14 AND#26 AND#35

references were also screened to ensure that all included references met the inclusion and exclusion criteria.

2.2 Research screening

The relevant inclusion criteria are as follows: 1) Population: patients with pathologically proven breast cancer with axillary lymph node metastasis; 2) Diagnosis method: including two or more ultrasound imaging methods; 3) Study result should include calculable indicators such as true-positive (TP), false-positive (FP), true-negative (TN), false-negative (FN) of describe the diagnostic performance of the study; 4) Type of study: diagnostic trial.

The relevant exclusion criteria include the following aspects: (1) The study population is non-human studies or studies with axillary lymph node metastases of breast cancer without pathological confirmation; (2) the diagnostic performance indicators in the studies are incomplete; (3) Editorials, reviews, case reports, meeting minutes, guidelines, etc.

The titles and abstracts of retrieved articles were read by two authors, respectively. studies that do not meet the inclusion criteria will be excluded according to the inclusion and exclusion criteria established in this study.

2.3 Data extraction

Data extraction was performed on the originally included studies and was independently extracted by two investigators. The extracted data included: 1) The first author; 2) Research publication time; 3) Country of the first author; 4) The mean of the patient's age; 5) Diagnostic method; 6) Sample size; 7) The results of the study were TP, FP, TN, FN.

2.4 Statistical analysis

This meta-analysis has been registered on the PROSPERO website with registration number CRD42022336701.We divided the different ultrasound diagnostic methods in the included study into five groups, namely US, UE, CEUS, US+UE, and US+CEUS, and used NMA to analyze the diagnostic performance of the five groups in the diagnosis of axillary lymph node metastasis in breast cancer. According to the PRISMA NMA list, Stata's(version-15.1) -Markov chain Monte Carlo model was used. The NMA was aggregated and analyzed in a Bayes-based framework, and the five groups of data were compared directly and indirectly. The diagnostic performance of each diagnostic method was judged by analyzing its SEN, SPE, PPV, NPV, and ACC indicators, and using the P value or I^2 to evaluate heterogeneity. P value <0.05 or I^2>90% indicates that the heterogeneity was large.

We also use the nodal method to evaluate the inconsistency in NMA, using the surface under the cumulative ranking curve (SUCRA) to calculate the probability of each imaging mode. The value of SUCRA is between 0 and 1($0 \leq SUCRA \leq 1$), when SUCRA is 1, it indicates that the intervention is absolutely effective, and when SUCRA is 0, it indicates that the intervention is absolutely ineffective. According to the value of SUCRA, the pros and cons of the diagnostic methods can be sorted, so as to screen out the most effective diagnostic methods.

This study used funnel plots to detect possible publication bias, and the results showed that the distribution of funnel plots was roughly symmetric, suggesting that there was no publication bias or other bias in the study (Figure 1).

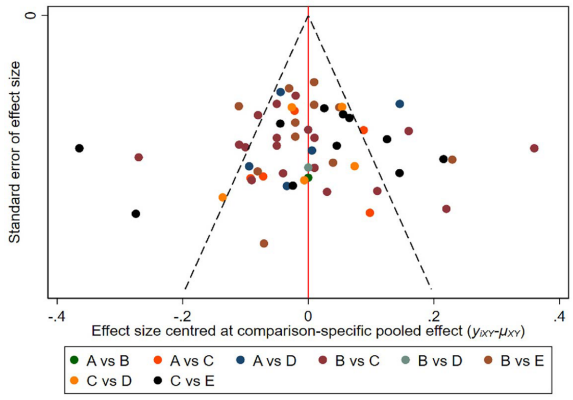


FIGURE 1
Funnel plot on publication bias (A=CEUS, B=UE, C=US, D=US +CEUS, E=US+UE).

3 Results

3.1 Literature selection

This study found 8072 studies from the database based on keywords, of which 1999 articles were extracted from PubMed, 3502 articles were extracted from Web of Science,1214 articles were extracted from Wan Fang, and 1357 articles were extracted from CNKI. A total of 8050 studies that did not meet the inclusion criteria were excluded from this study, and 22 studies were finally included (3, 10, 12–31) (Table 2). We included published studies using two or more ultrasound imaging methods, and analyzed and evaluated the extracted diagnostic indicators.

3.2 Study characteristics

A total of 7776 patients (range, 42-313) were included in 22 studies (3, 10, 12–31), all of whom were pathologically confirmed to have lymph node metastases of breast cancer. Among these studies, there were 2 retrospective studies and 20 randomized controlled studies. There were many studies on the US, UE, and US+UE in the included literature, among which 18 studies compared US vs UE,11 studies compared US vs US+UE, 11 studies compared UE vs US+UE, 5 studies compared US vs CEUS. Five studies compared US vs US+CEUS (Table 3). The quality assessment of the literature was based on QUADAS-2 scale to evaluate 22 studies from four aspects: Patient Selection, Reference Standard, Index Test, and Flow Timing. The results show that the overall quality of the included studies was relatively satisfactory (Figure 2). Among the 22 articles, 5 had an unclear risk of bias in the Index Test, which may be due to the differences in the operators performing the tests and their experience levels.

3.3 Network meta-analysis

The Network evidence diagram was shown in Figure 3. In this study, the consistency of direct comparison and indirect comparison of the included studies was analyzed, and the results showed that all studies were P > 0.05, indicating that the studies had good consistency.

3.3.1 SEN

NMA showed that US+CEUS [MD=0.15, 95%CI (0.02, 0.28)] was superior to the control group (CEUS) in diagnosing SEN in axillary lymph node metastasis of breast cancer (Table 4A). US+CEUS ranked first in SEN for axillary lymph node metastasis of breast cancer in different methods(SUCRA: 87.4% as shown in Table 5) (Figure 4).

3.3.2 SPE

NMA showed that US+CEUS [MD=0.16, 95% CI (0.01, 0.31)] was superior to the control group (UE) in diagnosing of SPE in axillary lymph node metastasis in breast cancer. US +CEUS [MD=0.21, 95%CI (0.07, 0.35)] and CEUS [MD=0.17, 95%CI (0.03, 0.31)] were superior to the control group (US) in diagnosing of SPE in axillary lymph node metastasis in breast cancer (Table 4B). US+CEUS ranked first in SPE for axillary lymph node metastasis of breast cancer in different methods (SUCRA: 90.8% as shown in Table 5) (Figure 5).

3.3.3 PPV

NMA showed that US+CEUS [MD=0.18, 95%CI (0.05, 0.31)] was superior to the control group (US+UE) in diagnosing of PPV in axillary lymph node metastasis of breast cancer. US+CEUS [MD=0.20, 95%CI (0.08, 0.34)] was better than control group (UE) in diagnosing of PPV in axillary lymph node metastasis of breast cancer. US+CEUS [MD=0.22, 95%CI (0.11, 0.33)] and CEUS [MD=0.15, 95%CI (0.04, 0.26)] were superior to the control group (US) in the diagnosing of PPV in axillary lymph node metastasis of breast cancer (Table 4C). US +CEUS ranked first in PPV for axillary lymph node metastasis of breast cancer in different methods (SUCRA: 97.3% as shown in Table 5) (Figure 6).

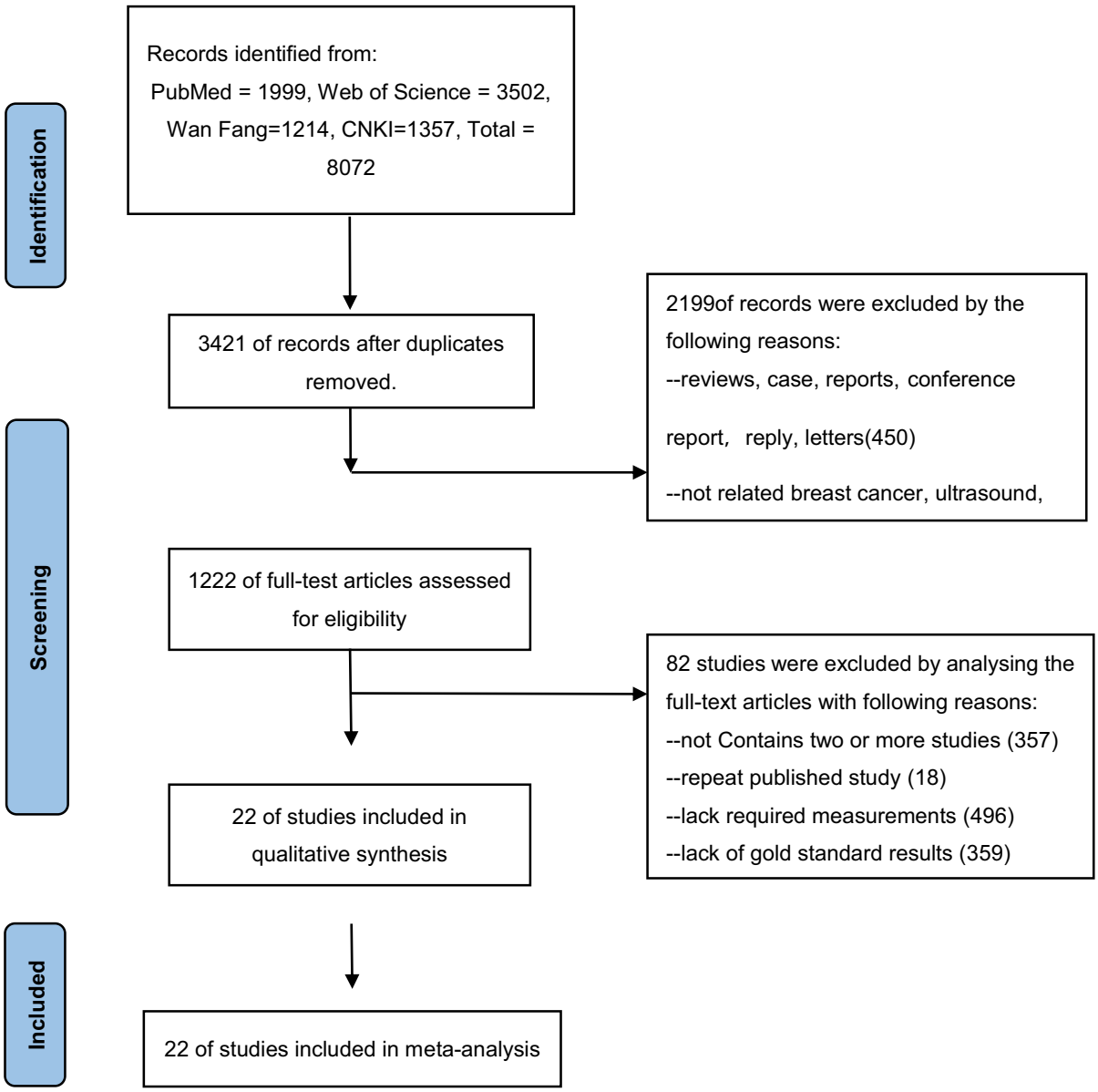
3.3.4 NPV

NMA showed that US+CEUS [MD=0.10, 95%CI (0.01, 0.19)] and US+UE [MD=0.08, 95%CI (0.02, 0.14)] were superior to the control group (US+UE) in diagnosing of NPV in axillary lymph node metastasis of breast cancer (Table 4D). US+CEUS ranked first in NPV for axillary lymph node metastasis of breast cancer in different methods (SUCRA:87.6% as shown in Table 5) (Figure 7).

3.3.5 ACC

NMA showed that US+CEUS was superior to the control group in diagnosing of ACC in axillary lymph node metastasis of

TABLE 2 Flow diagram of literature selection.



breast cancer (US+UE, UE, US) (Table 4E). US+CEUS ranked first in ACC for axillary lymph node metastasis of breast cancer in different methods(SUCRA:99.0% as shown in Table 5) (Figure 8).

4 Discussion

Early identification of axillary lymph node metastasis in breast cancer is crucial for the prognosis and treatment of breast cancer patients, and SLNB is a necessary means to detect whether breast cancer has lymph node metastasis (32). However, SLNB usually carries a risk of acute or long-term complications including nerve damage, lymphedema, and wound infection etc (33). Therefore, accurate prediction of axillary lymph node metastasis in breast cancer by non-invasive diagnosis is an urgent problem to be solved. This study evaluated the diagnostic performance of US, UE, CEUS, US+UE, and US +CEUS of axillary lymph node metastasis in breast cancer patients with in detail from five aspects: SEN, SPE, PPV, NPV, and ACC. This is the first systematic review and NMA of non-invasive imaging modalities of ultrasound diagnostic methods in patients with pathologically confirmed breast cancer with axillary lymph node metastases. A total of 22 articles were included in this study, with a total of 7776 patients (range, 42-313), The combined ultrasound method was significantly better than the single

TABLE 3 Overview of characteristics of all included studies.

Author	Year	country	Design	Age, Mean (Range)	Patient number	Gold standard	Diagnostic method		
ZHAO Q.	2018	China	Pro	53.1 (31-77)	313	Pathologic	①	②	
Choi J. J.	2011	Korea	RCT	53 (27-81)	62	Pathologic	①	②	④
Zhou J.	2022	China	RCT	/	160	Pathologic	①	②	
Wojcinski S.	2012	Germany	RCT	/	180	Pathologic	①	②	④
TSAI W. C.	2013	China (Taiwan)	RCT	51 (20-84)	89	Pathologic	①	②	④
Chang W. Y.	2018	China	RCT	55.3 (21-85)	140	Pathologic	①	②	④
Xu Y. J.	2018	China	RCT	/	97	Pathologic	①	②	④
Park Y. M.	2013	American	RCT	55 (33-99)	101	Pathologic	①	②	
Zhao Q. L.	2017	China	RCT	52.47 (27-79)	78	Pathologic	①	②	④
Luo C. Y.	2022	China	RCT	49.5 (41-58)	114	Pathologic	①	②	④
Lan M.	2019	China	RCT	50.5 (22-78)	107	Pathologic	①	②	
Vishnu P. P.	2022	India	RCT	46.3 (34-58)	54	Pathologic	①	②	④
Wei L. N.	2021	Malaya	RCT	58 (33-82)	107	Pathologic	①	②	④
Wang J.	2021	China	RCT	42.4 (35-78)	85	Pathologic	①	②	④
Seo M.	2018	Korea	RCT	54.7 (33-80)	66	Pathologic	①	②	
Youk J. H.	2017	Korea	RCT	/	130	Pathologic	①	②	
Luo S. Y.	2019	China	RCT	46.68 (27-69)	158	Pathologic	①	②	④
Du L. W.	2020	China	RCT	49.4 (24-84)	234	Pathologic	①	③	⑤
Zhang Q.	2021	China	RCT	50.5 (32-77)	120	Pathologic	①	②	③
Du L. W.	2020	China	RCT	49.4 (24-85)	234	Pathologic	①	③	⑤
Zhao Y. D.	2019	China	RCT	44.4 (28-59)	42	Pathologic	①	③	⑤
Wang S. F.	2021	China	RCT	48.4 (25-70)	120	Pathologic	①	③	⑤

①:Ultrasound; ②:Ultrasonic elasticity; ③:Contrast-enhance ultrasound; ④:Ultrasound+ Ultrasonic elasticity; ⑤:Ultrasound+ Contrast-enhance ultrasound.

ultrasound method in the diagnosis of axillary lymph node metastasis in breast cancer. Compared with other diagnostic methods, US+CEUS showed obvious advantages in predicting axillary lymph node metastasis in breast cancer in all aspects. The SUCRA values showed that CEUS had higher SEN and higher accuracy than US and UE alone in a single diagnostic method. Our analysis showed that US+CEUS could be an effective non-invasive diagnostic method for clinical diagnosis of axillary lymph node metastasis in breast cancer.

The US is considered to be a routine non-invasive diagnostic method for diagnosing axillary lymph node metastasis in breast cancer. The status of axillary lymph node s is usually assessed by blood flow, size, and shape. However, US diagnosis usually relies on the doctor's own experience and skills, and there may be a higher misdiagnosis rate, and its SEN and SPE are quite different (27). The SPE and SEN of this diagnostic method in this study were 70% and 86%, respectively, similar to the results of Qing.Z et al. (28). UE is widely used in the diagnosis of superficial organs and lymph node metastases. Wang J et al. (24) believed that traditional two-dimensional ultrasound technology is not ideal for the differential diagnosis of breast cancer axillary lymph node metastases, while UE can accurately reflect tissue stiffness. Thus, the types of breast cancer axillary lymph node metastasis can be identified semi-quantitatively. We analyzed the 12 included articles and found that the SEN and SPE of UE for breast cancer axillary lymph node metastasis were 83% and86%, respectively, which were consistent with the results of Choi J.J et al (15).

The morphology of lymph nodes and blood flow distribution are studied using conventional ultrasound, although it is difficult to identify small infiltrative foci that do not result in morphological changes in lymph nodes; Doppler ultrasound is unable to detect anterior lymph nodes because of its low signal-to-noise ratio, inability to see microvessels, and difficulty displaying tissue perfusion. The examination of abdominopelvic and superficial organ lesions as well as the detection of SLN in breast cancer have all benefited from the widespread use of CEUS, a novel technology for the dynamic assessment of tissue perfusion utilizing ultrasonic contrast agent (UCA). It has been commonly used for the diagnosis of benign and malignant breast cancer and the assessment of axillary lymph node metastasis. Ultrasonography under enhanced conditions can reveal some of the new and immature tissues around the tumor, and the boundary and internal blood flow of the primary breast cancer are more clearly shown compared to conventional ultrasound. It is mainly by injecting a contrast agent into the patient's body to enhance the outline of the axillary lymph node according to the concentration of the contrast agent in the patient's axillary lymph nodes. compared with normal lymph nodes, metastatic lymph nodes showed longer duration of enhancement as well as higher imaging intensities. CEUS has been shown to be more accurate than other ultrasound

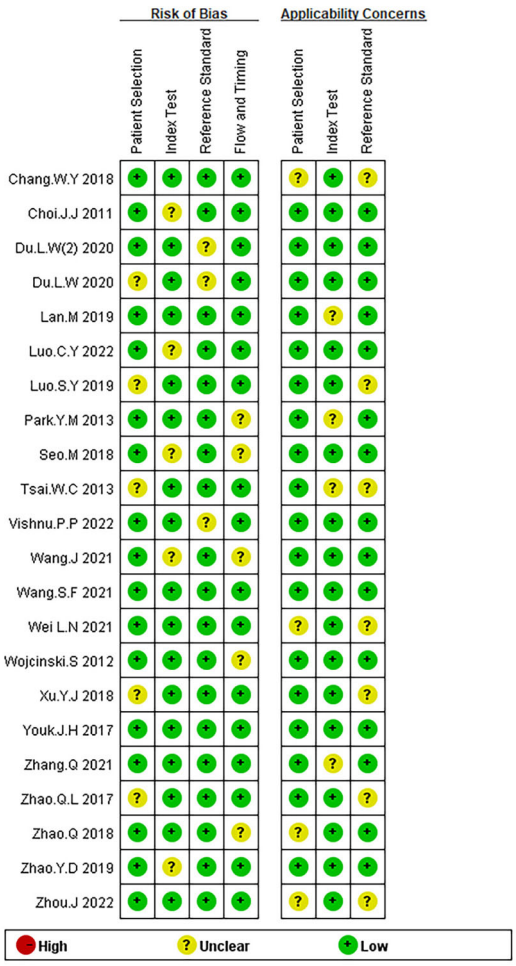


FIGURE 2
Bias risk of the included studies (QUADAS 2 criteria).

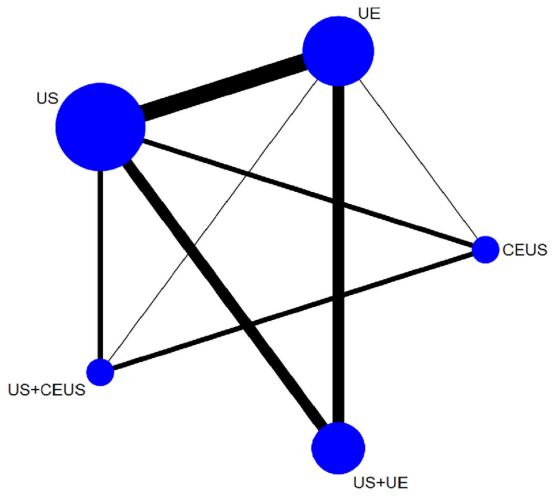


FIGURE 3
Network Mate-Analysis Figure.

methods in previous studies, and our study showed the same results with a SEN and SPE of 82% and 88%, respectively.

Most of the current clinical prediction models of axillary lymph node metastasis of breast cancer are based on clinicopathological characteristics such as age, tumor size, and histological grade. However, these clinicopathological features are usually acquired intraoperatively or postoperatively, and the diagnostic performance of single diagnostic imaging is not ideal. Therefore, we analyzed combined diagnostic methods, such as US+CEUS, and US+UE. Compared with previous studies, the combined diagnostic method was significantly higher than the single diagnostic method in terms of diagnostic performance, especially the US+CEUS combined diagnostic method showed satisfactory predictive results in terms of SEN and SPE, the mean reason is that conventional ultrasound must first locate lymph nodes in order to distinguish between benign and malignant ones; however, some lymph nodes are challenging to distinguish from nearby tissues and are frequently missed. However, some lymph nodes are hard to spot in the tissues around them and are frequently missed. By using enhanced microbubbles to detect these occult lymph nodes, CEUS can aid in their detection. It can also correct some lymph nodes that conventional ultrasonography incorrectly labeled as benign due to minor metastases. Traditional ultrasonography misdiagnoses lymph nodes as benign because of minor metastases. The combined diagnosis of the two can offer a thorough assessment of the lymph nodes' size, shape, internal structure, and lymphatic drainage, and evaluation of the internal anatomy, lymphatic drainage, size, morphology, and diagnostic value of axillary lymph nodes. There were still some limitations in the study. First, this study needs to include kinds of literature containing two or more diagnostic methods. However, it is found that the number of such articles is limited through search, resulting in an uneven number of studies on each diagnostic method. Second, some of the results of this study may have an impact on the results of the study due to differences in the number of patients between studies. Third, due to the differences in the experience level of the radiologist in the diagnosis of diseases, there are potential differences in the studies. In view of the above deficiencies, it is suggested that readers should reasonably refer to and select the diagnostic method of this study according to clinical practice and actual results.

In conclusion, the analysis of this study showed that single US, UE, and CEUS have limited diagnostic performance in diagnosing axillary lymph nodes metastases in breast cancer. Compared with single ultrasound imaging, US + CEUS have highest diagnostic performance of axillary lymph nodes

10.3389/fonc.2022.1043185

TABLE 4 League table on five kinds of diagnostic efficacy.

US+CEUS	US+UE	UE	US	CEUS
A. League table on SEN				
US+CEUS	-0.04 (-0.19,0.12)	-0.08 (-0.23,0.06)	-0.12 (-0.25,0.00)	-0.15 (-0.28, -0.02)
0.04 (-0.12,0.19)	US+UE	-0.05 (-0.13,0.04)	-0.09 (-0.18, -0.00)	-0.11 (-0.27,0.04)
0.08 (-0.06,0.23)	0.05 (-0.04,0.13)	UE	-0.04 (-0.11,0.03)	-0.07 (-0.21,0.08)
0.12 (-0.00,0.25)	0.09 (0.00,0.18)	0.04 (-0.03,0.11)	US	-0.02 (-0.15,0.11)
0.15 (0.02,0.28)	0.11 (-0.04,0.27)	0.07 (-0.08,0.21)	0.02 (-0.11,0.15)	CEUS
B. League table on SPE				
US+CEUS	CEUS	US+UE	UE	US
US+CEUS	-0.04 (-0.18,0.10)	-0.14 (-0.30,0.02)	-0.16 (-0.31, -0.01)	-0.21 (-0.35, -0.07)
0.04 (-0.10,0.18)	CEUS	-0.10 (-0.27,0.06)	-0.12 (-0.28,0.03)	-0.17 (-0.31, -0.03)
0.14 (-0.02,0.30)	0.10 (-0.06,0.27)	US+UE	-0.02 (-0.11,0.07)	-0.07 (-0.16,0.02)
0.16 (0.01,0.31)	0.12 (-0.03,0.28)	0.02 (-0.07,0.11)	UE	-0.05 (-0.12,0.03)
0.21 (0.07,0.35)	0.17 (0.03,0.31)	0.07 (-0.02,0.16)	0.05 (-0.03,0.12)	US
C. League table on PPV				
US+CEUS	CEUS	US+UE	UE	US
US+CEUS	-0.07 (-0.18,0.04)	-0.18 (-0.31, -0.05)	-0.20 (-0.32, -0.08)	-0.22 (-0.33, -0.11)
0.07 (-0.04,0.18)	CEUS	-0.11 (-0.24,0.02)	-0.13 (-0.25, -0.00)	-0.15 (-0.26, -0.04)
0.18 (0.05,0.31)	0.11 (-0.02,0.24)	US+UE	-0.02 (-0.09,0.05)	-0.04 (-0.11,0.03)
0.20 (0.08,0.32)	0.13 (0.00,0.25)	0.02 (-0.05,0.09)	UE	-0.02 (-0.08,0.04)
0.22 (0.11,0.33)	0.15 (0.04,0.26)	0.04 (-0.03,0.11)	0.02 (-0.04,0.08)	US
D. League table on NPV				
US+CEUS	US+UE	UE	CEUS	US
US+CEUS	-0.02 (-0.13,0.09)	-0.06 (-0.16,0.04)	-0.09 (-0.19, -0.00)	-0.10 (-0.19, -0.01)
0.02 (-0.09,0.13)	US+UE	-0.04 (-0.11,0.02)	-0.07 (-0.18,0.04)	-0.08 (-0.14, -0.02)
0.06 (-0.04,0.16)	0.04 (-0.02,0.11)	UE	-0.03 (-0.13,0.07)	-0.03 (-0.09,0.02)
0.09 (0.00,0.19)	0.07 (-0.04,0.18)	0.03 (-0.07,0.13)	CEUS	-0.01 (-0.10,0.09)
0.10 (0.01,0.19)	0.08 (0.02,0.14)	0.03 (-0.02,0.09)	0.01 (-0.09,0.10)	US
E. League table on ACC				
US+CEUS	CEUS	US+UE	UE	US
US+CEUS	-0.08 (-0.16, -0.00)	-0.11 (-0.21, -0.01)	-0.14 (-0.23, -0.05)	-0.16 (-0.24,-0.08)
0.08 (0.00,0.16)	CEUS	-0.03 (-0.13,0.07)	-0.06 (-0.15,0.04)	-0.08 (-0.16,0.00)
0.11 (0.01,0.21)	0.03 (-0.07,0.13)	US+UE	-0.03 (-0.08,0.03)	-0.05 (-0.11,0.00)
0.14 (0.05,0.23)	0.06 (-0.04,0.15)	0.03 (-0.03,0.08)	UE	-0.02 (-0.07,0.02)
0.16 (0.08,0.24)	0.08 (-0.00,0.16)	0.05 (-0.00,0.11)	0.02 (-0.02,0.07)	US

The values in red have been expounded in the *Results* section.

TABLE 5 SUCRA values of preoperative detection of axillary lymph node metastases in breast cancer patients by 5 different ultrasonic diagnostic methods.

Method	SEN	SPE	PPV	NPV	ACC
US C	20.5	.4.7	8.7	13.9	5.7
UE B	49.1	32.7	28.1	44.9	27.7
CEUS A	15.8	78.1	75.7	24.1	64.8
US+UE E	77.2	43.8	40.1	79.5	22.7
US+CEUS D	87.4	90.8	97.3	87.6	99.0

SEN, Sensitivity; SPE, Specificity; PPV, Positive predictive value; NPV, Negative predictive value; ACC, Accuracy.

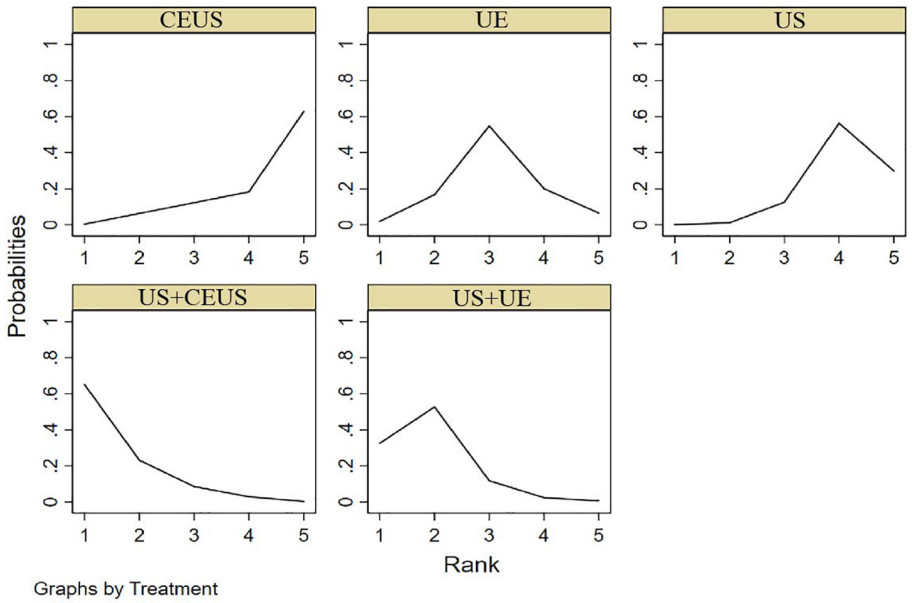


FIGURE 4
SUCRA plot for SEN.

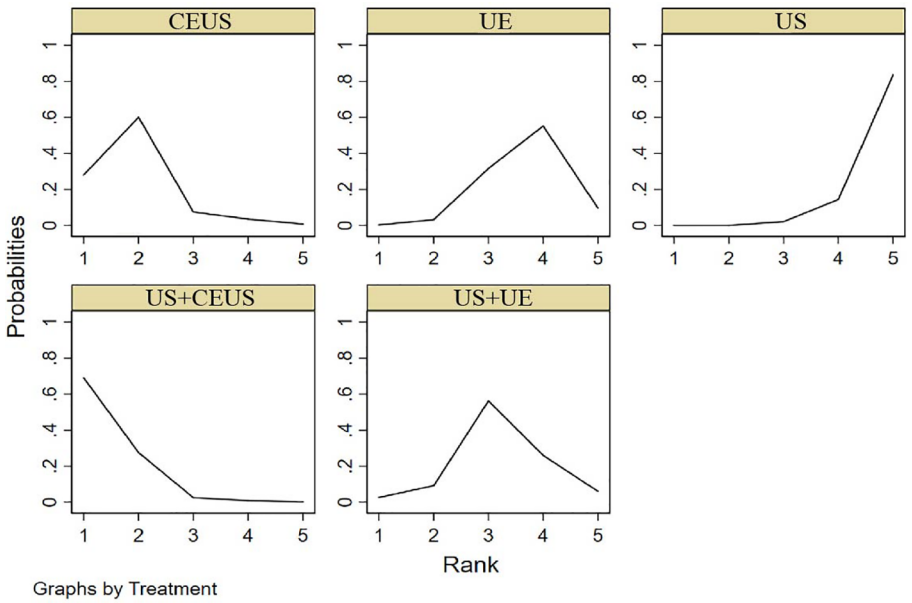


FIGURE 5
SUCRA plot for SPE.

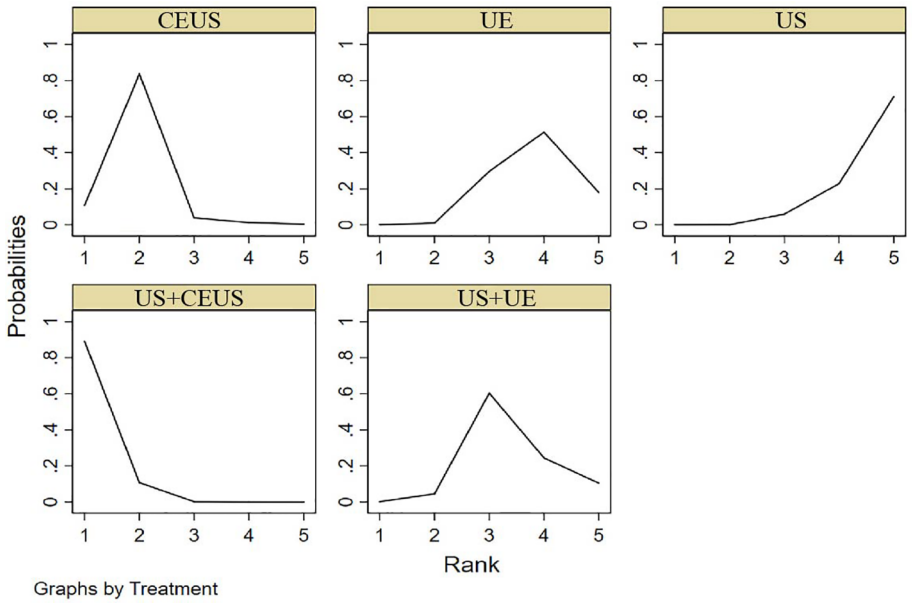


FIGURE 6
SUCRA plot for PPV.

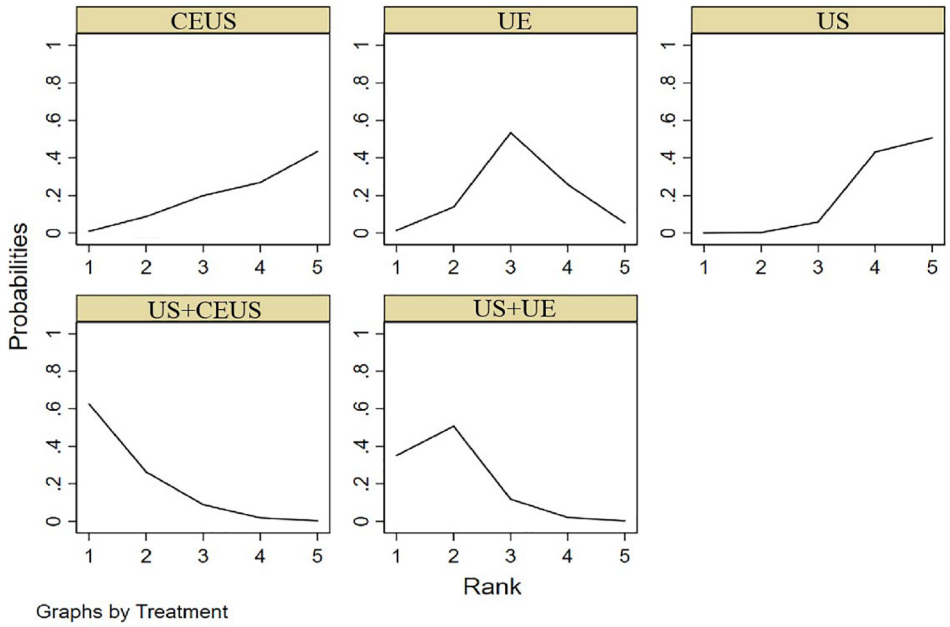


FIGURE 7
SUCRA plot for NPV.

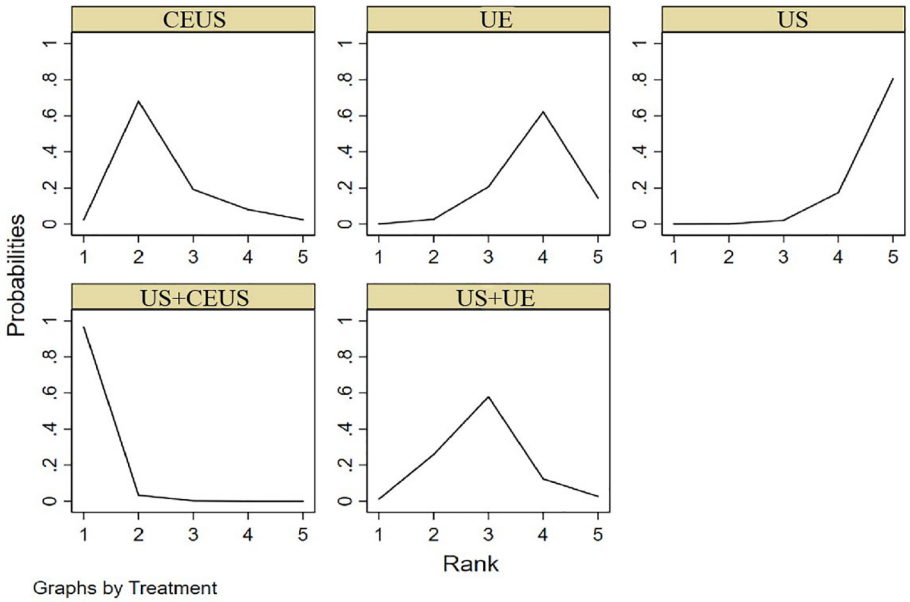


FIGURE 8
SUCRA plot for ACC.

metastasis in breast cancer in the combined diagnosis, which can provide a reliable basis for breast cancer axillary lymph nodes metastasis, However, due to the lack of literature, more prospective studies are still needed to confirm this conclusion.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

Study concept and design: S-RW, Q-LL. Acquisition of data: Q-LL, MC, S-RW, TZ. Analysis and interpretation of data: S-RW, TZ. Drafting of the manuscript: TZ, P-SZ. Critical revision of the manuscript for important intellectual content: JL. Approval of the final manuscript: JL, S-RW. Study supervision: JL. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences(2020-PT330-003); Open Research Fund of NHC Key Laboratory of Prevention and Treatment of Central Asia High Incidence Diseases; The First Affiliated Hospital of Shihezi University School of Medicine Youth Fund Project (QN202107); The First Affiliated Hospital of Shihezi University School of Medicine Youth Fund Project (QN202126); Supported by the National Natural Science Foundation of China (82060318).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer MX declared a shared parent affiliation with the author X-WC to the handling editor at the time of review.

Publisher's note

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654

3. Chang JM, Leung JWT, Moy L, Ha SM, Moon WK. Axillary nodal evaluation in breast cancer: State of the art. *Radiology* (2020) 295(3):500–15. doi: 10.1148/radiol.2020192534

4. Balasubramanian I, Fleming CA, Corrigan MA, Redmond HP, Kerin MJ, Lowery AJ. Meta-analysis of the diagnostic accuracy of ultrasound-guided fine-needle aspiration and core needle biopsy in diagnosing axillary lymph node metastasis. *Br J Surg* (2018) 105(10):1244–53. doi: 10.1002/bjs.10920

5. Valente SA, Levine GM, Silverstein MJ, Rayhanabad JA, Weng-Grumley JG, Ji L, et al. Accuracy of predicting axillary lymph node positivity by physical examination, mammography, ultrasonography, and magnetic resonance imaging. *Ann Surg Oncol* (2012) 19(6):1825–30. doi: 10.1245/s10434-011-2200-7

6. Suvannarerg V, Chitchumnong P, Apiwat W, Lertdamrongdej L, Tretipwanit N, Pisarnturakit P, et al. Diagnostic performance of qualitative and quantitative shear wave elastography in differentiating malignant from benign breast masses, and association with the histological prognostic factors. *Quant Imaging Med Surg* (2019) 9(3):386–98. doi: 10.21037/qims.2019.03.04

7. Qiu SQ, Zeng HC, Zhang F, Chen C, Huang WH, Pleijhuis RG, et al. A nomogram to predict the probability of axillary lymph node metastasis in early breast cancer patients with positive axillary ultrasound. *Sci Rep* (2016) 6:21196. doi: 10.1038/srep21196

8. Choi YJ, Ko EY, Han BK, Shin JH, Kang SS, Hahn SY. High-resolution ultrasonographic features of axillary lymph node metastasis in patients with breast cancer. *Breast* (2009) 18(2):119–22. doi: 10.1016/j.breast.2009.02.004

9. Alvarez S, Añorbe E, Alcorta P, López F, Alonso I, Cortés J. Role of sonography in the diagnosis of axillary lymph node metastases in breast cancer: A systematic review. *AJR Am J Roentgenol* (2006) 186(5):1342–8. doi: 10.2214/AJR.05.0936

10. Wojcinski S, Dupont J, Schmidt W, Cassel M, Hillemanns P. Real-time ultrasound elastography in 180 axillary lymph nodes: elasticity distribution in healthy lymph nodes and prediction of breast cancer metastases. *BMC Med Imaging* (2012) 12:35. doi: 10.1186/1471-2342-12-35

11. Sadigh G, Carlos RC, Neal CH, Dwamena BA. Ultrasonographic differentiation of malignant from benign breast lesions: A meta-analytic comparison of elasticity and BIRADS scoring. *Breast Cancer Res Treat* (2012) 133(1):23–35. doi: 10.1007/s10549-011-1857-8

12. Park YM, Fornage BD, Benveniste AP, Fox PS, Bassett RLJr., Yang WT. Strain elastography of abnormal axillary nodes in breast cancer patients does not improve diagnostic accuracy compared with conventional ultrasound alone. *AJR Am J Roentgenol* (2014) 203(6):1371–8. doi: 10.2214/AJR.13.12349

13. Tsai WC, Lin CK, Wei HK, Yu BL, Hung CF, Cheng SH, et al. Sonographic elastography improves the sensitivity and specificity of axilla sampling in breast cancer: a prospective study. *Ultrasound Med Biol* (2013) 39(6):941–9. doi: 10.1016/j.ultrasmedbio.2012.12.013

14. Zhao QL, Xia XN, Zhang Y, He JJ, Sheng W, Ruan LT, et al. Elastosonography and two-dimensional ultrasonography in diagnosis of axillary lymph node metastasis in breast cancer. *Clin Radiol* (2018) 73(3):312–8. doi: 10.1016/j.crad.2017.09.013

15. Choi JJ, Kang BJ, Kim SH, Lee JH, Jeong SH, Yim HW, et al. Role of sonographic elastography in the differential diagnosis of axillary lymph nodes in breast cancer. *J Ultrasound Med* (2011) 30(4):429–36. doi: 10.7863/jum.2011.30.4.429

16. Zhou J, Zhang Q, Zhang Q, Yan L, Gao Q. Evaluation of the property of axillary lymph nodes and analysis of lymph node metastasis factors in breast cancer by ultrasound elastography. *Comput Math Methods Med 2022* (2022), 8066289. doi: 10.1155/2022/8066289

17. Xu Y, Bai X, Chen Y, Jiang L, Hu B, Hu B, et al. Application of real-time elastography ultrasound in the diagnosis of axillary lymph node metastasis in breast cancer patients. *Sci Rep* (2018) 8(1):10234. doi: 10.1038/s41598-018-28474-y

18. Zhao Q, Sun JW, Zhou H, Du LY, Wang XL, Tao L, et al. Pre-operative conventional ultrasound and sonoelastography evaluation for predicting axillary lymph node metastasis in patients with malignant breast lesions. *Ultrasound Med Biol* (2018) 44(12):2587–95. doi: 10.1016/j.ultrasmedbio.2018.07.017

19. Luo S, Yao G, Hong Z, Zhang S, Wang W, Zhang J, et al. Qualitative classification of shear wave elastography for differential diagnosis between benign and metastatic axillary lymph nodes in breast cancer. *Front Oncol* (2019) 9:533. doi: 10.3389/fonc.2019.00533

20. Meng L, Ailian L, Tianxiang L, Fenging F, Dan G, Xuan S, et al. The value of acoustic palpation tissue and quantitative techniques and conventional ultrasound in qualitative diagnosis axillary lymph nodes in breast cancer. *Chin J Clin* (2019) 13 (04):281–5.

21. Luo C, Lu L, Zhang W, Li X, Zhou P, Ran Z. The value of shear wave elastography in the diagnosis of breast cancer axillary lymph node metastasis and its correlation with molecular classification of breast masses. *Front Oncol* (2022) 12:846568. doi: 10.3389/fonc.2022.846568

22. Pulappadi VP, Paul S, Hari S, Dhamija E, Manchanda S, Kataria K, et al. Role of shear wave elastography as an adjunct to axillary ultrasonography in predicting nodal metastasis in breast cancer patients with suspicious nodes. *Br J Radiol* (2022) 95(1134):20220055. doi: 10.1259/bjr.20220055

23. Ng WL, Omar N, Ab Mumin N, Ramli Hamid MT, Vijayananthan A, Rahmat K. Diagnostic accuracy of shear wave elastography as an adjunct tool in detecting axillary lymph nodes metastasis. *Acad Radiol* (2022) 29 Suppl 1:S69–s78. doi: 10.1016/j.acra.2021.03.018

24. Wang J, Ben Z, Gao S, Lyu S, Wei X. The role of ultrasound elastography and virtual touch tissue imaging in the personalized prediction of lymph node metastasis of breast cancer. *Gland Surg* (2021) 10(4):1460–9. doi: 10.21037/gs-21-199

25. Seo M, Sohn YM. Differentiation of benign and metastatic axillary lymph nodes in breast cancer: Additive value of shear wave elastography to b-mode ultrasound. *Clin Imaging* (2018) 50:258–63. doi: 10.1016/j.clinimag.2018.04.013

26. Youk JH, Son EJ, Kim JA, Gweon HM. Pre-operative evaluation of axillary lymph node status in patients with suspected breast cancer using shear wave elastography. *Ultrasound Med Biol* (2017) 43(8):1581–6. doi: 10.1016/j.ultrasmedbio.2017.03.016

27. Du LW, Liu HL, Gong HY, Ling LJ, Wang S, Li CY, et al. Adding contrast-enhanced ultrasound markers to conventional axillary ultrasound improves specificity for predicting axillary lymph node metastasis in patients with breast cancer. *Br J Radiol* (2021) 94(1118):20200874. doi: 10.1259/bjr.20200874

28. Zhang Q, Agyekum EA, Zhu L, Yan L, Zhang L, Wang X, et al. Clinical value of three combined ultrasonography modalities in predicting the risk of metastasis to axillary lymph nodes in breast invasive ductal carcinoma. *Front Oncol* (2021) 11:715097. doi: 10.3389/fonc.2021.715097

29. Liwen D, Haiyan G, Jing D, Hui W, Chunbei Y, Cuiying L. The diagnostic value of conventional ultrasonography combined with contrast-enhanced ultrasonography in evaluating axillany lymph nodes in breast cancer patients. *Cancer Imaging* (2020) 29(04):397–405.

30. Shaofen W, Junging L. The diagnostic value of conventional ultrasonography combined with contrast-enhanced ultrasonography in metastatic sentinel lymph nodes of breast cancer. *Clinical Medicine Research and Practice* (2021) 6(35):114–6.

31. Xiaodan Z, Xinjia W. Application analysis of ultrasonography combined with contrast-enhanced ultrasonography in the diagnosis of metastatic sentinel lymph nodes in breast cancer. *Electronic J Clin Med Literature* (2019) 6(13):165.

32. Garcia-Etienne CA, Ferrari A, Della Valle A, Lucioni M, Ferraris E, Di Giulio G, et al. Management of the axilla in patients with breast cancer and positive sentinel lymph node biopsy: An evidence-based update in a European breast center. *Eur J Surg Oncol* (2020) 46(1):15–23. doi: 10.1016/j.ejso.2019.08.013

33. Lyman GH, Somerfield MR, Bosserman LD, Perkins CL, Weaver DL, Giuliano AE. Sentinel lymph node biopsy for patients with early-stage breast cancer: American society of clinical oncology clinical practice guideline update. *J Clin Oncol* (2017) 35(5):561–4. doi: 10.1200/JCO.2016.71.0947

Frontiers in Oncology

Advances knowledge of carcinogenesis and tumor progression for better treatment and management

The third most-cited oncology journal, which highlights research in carcinogenesis and tumor progression, bridging the gap between basic research and applications to imrpove diagnosis, therapeutics and management strategies.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact