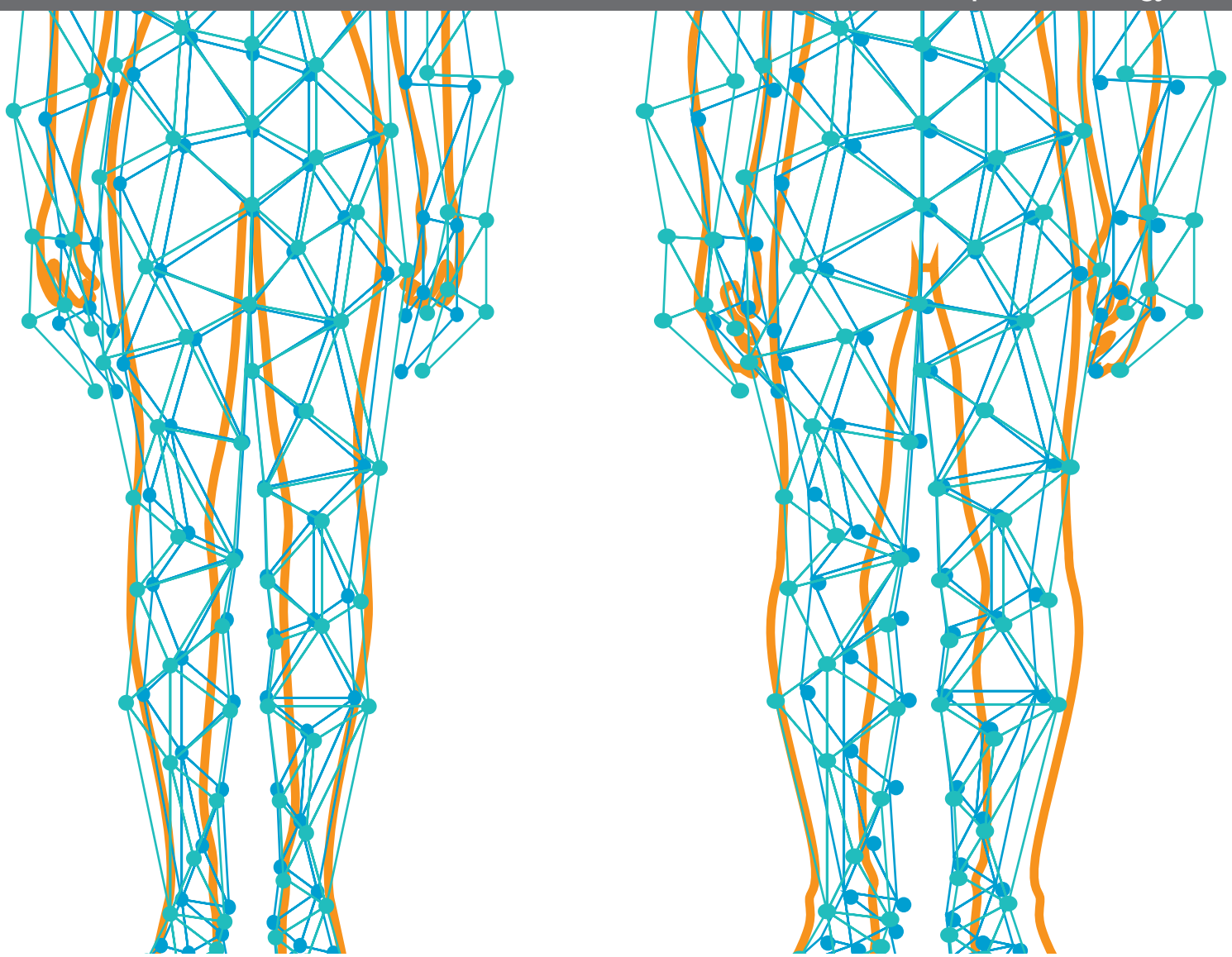
PERSPECTIVES IN PRIMARY PREVENTION RESEARCH FOR BREAST CANCER: A FOCUS ON GENE—ENVIRONMENT INTERACTIONS

EDITED BY: Sophie A. Lelièvre, Rabih Shakib Talhouk, Victoria Seewaldt, Mary Beth Terry and Martine Marie Bellanger

PUBLISHED IN: Frontiers in Medicine, Frontiers in Oncology, Frontiers in Public Health, Frontiers in Genetics and Frontiers in Cell and Developmental Biology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88966-698-0
DOI 10.3389/978-2-88966-698-0

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

PERSPECTIVES IN PRIMARY PREVENTION RESEARCH FOR BREAST CANCER: A FOCUS ON GENE—ENVIRONMENT INTERACTIONS

Topic Editors:
Sophie A. Lelièvre, Purdue University, United States
Rabih Shakib Talhouk, American University of Beirut, Lebanon
Victoria Seewaldt, Beckman Research Institute, United States
Mary Beth Terry, Columbia University, United States
Martine Marie Bellanger, UniCancer Group, France

Citation: Lelièvre, S. A., Talhouk, R. S., Seewaldt, V., Terry, M. B., Bellanger, M. M., eds. (2021). Perspectives in Primary Prevention Research for Breast Cancer: A Focus on Gene—Environment Interactions. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88966-698-0

Table of Contents

05 ***Editorial: Perspectives in Primary Prevention Research for Breast Cancer: A Focus on Gene—Environment Interactions***
Sophie A. Lelièvre, Martine Bellanger, Victoria Seewaldt, Rabih S. Talhouk and Mary Beth Terry

08 ***Epigeneti-What? Approaches on Translating Research for Primary Breast Cancer Prevention***
Evan K. Perrault, Grace M. Hildenbrand and Robert G. Nyaga

14 ***Connexin43 as a Tumor Suppressor: Proposed Connexin43 mRNA-circularRNAs-microRNAs Axis Towards Prevention and Early Detection in Breast Cancer***
Nataly Naser Al Deen, Mounir AbouHaidar and Rabih Talhouk

22 ***Breast Tissue Biology Expands the Possibilities for Prevention of Age-Related Breast Cancers***
Tara Fresques, Arrianna Zirbes, Sundus Shalabi, Susan Samson, Sandy Preto, Martha R. Stampfer and Mark A. LaBarge

33 ***The Many Faces of Obesity and Its Influence on Breast Cancer Risk***
Tanya Agurs-Collins, Sharon A. Ross and Barbara K. Dunn

47 ***Glyphosate Primes Mammary Cells for Tumorigenesis by Reprogramming the Epigenome in a TET3-Dependent Manner***
Manon Duforestel, Arulraj Nadaradjane, Gwenola Bougras-Cartron, Joséphine Briand, Christophe Olivier, Jean-Sébastien Frenel, François M. Vallette, Sophie A. Lelièvre and Pierre-François Cartron

61 ***Mobilizing Breast Cancer Prevention Research Through Smartphone Apps: A Systematic Review of the Literature***
Lauren C. Houghton, Renata E. Howland and Jasmine A. McDonald

76 ***Nutrition in the Prevention of Breast Cancer: A Middle Eastern Perspective***
Farah Naja, Lara Nasreddine, Sara Awada, Raeda El Sayed Ahmad and Nahla Hwalla

84 ***Radial Profile Analysis of Epithelial Polarity in Breast Acini: A Tool for Primary (Breast) Cancer Prevention***
Lawton Manning, Julia Holmes, Keith Bonin and Pierre-Alexandre Vidi

95 ***The Menstrual Cycle and Risk of Breast Cancer: A Review***
Håkan Lars Olsson and Mona Landin Olsson

99 ***Cost-Effectiveness of Lifestyle-Related Interventions for the Primary Prevention of Breast Cancer: A Rapid Review***
Martine Bellanger, Katharine Barry, Juwel Rana and Jean-Philippe Regnaux

109 ***Peripheral Blood-Based Biopsy for Breast Cancer Risk Prediction and Early Detection***
Farah J. Nassar, Ghada Chamandi, Mohamad Ali Tfaily, Nathalie Khoueiry Zgheib and Rihab Nasr

117 ***Breast Cancer and Nutrition: A Paradigm for Prevention in 3D Across the Life Course***
Michele R. Forman

123 ***Current and Emerging Magnetic Resonance-Based Techniques for Breast Cancer***
Apekshya Chhetri, Xin Li and Joseph V. Rispoli

140 ***Metformin and Chemoprevention: Potential for Heart-Healthy Targeting of Biologically Aggressive Breast Cancer***
Veronica C. Jones, Eric C. Dietze, Tijana Jovanovic-Talisman, Jeannine S. McCune and Victoria L. Seewaldt

frontiers in Medicine

EDITORIAL
published: 11 December 2020
doi: 10.3389/fmed.2020.621959

Editorial: Perspectives in Primary Prevention Research for Breast Cancer: A Focus on Gene—Environment Interactions

Sophie A. Lelièvre[1,2*], *Martine Bellanger*[3], *Victoria Seewaldt*[4], *Rabih S. Talhouk*[5] *and Mary Beth Terry*[6]

[1] Department of Basic Medical Sciences, Purdue University, West Lafayette, IN, United States, [2] Purdue University Center for Cancer Research, West Lafayette, IN, United States, [3] Institut de Cancérologie de L'Ouest, St Herblain, France, [4] Department of Population Sciences, Beckman Research Institute, City of Hope, Pasadena, CA, United States, [5] Department of Biological Sciences, American University of Beirut, Beirut, Lebanon, [6] Department of Epidemiology, Columbia University, New York, NY, United States

Keywords: economy, communication, epidemiology, aging, tissue homeostasis, chemoprevention, epigenome, biomarker

Editorial on the Research Topic

Perspectives in Primary Prevention Research for Breast Cancer: A Focus on Gene—Environment Interactions

INTRODUCTION

We initiated the collaborative research program "international breast cancer & nutrition" (IBCN) in 2010 (1), responding to the increasing trends in breast cancer (BC) incidence globally (2–4). World-wide there are many similarities, including the increasing incidence of BC in young women, which demands more research on changing environmental exposures and transitions, such as increasing obesity, shifting diets and lower fertility. This special issue has been dedicated to what the IBCN considers at the heart of the problem, namely the interplay between BC susceptibility genes and the environment (5). The articles outlined below illustrate the importance of transdisciplinary approaches and networks and fall into four categories that warrant attention to reduce the global burden of disease: (1) lifestyle modifiers of risk; (2) early detection and risk reduction; (3) new avenues in research; and (4) economic benefits of global BC prevention.

LIFESTYLE MODIFIERS OF RISK

The global increase of BC is especially high in the Middle East, and Naja et al. comprehensively summarize reasons for the increase with a specific focus on nutrition and obesity. Their detailed review offers hope of possible reversal of the incidence trends, as many of the risk factors that they outline are modifiable. Complementing this article is a thorough review by Agurs-Collins et al. on the mechanisms and metabolic pathways with which changes in body fat and nutrition can affect BC risk. Considering biomarkers in future etiologic studies as well as potential targets for intervention studies are important perspectives highlighted by these authors. Key to understanding how to modify BC risk is to grasp that just like breast tumors are 3D, it is helpful to think about their causes as 3D. Forman presents a novel framework to understand BC trends and etiology through the 3D lens of (1) windows of susceptibility, (2) duration and intensity of exposures, and (3) pace of development and trajectories. All risk factors are affected by issues of timing, even one of the

OPEN ACCESS

Edited and reviewed by:
Arch Mainous,
University of Florida, United States

***Correspondence:**
Sophie A. Lelièvre
lelievre@purdue.edu

Specialty section:
This article was submitted to Family Medicine and Primary Care, a section of the journal Frontiers in Medicine

Received: *27 October 2020*
Accepted: *19 November 2020*
Published: *11 December 2020*

Citation:
Lelièvre SA, Bellanger M, Seewaldt V, Talhouk RS and Terry MB (2020) Editorial: Perspectives in Primary Prevention Research for Breast Cancer: A Focus on Gene—Environment Interactions. Front. Med. 7:621959. doi: 10.3389/fmed.2020.621959

long-established risk factors for BC—age at menarche. Olsson and Olsson in an insightful mini-review remind us to think about the meaning of constructs and, rather than merely continuing to use age at menarche as a BC predictor, to focus more on menstrual activity (i.e., number of cycles before first pregnancy for premenopausal BC, and lifetime number of cycles for post-menopausal BC). This recommendation to focus on menstrual activity may be particularly useful for prediction as age at menarche is becoming more similar between countries, but there are still large gaps based on menstrual activity.

EARLY DETECTION AND RISK REDUCTION

There are recent technological advances and risk reduction efforts for BC. For example, Houghton et al. conducted a systematic review of BC management efforts that employ smartphone apps and identified two common themes of utilization, (1) clinical care coordination and (2) health care quality of life during and after a BC diagnosis. Moreover, an emerging interest in primary prevention is evidenced by apps that help predict BC risk and provide information related to primary BC prevention. There remain many opportunities to include for global use. The increase of BC incidence world-wide has spurred the need for cost-effective and minimally invasive early detection methods. Mammography screening is difficult to set up and less sensitive in "at high risk" young women with denser breasts. Nassar et al. discuss clinically easily accessible peripheral blood-based analysis of "liquid-biopsy." Specifically, they evaluate emerging biomarker strategies that include circulating miRNA, proteins and nucleic acids, with methylation patterns for the latter, as well as exosomes that might augment routine screening tests. However, biomarkers will only be truly valuable if risk reduction can be implemented. An example of promising chemoprevention is low toxicity metformin, for which Jones et al. discuss some of the mechanisms of action. Noteworthy, their review emphasizes the importance of integrating the use of safe medications with other aspects of an intervention aimed to "make the whole person healthy."

NEW AVENUES IN RESEARCH

Focusing on mechanisms that control tissue homeostasis is a widespread approach to study risk factors and identify targets to inhibit cancer onset. This goal necessitates 3D cell culture models of phenotypically normal breast tissue, since normal breast biopsies are seldom accessible in most countries. The recent connection, using such models, between increased body mass and loss of epithelial polarity demonstrates how biology and epidemiology may be merged to identify markers of risk (6). Models that recapitulate breast polarity will be a useful resource for *in vitro* screening of modulators of risk. To ease the screening method, Manning et al. are presenting the radial profile analysis, an algorithm that objectively quantifies polarity in epithelial glandular structures from immunofluorescence images (available on the Open Science Framework).

Transposing tissue alterations measurable *in vitro* to the real organ for risk detection purposes is one of the many challenges of primary prevention. Building from the recent demonstration that tumor suppressor connexin-43 (Cx43) controls breast epithelial polarity and cell multilayering (7, 8), in their minireview Naser Al Deen et al. propose that Cx43-derived circRNA and associated sponged miRNA might be attractive liquid biopsy biomarkers indicative of Cx43 mRNA levels in tissues, hence serving as a signature axis for BC risk. Another area of excitement is magnetic resonance (MR) with which methodology progress made on breast tumors paves the way for potential risk assessment. Imaging with MR assesses breast density, the increase of which is an aggravating factor of cancer risk (9). Chhetri et al. provide an insightful discussion of MR methods available for the breast and on how techniques, like contrast enhanced perfusion MR imaging and MR spectroscopy, might be applied to detect microstructural and physiological alterations that are signs of increased risk for cancer. Tissue integrity via maintenance of homeostasis is also the topic covered by Fresques et al. who propose sustaining tissue organization by driving progenitor cells to terminally differentiate, or alternatively reducing or delaying the innate age-related immune changes in the breast that include chronic low-grade sterile inflammation, known as inflammaging. A case is made for repurposing warfarin and metformin for prevention, since both drugs act in part by modulating aging-associated changes at the tissue level.

The ultimate demonstration of sustained deleterious impact of risk factors on tissue homeostasis is an alteration of the epigenome. Duforestel et al. bring evidence that the pesticide/herbicide glyphosate specifically alters the expression of genes controlled by the epigenetic enzyme TET3 and synergizes with miR-182 (part of oxidative stress associated with aging tissues) to trigger mammary tumors. This first demonstration that a pollutant can synergize with a physiological alteration of cells is a powerful illustration of the concept of multifactorial disease relevant to cancer. The detection of DNA methylation changes characteristic of glyphosate in the blood opens new directions for epigenetic biomarkers to reveal potentially persistent effects of exposures.

ECONOMIC BENEFITS OF GLOBAL BC PREVENTION

The effects of prevention are measurable in the future despite the perceived concern about the current value of any action. Bellanger et al. provide evidence of the cost-effectiveness of lifestyle related interventions for BC. From a societal perspective physical activity programs are highly cost effective for BC and other major non-communicable diseases, and low-fat diet programs for post-menopausal women are cost-effective for breast and ovarian cancers. These encouraging findings on healthier lifestyle influence deserve attention from both individuals and public decision makers. However, the inherent link between people's environment and the epigenome requires urgent efforts in communication to better translate research on primary prevention to the public and policymakers, as clearly

demonstrated by Perrault et al. Their core message is simple; researchers willing to advance their scientific knowledge have to be concomitantly willing to translate and disseminate their work to the public who will be able to act ultimately.

In sum, the articles in this issue highlight the importance of prevention to reverse the global rise in BC. In comparison to the large investment in BC therapies and detection, investment in primary prevention research has been much more limited. Prevention options like risk-reducing surgeries and chemoprevention for women at higher risk are restricted to certain countries and bring challenges like genetic testing and invasive interventions. In contrast, a focus on gene-environment interactions expands the perspectives in primary prevention research by adopting a holistic paradigm and promises a much wider population impact. The integration of environmental impact in health risk can be used extensively by policymakers while the world is currently facing many pandemics from COVID19 to climate change to widespread health inequities. A transdisciplinary approach through international collaborative networks like IBCN will be essential to continue to move forward and reduce the global burden of BC.

AUTHOR CONTRIBUTIONS

SL and MT have edited the different versions of the manuscript. All authors contributed to the writing of the manuscript.

ACKNOWLEDGMENTS

All coauthors are part of the International Breast Cancer and Nutrition-IBCN Project (www.purdue.edu/discoverypark/ibcn/). We thank all IBCN contributors and the IBCN External Advisory Board for their support of the Project.

REFERENCES

1. Lelièvre SA, Weaver CM. Global nutrition research: nutrition and breast cancer prevention as a model. *Nutrition Rev.* (2013) 71:742–52. doi: 10.1111/nure.12075
2. Torre LA, Islami F, Siegel RL, Ward EM, Jemal A. Global cancer in women: burden and trends. *Cancer Epidemiol Biomarkers Prev*. (2017) 26:444–57. doi: 10.1158/1055-9965.EPI-16-0858
3. Bellanger M, Zeinomar N, Tehranifar P, Terry MB. Are global breast cancer incidence and mortality patterns related to country-specific economic development and prevention strategies? *J Glob Oncol.* (2018) 4:1–16. doi: 10.1200/JGO.17.00207
4. 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
5. Teegarden D, Romieu I, Lelièvre SA. Redefining the impact of nutrition on breast cancer incidence: Is epigenetics involved? *Nutrition Res Rev.* (2012) 25:68–95. doi: 10.1017/S0954422411000199
6. Tenvooren I, Jenks MZ, Rashid H, Cook KL, Muhleman JK, Sistrunk C, et al. Elevated leptin disrupts epithelial polarity and primes cancer initiation in the mammary gland. *Oncogene.* (2019) 38:3855–70. doi: 10.1038/s41388-019-0687-8
7. Bazzoun D, Adissu H, Wang L, Urazaev A, Tenvooren I, Fostok SF, et al. Connexin 43 maintains tissue polarity and regulates mitotic spindle orientation in the breast epithelium. *J Cell Sci.* (2019) 132:jcs223313. doi: 10.1242/jcs.223313
8. Fostok SF, El-Sibai M, Bazzoun D, Lelièvre SA, Talhouk RS. Connexin 43 loss triggers cell cycle entry and invasion in non-neoplastic breast epithelium: a role for noncanonical Wnt signaling. *Cancers.* (2019) 11:E339. doi: 10.3390/cancers11030339
9. Bissell MCS, Kerlikowske K, Sprague BL, Tice JA, Gard CC, Tossas KY, et al. Breast cancer population attributable risk proportions associated with body mass index and breast density by race/ethnicity and menopausal status. *Cancer Epidemiol Biomarkers Prev.* (2020) 29:2048–56. doi: 10.1158/1055-9965.EPI-20-0358

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 in Oncology

PERSPECTIVE
published: 12 April 2019
doi: 10.3389/fonc.2019.00267

Epigeneti-What? Approaches on Translating Research for Primary Breast Cancer Prevention

Evan K. Perrault, Grace M. Hildenbrand and Robert G. Nyaga*

Brian Lamb School of Communication, Purdue University, West Lafayette, IN, United States

In fiscal year 2017, the National Cancer Institute devoted more than a half billion dollars to breast cancer research. Since 2012, the total investment has been more than $3 billion. Despite this significant investment, breast cancer still has no known immediate causes as it generally develops over the life course. Therefore, research is unable to provide the public any sort of magic bullet, or conclusive link between certain environmental exposures and the development of breast cancer later in life. What research is only able to report are likelihoods—possible links—things people might want to consider avoiding or doing in their everyday lives to reduce their future risks of developing breast cancer. This abundance of rigorously performed, albeit causally inconclusive, research focused on "plausible" links poses a challenge for health communicators who are tasked with seeking to find ways to translate this science into advice that people can act upon today. However, if society must wait for the science to provide 100% conclusive evidence before anyone ever takes action, how many lives could have been saved in the interim? Therefore, we advocate a two-pronged approach to translating scientific findings regarding environmental exposures and breast cancer prevention: a bottom-up approach—focused on informing the lay public and individuals, while simultaneously performing a top-down approach—focused on influencing policymakers. The current perspective analyzes the strengths and weaknesses to both of these approaches, and encourages scientists to work closely with health communicators to develop theoretically-driven strategies to drive positive changes over time.

Keywords: breast cancer, translation, prevention, health communication, epigenetics

OPEN ACCESS

Edited by:
Martine Marie Bellanger, École des Hautes Etudes en Santé Publique, France

Reviewed by:
Galya Bigman, University of Maryland, Baltimore, United States
Jessica Mudry, Ryerson University, Canada

****Correspondence:***
Evan K. Perrault
eperraul@purdue.edu

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

Received: *03 January 2019*
Accepted: *25 March 2019*
Published: *12 April 2019*

Citation:
Perrault EK, Hildenbrand GM and Nyaga RG (2019) Epigeneti-What? Approaches on Translating Research for Primary Breast Cancer Prevention. Front. Oncol. 9:267. doi: 10.3389/fonc.2019.00267

INTRODUCTION

The purpose of science is often described as a pursuit of new knowledge and understanding. This pursuit is awarded billions of publicly-funded dollars each year by governments around the globe. While much of this research is likely to find a home in pay-walled peer-reviewed publications for other scientists to read, very little is likely to make its way directly to the public—those who actually funded this research in the first place. New knowledge—knowledge that if placed in the right hands could potentially save lives—does no one any good if it sits on a shelf and is not shared with others. Given the enormous economic burden that cancer, especially breast cancer, places on society (1), it is imperative research findings that may provide a window into preventing cancers from ever occurring in populations be translated for public consumption.

One of these areas of research receiving significant support is the area of epigenetics, the study of how chemicals present in foods and drinks we all consume may alter gene expression through hormonal disruptions and increase breast cancer risks (2). While the term "epigenetics" has received a lot of attention in the media, most lay individuals do not adequately understand the term (3). This could be because the term is not all that easy to explain in a simple sound bite—often using highly complex multisyllabic vocabulary to discuss how it actually effects a person's biology [e.g., "methylation epigenetic modification;" (4)]. Unlike simply translating information from one language to another using electronic translators or dictionaries, there is no magic elixir for translating complex scientific information into ideas that are easily digestible for the lay public or policymakers to act upon.

Translating the latest epigenetic research simultaneously for these two primary target audiences is essential if we ever hope to achieve zero prevalence of breast cancer in society. Individuals have the power to make localized changes within their own personal spheres, while policymakers have the power to change entire societies through the enactment of new laws. However, reaching and changing these two very different groups will require shifts in standard tactics and strategies, true interdisciplinary collaborations between the biological and social sciences, and likely a good dose of patience. The following perspective labels these approaches bottom-up (focusing on reaching individuals directly) and top-down (focusing on reaching policymakers), and discusses the unique benefits and challenges to embarking on each of these approaches.

INDIVIDUAL FOCUS: THE BOTTOM-UP APPROACH

Going Beyond Traditional News Media to Reach Individuals

General news outlets have commonly been utilized as a popular means to help scientists spread their research beyond the walls of their laboratories to reach individuals in their homes. This is because, for usually very little cost and effort, institutions can write press releases about new research that sometimes get picked-up by the media, and have the potential to reach large segments of the lay public (5). However, while these news outlets can serve as a means to get stories out to large audiences, this dissemination often comes with a loss of message fidelity.

Only 28% of Americans state they think news outlets get science facts right most of the time (6). News stories on genetic research about cancer tend to not be as accurate as the press releases from which they obtain their information (7), and often shy away from reporting on cancer prevention-related topics (8). For example, only about 4% of news stories about breast cancer analyzed by Atkin et al. (8) covered environmental hazards such as risks connected to chemical contaminants. There are a host of potentially carcinogenic chemical compounds found in everyday household products that could be related to increased breast cancer risk later in life; for example: bisphenol A (BPA) found in plastics, butyl benzyl phthalate (BBP) used in food packages and cosmetics, and perfluoroactanoic acid (PFOA) which is contained in some industrial and consumer goods (9). These chemicals often consist of a confusing string of jargon for both journalists and the lay public to comprehend, making it daunting to determine which products to avoid, but more importantly the epigenetic science behind why individuals may want to refrain from using them in their daily lives.

Therefore, it is no wonder why the media tend to overgeneralize results from epigenetic scientific studies (e.g., stating cause-and-effect relationships) that instead should be approached with more nuance and tentative language (10). "The simplification that is often necessary for good, clear journalism can foster inferences that go far beyond the original observation from which the inferences were drawn" (10; p. 4). This is why medical professionals and researchers should actively seek collaborations with health-beat reporters to help them to see which risks should receive attention in stories, and also potentially serve as fact-checkers to help ensure the accuracy of the information ultimately shared with the public (11).

However, instead of relying on news personnel to essentially act as mediators between scientists and the lay public—who may inadvertently get the science incorrect—breast cancer researchers should be seeking to reach the public directly to educate them on the latest scientific research to reduce their breast cancer risks.

Communicating Scientific Uncertainty via the Precautionary Principle

Breast cancer researchers might be hesitant to advocate that individuals make various changes in their lives to reduce their breast cancer risks simply because no research regarding chemical influences on breast cancer and the human epigenome is 100% certain. While true, scientists need to realize that the science will never be 100% conclusive, and should therefore frame their research to the public around the precautionary principle. In other words, even though breast cancer prevention research continues to be ongoing, with findings that will likely never be able to truly find cause-and-effect relationships, letting the public at least know these findings may still make a difference by saving lives at an individual level (12). Research indicates that communicating this scientific uncertainty does not negatively influence the public's interest in science or perceptions of the trustworthiness of scientists (13), suggesting it is not detrimental for scientists to express that findings are uncertain.

Finding Allies in Health Communication

Scientists seeking ways to communicate their research to the lay public should look no further than colleagues they may have across campus in the liberal arts or humanities within the discipline of health communication. Health communicators are social scientists trained in the study of using evidence- and theory-based approaches to effectively change individuals' knowledge, attitudes and behaviors surrounding health topics (14). For example, projects stemming from health communicators embedded within the National Cancer Institute funded Breast Cancer and Environment Research Program (BCERP) found that higher literacy-level translated research regarding progesterone's potential impact on breast cancer was

actually more effective at increasing the public's perceptions of risk than messages translated to a lower literacy-level (15). Silk et al. (16) also found that the public does desire some level of scientific complexity in breast cancer prevention messaging in order to help them better understand the relevance of the research to their daily lives.

Health communication scholars are able to utilize a large toolbox of formative research skills (e.g., survey design, in-depth interviewing, data analysis) in order to determine which elements of theory should be emphasized in messages targeted to the lay public (17). For instance, Smith et al. (18) conducted research guided by the Heuristic Systematic Model to determine the way capability, motivation, and different types of processing result in particular beliefs and attitudes about environmental breast cancer risks from PFOA. Such theoretical guidance is essential to not only guarantee that resources are well spent, but also to ensure the public is motivated by messages that are developed to make well-informed decisions.

Strategies for Communicating Environmental Risk Factors

Developing highly tailored campaigns and interventions for specific target audiences is likely to yield the most promising results in changing the publics' knowledge, attitudes, and behaviors toward potential environmental breast cancer risks (19). When communicating uncertain scientific findings to the public, one effective strategy is to present multiple claims and then state how many experts believe each claim, generating perceptions of certainty about a scientific claim (20). If a breast cancer risk message conveys the number of scientists who believe there is a need to take environmental risks seriously, this might motivate members of the public to engage in precautionary behaviors such as avoiding consumer products that contain chemical toxins.

Scientists must also move beyond scholarly outlets to reach lay audiences (14). While members of the public mainly obtain scientific information from the media, they rely on multiple sources, using both online and traditional communication channels (21). Thus, a majority of health campaigns make use of multiple channels in order to reach the greatest number of people (22). In translating scientific information, communicators should select channels that are easily accessible by the public, and that capture their attention (23). Personal communication in the form of interpersonal influence is a valuable supplement to mass communication and a strong contributor to behavior change. Campaign managers would be wise to find key influencers in particular communities and persuade them to influence others (24). Another strategy that is effective at informing a lay audience about scientific information is using website videos to discuss possible environmental risks for breast cancer (25). Channels should be selected based on preferences of the target audience—not on the personal preferences of scientists.

Strengths and Weaknesses of a Bottom-Up Approach

One major strength of developing communication geared directly toward individual-level behavior change—compared to policies—is that individual-level change is rarely controversial. No one gets outraged when individuals decide to voluntarily change their diets, purchase behaviors, or exercise habits. Communication targeting individuals is also likely to lead to quicker changes (e.g., changing knowledge, attitudes, or behaviors) than communication seeking policy change, which can take years to pass and even longer to enact. However, one key weakness of this bottom-up approach is that these strategies tend to have only small to medium effects on influencing knowledge, attitudes, and behaviors (22). Reaching all members of a population is difficult, if not impossible, and even if people receive a message, this does not mean the amount of exposure was sufficient to fuel behavior change.

Therefore, a multi-pronged approach is advocated. While attempts are developed to help change the public at the individual level, scientists should simultaneously be working to change the minds of lawmakers to develop policies that could allow for a much more substantial impact on populations.

FOCUSING ON POLICYMAKERS: A TOP-DOWN APPROACH

Beyond communicating cancer research effectively to individuals and the public, there is also a need to anchor interventions on policies that protect their safety from carcinogens, and ensure penalties for industries whose products expose the public to cancer-related risks. The recent revelation that Johnson & Johnson may have known for decades that its talcum baby powder may have contained asbestos (26) showcases a need for policies to control potentially carcinogenic substances and highlight objective research that is devoid of potential influence by profit-driven industry players. To achieve such goals, it is important for scientists and policymakers to work together to formulate evidence-based cancer policies.

However, so far, policymakers and scientists seem disconnected (27, 28), and often do not share the same priorities and values (29–31). These tensions undermine the role of research in policymaking and attest to the need of dialogue between the two groups as a way of bolstering the progress made so far in the war on cancer.

Why Should Breast Cancer Researchers and Policymakers Work Together?

It is important for the policymaking and scientific communities to work closely together to ensure robust policies that address salient issues associated with breast cancer, such as exorbitant treatment costs, reduction of quality life years and loss of productivity due to employment disability, missed work days, and days spent in bed (32). This is particularly important because by 2020, the loss of present value of lifetime earnings (PVLE) due to cancer is estimated to be $147.6 billion, with breast cancer leading in the loss of PVLE among women below 55 years of age (33). Additionally, the caregiving costs associated with cancer in 2000 were estimated at $232.4 billion and are expected to rise to $308 billion by 2020 (33). In the non-elderly population, breast cancer has the second highest adjusted annual economic burden estimated at $14,167 after colorectal cancer (32). These high costs associated with breast cancer treatment point to the need

for epigenetic breast cancer prevention researchers to begin to advocate for policy changes that could lead to substantial benefits for populations decades and centuries into the future.

Effective Communication of Cancer Research to Policymakers

To enhance the effectiveness of breast cancer prevention research in informing policymaking, it is imperative that scientists communicate their research findings in a way that captures the attention of policymakers because some of them, especially legislators, are inundated by the volume of policy-related information they receive (34–36). One effective way to do this may not be by reaching out directly to policymakers, but instead by reaching them indirectly through the mass media—a strategy known as media advocacy (37). The goal of media advocacy is to use a mix of both paid (e.g., advertisements) and unpaid media (e.g., PSAs, grass roots organizing) to set the media's agenda and get a topic wide attention. When the topic is on the media's agenda (e.g., it is a lead story for multiple days), policymakers are sure to pay attention. For example, individual researchers, or organizations like the IBCN, could start by writing a series of Op-Eds regarding policy changes that could have an impact on reducing breast cancer (38). Researchers could also come out with a series of policy statements, and generate news coverage through manufactured press events (e.g., rallies, community demonstrations) that would appeal to news outlets. To enhance their persuasiveness, researchers could also make arguments for the wider relevance of their research by extrapolating their findings across states and/or countries (30, 39) thereby helping policymakers to understand the potential impact of their research and how novel policies could help to ameliorate the effects associated with breast cancer.

Researchers may also want to initially aim small in trying to achieve policy changes. Changes at the local level (e.g., city, county) are likely to take place much quicker than at a national level. These local level changes could also ultimately lead to much more significant changes. For example, products required in California through Proposition 65 to carry a message stating they contain chemicals known to the state to cause cancer, can oftentimes be found across the United States—thereby extending the impact of this local policy. Similarly, researchers could strive to enact a policy at one elementary school, one university, or in one city, banning the sale of certain foods or products that contain chemicals known to detrimentally effect the epigenome. This ban could then have ripples across the supply chain in a region, thereby essentially eliminating a potentially hazardous product in more than just the municipality with the ban.

Overall, to bridge the gap between scientists and policymakers, it is necessary for these two groups to build relationships and create avenues for effective deliberations. This participatory approach might encompass scholars inviting policymakers to their classes, or policymakers inviting researchers to their forums to offer input on cancer policies (28). Although researchers and policymakers have working differences, when policymakers are faced with dilemmas, they turn to academics for alternative agendas (40). Therefore, the role of scholars in generating policy issues cannot be underestimated. Kingdom (40) also advises that researchers join policy communities, which are composed of specialists in a given policy area. Such communities are important because they can help scientists to build networks with advocacy groups, enhance their understanding of the information needs of policymakers, and have opportunities to learn health policy language (34, 41).

Strengths and Weaknesses of a Top-Down Approach

The clear strength of the top-down approach is that changing policy is likely to have long-lasting effects on society. For example, enacting policies to fluoridate public water supplies has led to significant reductions in cavities over the last 70 years, and is cited as one of the top-10 public health achievements of the 20th century (42). However, changing policies is likely to be a much lengthier endeavor than seeking to change individual behaviors through campaign efforts. Therefore, advocating policy change should be seen as part of a comprehensive strategy—alongside individual behavior change—to achieve breast cancer prevention.

CONCLUSION

In conclusion, neither the bottom-up nor top-down strategy should be used in isolation. While utilizing the bottom-up approach researchers are likely to see effects rather quickly, but these effects will likely be limited to small pockets of populations, and potentially not very long lasting. Utilizing the top-down approach is likely to yield much larger dividends, but it also comes with a much longer time commitment, and no guarantee of success after years of advocacy work. To maximize return-on-investment, breast cancer prevention researchers should seek to translate their findings simultaneously along both of these routes, and seek guidance from interdisciplinary colleagues trained in their intricacies—those in the health communication discipline.

If researchers truly want to advance knowledge, part of that advancement has to be translating and disseminating their work to the public to help them act on it in meaningful ways. Until breast cancer prevention researchers are ready to work comprehensively and share resources across disciplinary boundaries with those in communication, it is likely researchers' advancement of knowledge will stop at the peer-reviewed publication of their work—relegated to a dusty shelf or seldom used online depository—and society will potentially be no better off for it.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

Thank you to International Breast Cancer and Nutrition (IBCN) for providing the funding for the open-access publication fee.

REFERENCES

1. Blumen H, Fitch K, Polkus V. Comparison of treatment costs for breast cancer, by tumor stage and type of service. *Am Health Drug Benefits.* (2016) 9:23–32.
2. Romagnolo DF, Daniels KD, Grunwald JT, Ramos SA, Propper CR, Selmin OI. Epigenetics of breast cancer: modifying role of environmental and bioactive food compounds. *Mol Nutr Food Res.* (2016) 60:1310–29. doi: 10.1002/mnfr.201501063
3. Henikoff S, Greally JM. Epigenetics, cellular memory and gene regulation. *Curr Biol.* (2016) 26:R644–R648. doi: 10.1016/j.cub.2016.06.011
4. U.S. National Library of Medicine. *What is Epigenetics?* (2019). Retrieved from https://ghr.nlm.nih.gov/primer/howgeneswork/epigenome (accessed February 17, 2019).
5. Davis LS. Outreach activities by universities as a channel for science communication. In: Tan Wee Hin L, Subramaniam R, editors. *Communicating Science to the Public.* Dordrecht: Springer (2014). p. 161–81.
6. Pew Research Center. *Science News and Information Today: a Majority of Americans Rely on General Outlets for Science News but More Say Specialty Sources Get the Facts Right About Science.* (2017). Retrieved from http://www.journalism.org/2017/09/20/science-news-and-information-today/ (accessed February 17, 2019).
7. Brechman JM, Lee CJ, Cappella JN. Distorting genetic research about cancer: from bench science to press release to published news. *J Commun.* (2011) 61:496–513. doi: 10.1111/j.1460-2466.2011.01550.x
8. Atkin CK, Smith SW, McFeters C, Ferguson V. A comprehensive analysis of breast cancer news coverage in leading media outlets focusing on environmental risks and prevention. *J Health Commun.* (2008) 13:3–19.doi: 10.1080/10810730701806912
9. Hiatt RA, Brody JG. Environmental determinants of breast cancer. *Annu Rev Public Health.* (2018) 39:113–33.doi: 10.1146/annurev-publhealth-040617-014101
10. Yehuda R, Lehrner A, Bierer LM. The public reception of putative epigenetic mechanisms in the transgenerational effects of trauma. *Environ Epigenet.* (2018) 4:1–7.doi: 10.1093/eep/dvy018
11. Park H, Reber BH. Using public relations to promote health: a framing analysis of public relations strategies among health associations. *J Health Commun.* (2010) 15:39–54.doi: 10.1080/10810730903460534
12. Davis DL, Axelrod D, Bailey L, Gaynor M, Sasco AJ. Rethinking breast cancer risk and the environment: the case for the precautionary principle. *Environ Health Perspect.* (1998) 106:523–9.doi: 10.1289/ehp.98106523
13. Retzbach A, Maier M. Communicating scientific uncertainty: media effects on public engagement with science. *Communic Res.* (2015) 42:429–56.doi: 10.1177/0093650214534967
14. Kreps G. Translating health communication research into practice: the importance of implementing and sustaining evidence-based health communication interventions. *Atl J Commun.* (2012) 20:5–15.doi: 10.1080/15456870.2012.637024
15. Hitt R, Perrault E, Smith S, Keating DM, Nazione S, Silk K, et al. Scientific message translation and the heuristic systematic model: Insights for designing educational messages about progesterone and breast cancer risks. *J Cancer Educ.* (2016) 31:389–96.doi: 10.1007/s13187-015-0835-y
16. Silk KJ, Perrault EK, Neuberger L, Rogers A, Atkin C, Barlow J, et al. Translating and testing breast cancer risk reduction messages for mothers of adolescent girls. *J Health Commun.* (2014) 19:226–43.doi: 10.1080/10810730.2013.811322
17. Atkin CK, Freimuth VS. Formative evaluation research in campaign design. In: Rice RE, Atkin CK, editors. *Public Communication Campaigns.* 3rd ed. Thousand Oaks, CA: Sage (2001). p. 125–45.
18. Smith SW, Hitt R, Russell J, Nazione S, Silk K, Atkin CK, et al. Risk belief and attitude formation from translated scientific messages about PFOA, an environmental risk associated with breast cancer. *Health Commun.* (2017) 32:279–87.doi: 10.1080/10410236.2016.1138350
19. Wakefield MA, Loken B, Hornik RC. Use of mass media campaigns to change health behaviour. *Lancet.* (2010) 376:1261–71.doi: 10.1016/S0140-6736(10)60809-4
20. Dunwoody S, Kohl PA. Using weight-of-experts messaging to communicate accurately about contested science. *Sci Commun.* (2017) 39:338–57.doi: 10.1177/1075547017707765
21. Yi-Fan Su L, Akin H, Brossard D, Scheufele DA, Xenos MA. Science news consumption patterns and their implications for public understanding of science. *Journal Mass Commun Q.* (2015) 92:597–616.doi: 10.1177/1077699015586415
22. Noar SM. A 10-year retrospective of research in health mass media campaigns: where do we go from here? *J Health Commun.* (2006) 11:21–42.doi: 10.1080/10810730500461059
23. Field H, Powell P. Public understanding of science versus public understanding of research. *Public Underst Sci.* (2001) 10:421–6.doi: 10.1088/0963-6625/10/4/305
24. Silk KJ, Atkin CK, Salmon CT. Developing effective media campaigns for health promotion. In: Thompson TL, Parrott R, Nussbaum JF, editors. *The Routledge Handbook of Health Communication.* 2nd ed. New York, NY: Routledge (2011). p. 203–19.
25. Perrault EK, Silk KJ. Testing the effects of the addition of videos to a website promoting environmental breast cancer risk reduction practices: are videos worth it? *J Appl Commun Res.* (2014) 42:20–40.doi: 10.1080/00909882.2013.854400
26. Meyersohn N. Johnson and Johnson shares plunge after report that says it knew about asbestos in its baby powder. *CNN.* (2018, December 14). Retrieved from https://www.cnn.com/2018/12/14/business/johnson-and-johnson-stock-baby-powder-asbestos/index.html (accessed December 29, 2018).
27. Bogenschneider K, Corbett TJ. *Evidence-Based Policymaking. insights From Policy-Minded Researchers and Research-Minded Policymakers.* New York, NY: Routledge (2010).
28. Romich J, Fentress T. The policy roundtable model: encouraging scholar-practitioner collaborations to address poverty-related social problems. *J Soc Serv Res.* (2018) 45:76–86.doi: 10.1080/01488376.2018.1479343
29. Brownson RC, Dodson EA, Stamatakis KA, Casey CM, Elliott MB, Luke DA, et al. Communicating evidence-based information on cancer prevention to state-level policy makers. *J Natl Cancer Inst.* (2011) 103:306–16. doi: 10.1093/jnci/djq529
30. Jone E, Kreuter M, Pritchett S, Matulionis RM, Hann N. State health policy makers: what's the message and who's listening? *Health Promot Pract.* (2006) 7:280–6.doi: 10.1177/1524839906289583
31. Parker LA, Lumbreras B, Hernández-Aguado I. Health information and advocacy for "Health in All Policies": a research agenda. *J Epidemiol Community Health.* (2010) 64:114–6.doi: 10.1136/jech.2008.081976
32. Zheng Z, Yabroff R, Guy GP, Han X, Li C, Banegas MP, et al. Annual medical expenditure and productivity loss among colorectal, female breast, and prostate cancer survivors in the United States. *J Natl Cancer Inst.* (2016) 108:1–9.doi: 10.1093/jnci/djv382
33. Bradley CJ, Yabroff RK, Dahman B, Feuer EJ, Mariotto A, Brown ML. Productivity costs of cancer mortality in the United States: 2000–2020. . *J Natl Cancer Inst.* (2008) 100:1763–70.doi: 10.1093/jnci/djn384
34. McBride T, Coburn A, MacKinney C, Mueller K, Slifkin R, Wakefield M. Bridging health research and policy: Effective dissemination strategies. *J Public Health Manag Pract.* (2008) 14:150–4.doi: 10.1097/01.PHH.0000311893.80701.7a
35. Sorian R, Baugh T. Power of information: closing the gap between research and policy. When it comes to conveying complex information to busy policy-makers, a picture is truly worth a thousand words. *Health Aff.* (2002) 21:264–73.doi: 10.1377/hlthaff.21.2.264
36. Tabak RG, Eyler AA, Dodson EA, Brownson RC. Accessing evidence to inform public health policy: a study to enhance advocacy. *Public Health.* (2015) 129:698–704.doi: 10.1016/j.puhe.2015.02.016
37. Dorfman L, Wallack L. Putting policy into health communication. In: Rice RE, Atkin CK, editors. *Public Communication Campaigns.* 4th ed. Thousand Oaks, CA: Sage (2013). p. 335–48.
38. Coppock A, Ekins E, Kirby D. The long-lasting effects of newspaper op-eds on public opinion. *Quart J Polit Sci.* (2018) 13:59–87.doi: 10.1561/100.00016112

39. Brownson RC, Chriqui JF, Stamatakis KA. Understanding evidence-based public health policy. *Am J Public Health.* (2009) 99:1576–83.doi: 10.2105/AJPH.2008.156224
40. Kingdom JW. *Agendas, Alternatives, and Public Policies*. Harlow: Pearson Education Limited (2014).
41. Nutbeam D, Boxall A. What influences the transfer of research into health policy and practice? Observations from England and Australia. *Public Health.* (2008) 122:747–53.doi: 10.1016/j.puhe.2008.04.020
42. Centers for Disease Control and Prevention (CDC). *Community Water Fluoridation.* (2016). Retrieved from https://www.cdc.gov/fluoridation/index.html (accessed December 29, 2018).

Conflict of Interest Statement: 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 in Medicine

MINI REVIEW
published: 28 August 2019
doi: 10.3389/fmed.2019.00192

Connexin43 as a Tumor Suppressor: Proposed Connexin43 mRNA-circularRNAs-microRNAs Axis Towards Prevention and Early Detection in Breast Cancer

Nataly Naser Al Deen[1†], *Mounir AbouHaidar*[2] *and Rabih Talhouk*[1*†]

[1] *Department of Biology, Faculty of Arts and Sciences, American University of Beirut, Beirut, Lebanon,* [2] *Department of Cell and Systems Biology, Faculty of Arts and Sciences, University of Toronto, Toronto, ON, Canada*

OPEN ACCESS

Edited by:
Salvatore Piscuoglio, University Hospital of Basel, Switzerland

Reviewed by:
Brian D. Adams, Brain Institute of America, United States
Bhupinder Pal, Olivia Newton-John Cancer Research Institute, Australia

***Correspondence:**
Rabih Talhouk
rtalhouk@aub.edu.lb

[†]*Members of International Breast Cancer and Nutrition (IBCN) Project*

Specialty section:
This article was submitted to Pathology, a section of the journal Frontiers in Medicine

Received: *01 May 2019*
Accepted: *14 August 2019*
Published: *28 August 2019*

Citation:
Naser Al Deen N, AbouHaidar M and Talhouk R (2019) Connexin43 as a Tumor Suppressor: Proposed Connexin43 mRNA-circularRNAs-microRNAs Axis Towards Prevention and Early Detection in Breast Cancer. Front. Med. 6:192. doi: 10.3389/fmed.2019.00192

Breast cancer (BC) is a global public health burden, constituting the highest cancer incidence in women worldwide. Connexin43 (Cx43) is a member of a family of transmembrane proteins responsible in part for intercellular communication between adjacent breast epithelial cells, via gap junctions. Cx43 plays key role in mammary gland development and differentiation and its spatio-temporal perturbation contributes to tumorigenesis. Thus, Cx43 acts as a breast tumor-suppressor. Signaling pathways and phenotypes downstream of Cx43 mRNA loss/mis-localization in breast cells have been well-studied. However, axes parallel to Cx43 loss are less understood. microRNAs (miRNAs) are small endogenous non-coding RNAs that repress translation and circularRNAs (circRNAs) are a class of endogenous RNAs that originate from RNA splicing and act as miRNA "sponges". CircRNAs and miRNAs are dysregulated in cancers and are highly abundant and stable in the circulation. Thus, they present as attractive liquid biopsy cancer biomarkers. Here, an axis for Cx43 mRNA-circRNAs-miRNAs interactions along BC initiation (denoted by loss of breast epithelial polarity and development of hyperplastic phenotypes) is proposed to potentially serve as a signature biomarker toward BC early-onset detection and prevention.

Keywords: gap junctions, connexins, breast cancer, microRNAs, circularRNAs, tumor-suppressors, biomarkers, prevention

INTRODUCTION

BC registers the highest incidence and mortality rates in females and is the second most commonly diagnosed cancer (after lung cancer) (1). Incidence of early-onset BC in young women is alarming and has increased drastically (2–4). It is crucial to focus on non-invasive biomarkers and active players in BC early initiation processes, toward prevention and early detection (5). The mammary gland undergoes extensive remodeling during development, from prenatal to post lactation stages (6, 7). Lobules, milk ducts, connective tissues, and adipose tissues constitute the mature human female breast. Functional centers that link a lobule to its terminal duct and to the ductal system are terminal duct lobular units (TDLUs). Each lobule contains group of alveoli, responsible for milk secretion during lactation. Both ducts and alveoli are lined by luminal epithelial cells, forming

ductal and lobular epithelium, respectively, which in turn are lined by discontinuous layer of myoepithelial cells and are separated by a supporting basement membrane. The latter is underlain by the stroma, an extracellular matrix (ECM) and stromal cells, including fibroblasts, adipocytes, endothelial cells, and immune cells (8–10).

Mammary gland development requires well-orchestrated cell-cell and cell-ECM communication by gap junctions and systemic signals. Connexins (Cxs) are a family of transmembrane proteins. They are responsible for establishing gap junction intercellular communication (GJIC), capable of linking cytoplasm of two neighboring cells, allowing intercellular exchange of ions, second messengers, and metabolites (11–13). Each GJ channel is made up of two docked connexons, spanning the two membrane bilayers of adjacent cells, whereby each connexon forms by oligomerization of hexagonally arrayed connexins (14). GJs mediate channel-dependent and channel-independent functions. Any perturbations in Cxs expression/localization may alter the function of the gland and lead to tumorigenesis. Cxs act as tumor-suppressors, in a context-dependent manner, like Cx43, the focus of this review (8, 9) (**Figure 1**).

Recently, we revealed an apicolateral distribution/localization of Cx43 in luminal human breast epithelium, and that loss of Cx43 expression contributes to breast tumorigenesis by disrupting apical polarity and promoting cell multi-layering, a hallmark of tumor initiation (17). Furthermore, populations at higher risk of BC (like obese patients) exhibit loss of Cx43 apical distribution and cell multi-layering in an inflammatory microenvironment (21, 22). Studies from our group have characterized pathways and phenotypes downstream of Cx43 loss/mis-localization in 3D human breast epithelial HMT-3522 S1 cells (16, 17, 23–25). Hence, an axis that parallels Cx43 mRNA loss will be proposed. miRNAs

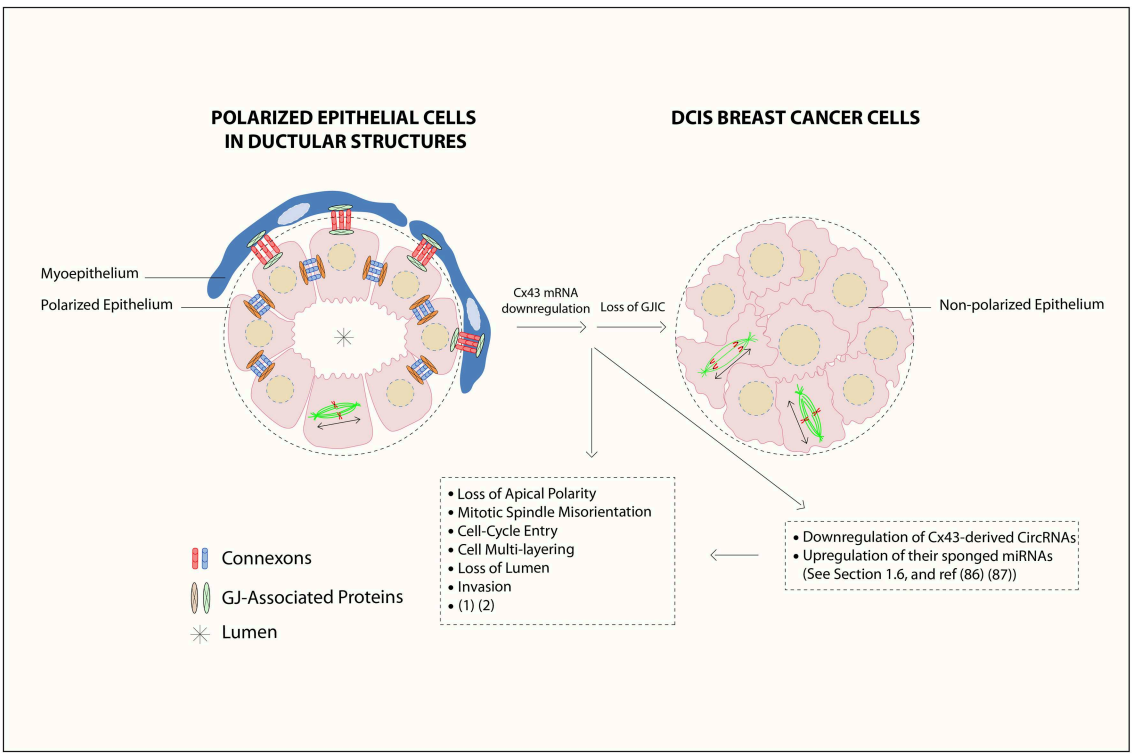


FIGURE 1 | Gap junction (GJ) complex dis-assembly in breast cancer initiation. In normal differentiated mammary epithelium, the cells polarize with apical, and basolateral domains and assemble membranous GJs between epithelial cells and between epithelial and myoepithelial cells. Mammary Cxs (), including Cx43, form a complex assembly with GJ-Associated Proteins () such as ZO-2, α- and β-catenins (15) in a differentiated epithelial cell. At the primary tumor site, the downregulation of Cx43 mRNA levels leads to loss of gap junction intercellular communication (GJIC) and dissociation of GJ-associated proteins complexes, which in turn causes loss of communication between neighboring cells, activation of cellular proliferation, and alteration in polarity protein distribution. Loss of apical polarity, mitotic spindle misorientation, cell cycle entry, cell multi-layering, loss of lumen (), and enhanced invasive capability in Cx43 knock out breast epithelial cells is also reported (16, 17). Mitotic-spindle orientation (MSO) is depicted based on the directionality of the α-tubulin poles, either parallel to the basement membrane [or tangential to the circumference of the growing acini], which is the proper MSO to maintain a monolayered epithelium (in polarized epithelial cells in ductular structures), in contrast to cell multilayering (in DCIS breast cancer cells). Double-headed arrows indicate MSO. Thus, Cx43 contributes to breast epithelial polarity and proper MSO in single layered mammary epithelial cells, whereas its loss contributes to disrupted polarity and MSO and multilayering, which are hallmarks of tumor initiation. In this review, an axis by Cx43-derived circRNAs and their sponged miRNAs is proposed during BC initiation stages, which almost parallels the roles of Cx43 mRNA down-regulation and GJIC loss. This is denoted by loss of breast epithelial polarity and development of hyperplastic phenotypes (18, 19). The axis might act as promising biomarker signature toward BC early-onset detection and prevention, as discussed in section Cx43 mRNA-circRNAs-miRNAs Axis [Figure is modified from El-Saghir et al. (20)].

are small non-coding RNAs that repress translation, and circRNAs originate from RNA splicing and act as miRNA "sponges" (26, 27). CircRNAs and miRNAs unique dysregulation signatures in cancers (in tissue- and development stage-specific manner), their tumor suppressive/oncogenic roles and stability and abundance in body fluids make them attractive non-invasive biomarkers in liquid biopsies (5, 27). Here, an axis by Cx43-derived circRNAs and their sponged miRNAs is proposed during BC initiation stages, which might act as promising biomarker signature toward BC early-onset detection and prevention, especially in patients at increased risk.

CX43 IN NORMAL MAMMARY GLAND DEVELOPMENT AND DIFFERENTIATION

GJs play major role in establishing communication between adjacent cells (20, 28–30) and studying mice made it possible to infer Cxs spatio-temporal expression patterns across mammary gland development (31). The mammary gland expresses Cx43 in myoepithelial and epithelial cells junction (23), whereby Cx43 mRNA levels drop half-way through gestation and lactation, while its active phosphorylated form is evident during lactation (9). Autosomal dominant Cx43 mutant mice ($Cx43^{I130T/+}$) exhibited delay in ductal elongation and atrophied glands pre-puberty (32). Myoepithelial contractility was inhibited upon Cx43 knockdown or GJIC blockage in primary mammary organoids of wild-type mice (33). Substituting Cx43 levels with Cx32 retarded growth and survival of ($Cx43^{Cx32/+}$) heterozygous knock-in pups, due to perturbation in milk ejection (34). These studies confirm Cx43 pivotal role along mammary gland development. We also demonstrated crucial roles for Cx43 in mammary epithelial differentiation, which relied on proper GJ complex assembly composed of Cx43, α-catenin, β-catenin, and ZO-2 (15). Thus, studying Cx43 perturbation is important in understanding early events in breast cellular transformation.

PERTURBATIONS IN CX43: CX43 AS TUMOR SUPPRESSOR/BIOMARKER IN BC

Since the mammary gland development is sensitive to perturbations in Cx43 expression, localization and function, Cx43 plays a tumor-suppressive role and contributes to breast tumorigenesis, in a context- and stage-dependent manner (35–39). Overexpression of Cx43 in MCF-7 and MDA-MB-231 BC cells significantly decreased cells proliferation and nuclear levels of β-catenin in 3D cultures, which was mediated by membranous Cx43 recruitment of α-catenin, β-catenin and ZO-2 (24). McLachlan et al. (40) linked an impedance of tumor growth to upregulation of Cx43 *in vivo*, by favoring a mesenchymal to epithelial transition. Recently, we showed for the first time an apicolateral distribution and localization of Cx43 in luminal breast epithelium. Further, we showed that silencing Cx43 expression contributes to breast tumorigenesis by enhancing proliferation and cell cycle progression and inducing mis-localization of membranous β-catenin, resulting in loss of apical polarity, misorientation of mitotic spindle, cell multi-layering, and loss of lumen (hallmarks of tumor initiation). Silencing Cx43 activates signaling pathways that promote invasion in non-tumorigenic breast epithelium (16, 17). Similarly, Lesko et al. (41) showed that disruption of epithelial polarity was a marker of epithelial-derived tumor initiation.

Teleki et al. (42, 43) conducted a meta-analysis on Cx isotype expression data in breast tissue microarray from patients from all tumor grades. Their results showed, both in normal and breast tissues, the expression of Cx43, Cx46, Cx26, Cx30, and Cx32. Of the detected Cxs, only Cx43 correlated with improved disease prognosis and served as better prognostic marker than vascular invasion or necrosis. High levels of Cx43 in grade 2 tumors marked them as good relapse free survival subgroups. Other microarray results from tissue samples of invasive breast carcinoma patients showed that Cx43 levels positively correlated with progesterone and estrogen receptor status, but negatively correlated with Ki67 (proliferation marker) expression (44). In contrast, high levels of Cx43 was detected in BC patient biopsies at later tumor stages, suggesting its potential role in inducing tumor progression (45, 46). This is since during invasion, the tumor epithelial cells may reactivate GJIC with endothelial cells to facilitate intravasation/extravasation (20). Thus, Cx43 acts as a tumor suppressor in normal breast tissues, its loss/mis-localization contributes to BC initiation, its high levels in the primary tumor serves as a good prognostic marker while its re-expression at later tumor-stages facilitates invasion and metastasis (20).

INTERACTIONS BETWEEN CONNEXINS AND microRNAs

Recent studies reported two possible modes of interaction/regulation between miRNAs and Cxs. The first through direct binding of miRNAs to 3'-UTR of mRNAs coding for Cxs and other junctional proteins, and the second via direct transfer of candidate miRNAs through gap junctions between neighboring cells. Lin et al. (47) correlated BC distant metastasis to opposite expression levels of miR-206 and Cx43 in triple-negative MDA-MB-231 cells via miR-206 direct binding to Cx43-3'UTR. Inhibition of miR-206 caused an increase in Cx43 levels with significant upregulation in cell proliferation, migration, and invasion. Chang et al. (48) showed that low expression levels of miR-30a increased BC invasion and metastasis, while rescuing miR-30a levels caused cancer cells to switch from mesenchymal to epithelial etiology, by inhibiting interactions between Slug and claudin promoter (tight junction proteins). Oligonucleotides (size of siRNAs) passed only through Cx43/Cx43 GJ channels (49) and transfer of miR-5096 between tumor and endothelial cells was mediated by GJs in co-cultures of glioblastoma (U87) and microvascular endothelial (HMEC) cells (50).

Cxs-miRNAs interactions are important not only for their regulatory roles, but also for their biomarker potential. Current

available BC prognostic and diagnostic tests exhibit limitations (26). Serum antigens like carcinoembryonic antigen (CEA) and cancer antigen 153 (CA153) exhibit low sensitivity (51). Other tests require patient tissue biopsies, like Oncotype DX test, which estimates recurrence likelihood, MammaPrint, a prognostic test, and Veridex 76-gene signature, a diagnostic test that predicts distant metastasis in ER+ patients (52). Furthermore, mammograms usually display high false positive rates and do not detect cancers in young patients (53, 54). Amongst the BC diagnostic miRNAs, onco-miR-21 was significantly upregulated in plasma/serum and in frozen/ Formalin-Fixed, Paraffin-Embedded BC tissues compared to their normal counterparts in various ethnic cohorts (55). miR-155 and miR-18a were upregulated in sera and tissues of different ethnic cohorts and in sera of ER+ BC patients, respectively (26). Among the prognostic biomarkers, miR-106b predicted risk of high recurrence and shorter overall survival, while miR-122 was over-expressed in sera of relapsed patients and predicted metastasis (56). miR-18b, miR-103, miR-107, and miR-652 predicted recurrence and decreased overall survival in triple-negative BC patients (57). Therefore, Cxs and miRNAs serve as promising biomarkers for BC initiation and progression.

CIRCULARRNAS BIOGENESIS, FUNCTIONS, AND BIOMARKER ROLES IN BC

CircRNAs are known to regulate miRNAs function and biogenesis and dysregulated mRNA-circRNAs-miRNAs axes may act as signatures in cancers (58–61). CircRNAs are generated from RNA splicing (conserved sequences AG GT) by back ligation. CircRNAs are covalently closed continuous loops without $5^{'}$ cap or $3^{'}$ polyadenylated tail and are resistant to exonucleases (e.g., RNase R), which degrade linear RNA. They are structurally stable and their isolation and purification is easy. CircRNAs are expressed in tissue- and- developmental stage-specific manner and primarily localize to the cytoplasm and function as miRNA sponges (sequestering miRNAs and enhancing mRNAs stability and translation) (62–64). Known functions of circRNAs are sponging miRNAs and RNA-binding proteins (RBP)s, regulating cell cycle (e.g., FOXO3 circRNA in BC) (65), translation of few exonic circRNAs with an open reading frame (66), acting as scaffolds in protein complexes assembly (66), protein sequestration from subcellular localization (67), modulating parental gene expression (68), and regulating alternative splicing (69, 70). CircRNAs are primarily located in the cytoplasm and are up to 10 times more abundant than their linear counterparts (71), are released from cell lines via exosomes and microvesicles (72), are differentially expressed in exosomes from mice with tumors compared to healthy controls (59) and hundreds of circRNAs are significantly upregulated in human blood compared to their linear counterparts (73).

Several studies have reported a role for circRNAs in the initiation and progression of BC through acting as competing endogenous miRNA sponges. Xie et al. (74) identified differentially expressed circRNAs in BC tissues, and described circ_0004771/miR-653/ZEB2 as potential regulatory feedback axis for treatment of BC. Knockdown of hsa_circ_0004771 and ZEB2 exhibited similar functions as using miR-653 mimics to promote growth inhibition and apoptosis in BC cells. Tang et al. (75) revealed that hsa_circ_0001982 was significantly overexpressed in tissues and cell lines, whereby circ_0001982 knockdown suppressed BC cell proliferation and invasion and induced apoptosis by targeting miR-143. Xu et al. (76) detected circTADA2A-E5 and circTADA2A-E6, among five most differentially expressed circRNAs in large cohort of triple-negative BC (TNBC) patients, whose downregulation associated with poor survival. Through sponging miR-203a-3p, and therefore restoring the expression of its target *SOCS3*, circTADA2A-E6 suppressed proliferation, migration, and invasion *in vitro* and possessed tumor-suppressive capability. Thus, circTADA2A-E6/miR-203a-3p/*SOCS3* might act as a promising prognostic biomarker in TNBC.

In a validation BC patient cohort, circ_103110, circ_104689, and circ_104821 levels were elevated and were predicted to sponge oncogenic miR-339-5p, miR-143-5p, miR-409-3p, miR-153-3p, and miR-145-5p. Moreover, circ_006054, circ_100219, and circ_406697 were downregulated and were predicted to sponge miR-298, miR-485-3p, and miR-100 (miRNAs involved in pathways in BC). Thus, these circRNAs are important promoters of carcinogenesis and may be useful biomarkers for BC (77). Nair et al. (78) identified 256, 288, and 411 tumor-specific circRNAs in triple negative, estrogen receptor positive, and HER2-positive BC subtypes, respectively, from 885 samples from The Cancer Genome Atlas. The tumor suppressor, circ-Foxo3, significantly downregulated in BC patients and cell lines (79), likely contributes to BC progression (71) and its levels significantly increase when cancer cells undergo apoptosis. Upon knockdown of endogenous circ-Foxo3, cell viability was enhanced, while its ectopic expression inhibited xenografts tumor growth and prompted stress-induced apoptosis by upregulating PUMA and downregulating p53 (79). Moreover, circ-ABCB10 was upregulated in BC and its knockdown *in vitro* suppressed proliferation and enhanced apoptosis through sponging miR-1271 (80, 81). The upregulation of circ-Amotl1 in cancer patients and cell lines exhibited tumorigenic capacity through interacting with proto-oncogene, c-myc (82).

Although there exists a correlation between obesity and loss of Cx43 apical distribution and cell multi-layering in breast epithelial tissues in an inflammatory micro-environment (21, 22), no studies have linked so far the involvement of adipocytes in regulating Cx43-derived circRNAs or their sponged miRNAs. However, few studies have reported the exchange of circRNAs between adipocytes and tumor cells in other cancers (83, 84). Through activating PRDM16 and suppressing miR-133, exosomes from gastric cancer cells shuttle ciRS-133 into pre-adipocytes, thus stimulating differentiation into brown-like cells (83). CircRNAs in exosomes secreted from adipocytes stimulated growth of hepatocellular carcinoma and decreased DNA damage by suppressing miR-34a and activating USP7/Cyclin A2 signaling pathway (84). CircRNAs thus serve as an attractive new class of cancer biomarker axes (85).

Cx43 mRNA-circRNAs-miRNAs AXIS

Cx43 acts as a tumor suppressor, its loss/mis-localization is an important player in breast tumor initiation (16), plays role in BC progression (17) and places some individuals (obese women) at increased risk of BC (21, 22). Follow-up on differential expression levels of Cx43 mRNA in breast tissues requires tissue biopsies. We thus predict that circulating Cx43-derived circRNAs and their sponged miRNAs could be indicative of Cx43 mRNA levels in tissues (86), and might serve as non-invasive biomarker signatures for breast cancer initiation and prevention.

To predict human circRNA isoforms that originate from linear Cx43 (GJA1) transcript, CircularRNA Interactome was used and three Cx43-derived circRNA isoforms (circ_0077753, circ_0077754, and circ_0077755) along with their sponged miRNAs were identified (66) (**Supplementary Table 1**). We propose that a drop in circulating Cx43-derived circRNAs levels might reflect downregulation of Cx43 expression in breast epithelial tissue. Most of the sponged miRNAs by all three Cx43-derived circRNAs isoforms are involved in cancer-related signaling pathways, as predicted by miRSystem database (87). These circRNAs associate with early events of breast tumorigenesis and are referred to hereafter as "initiation circRNAs." Thus, when Cx43-derived circRNAs levels drop, their sponged miRNAs are expected to be relieved, and might be free to induce downstream cancer-initiating pathways. Indeed, upregulation of predicted sponged miRNAs by the three "initiation circRNAs" is involved in oncogenic initiation pathways, cellular multi-layering, and loss in organization in BC (18, 19). For instance, of the predicted sponged miRNAs, miR-182, miR-375, and miR-203 were found up-regulated during lobular neoplasia progression and miR-375 associated with loss of breast cellular organization and development of hyperplastic phenotypes. These miRNAs were indicative of a transition from lobular carcinoma *in situ* (LCIS), a benign precursor lesion, to invasive breast lobular carcinoma (ILC) (18, 19). Overexpression of oncomiRs, miR-21, miR-155, miR-10b, miR-373, and miR-520 was observed in many breast tumors (19), of which oncomiRs, miR-520g, and miR-520h are potentially sponged by two "initiation circRNAs." Therefore, the axis parallel to Cx43 mRNA loss, denoted by "initiation" Cx43-derived circRNAs and their sponged miRNAs seems to recapitulate phenotypes along BC initiation.

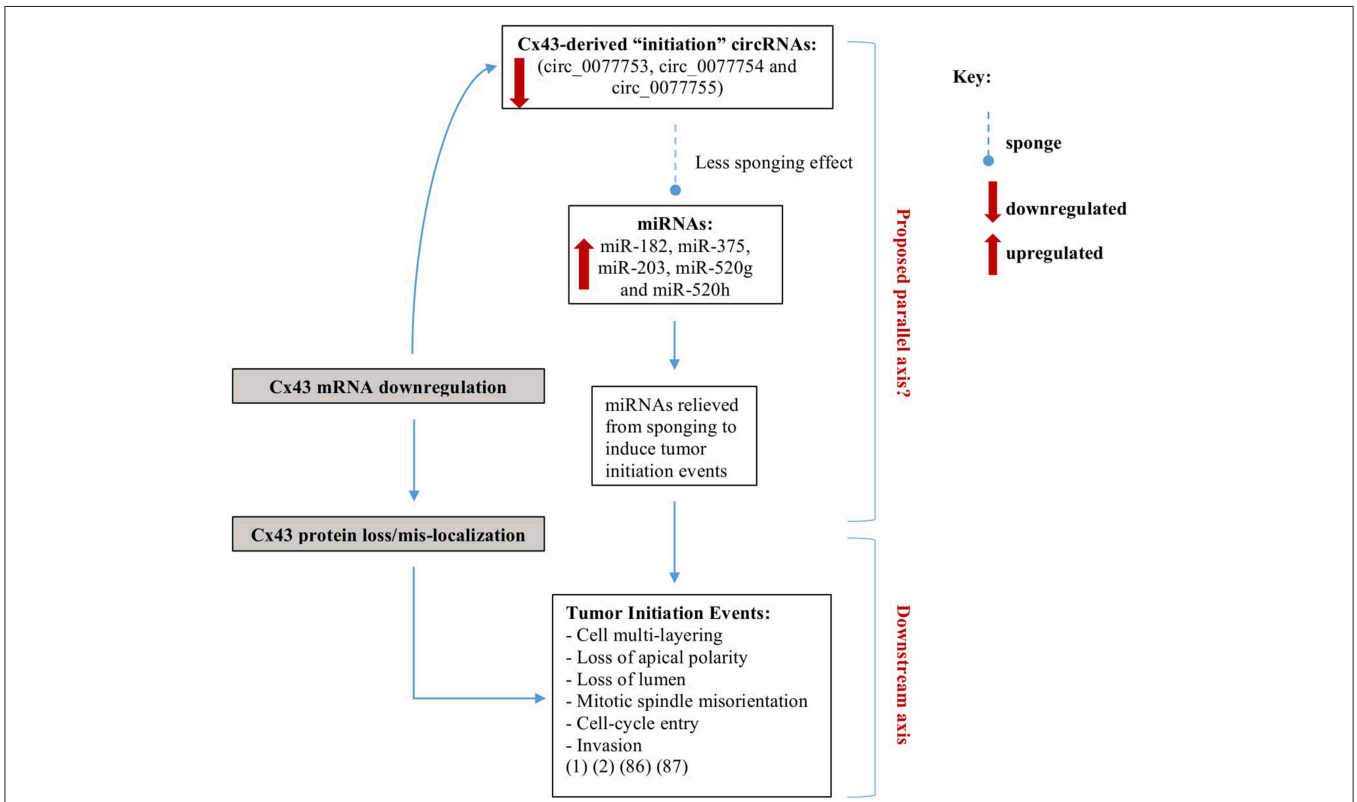


FIGURE 2 | Axes parallel to and downstream of Cx43 loss in breast cancer initiation. We recently showed that silencing Cx43 expression contributes to breast tumorigenesis by enhancing proliferation and cell cycle progression and inducing mis-localization of membranous β-catenin, resulting in loss of apical polarity, misorientation of mitotic spindle, cell multi-layering, and loss of lumen (hallmarks of tumor initiation) and by activating signaling pathways that promote invasion in non-tumorigenic breast epithelium (16, 17). We propose a possible parallel signature axis of Cx43 mRNA-circRNAs-miRNAs in BC early-onset for detection and prevention, which recapitulates the roles Cx43 loss plays along breast tumorigenesis. The Cx43 mRNA- "initiation circRNAs"-miRNAs axis is denoted by three "initiation circRNAs" (circ_0077753, circ_0077754, and circ_0077755) (66) and a panel of their sponged miRNAs, miR-182, miR-375, miR-203, miR-520g, and miR-520h. When the initiation Cx43-derived circRNAs levels drop, their sponged miRNAs are expected to be relieved, and might be free to induce downstream tumor-initiation pathways (18, 19).

CONCLUSION

In this review, we propose a possible biomarker signature axis of Cx43 mRNA-circRNAs-miRNAs in BC early-onset detection and prevention. We highlighted potential regulatory roles that Cx43-derived circulating circRNAs and their sponged miRNAs may play, which almost parallels the differential roles Cx43 plays along breast tumorigenesis. The Cx43 mRNA- "initiation circRNAs"-miRNAs axis is denoted by three "initiation circRNAs" and a panel of their sponged miRNAs (identified to date in the literature), miR-182, miR-375, miR-203, miR-520g, and miR-520h. This axis, when dysregulated in breast tissues, recapitulates phenotypes due to loss of Cx43 mRNA, associated with loss epithelial polarity and cell-multilayering during initiation stages of tumorigenesis (**Figure 2**) (16–19).

However, circRNAs and miRNAs present with few caveats that should be addressed. Interestingly, the proposed Cx43-derived circRNAs may circumvent them. First, miRNAs and circRNAs are highly expressed in circulating blood cells and their increased levels in blood might be due to high number of blood cells. Future studies thus should focus on defining actual abundance of circRNAs in different sub-populations of blood cells, characterize their mode of transportation in serum and plasma and devise markers that predict their origin (88). Cx43, however, is abundant in endothelial cells of large arteries (at aortic and coronary arteries branch points) but not in circulating blood cells (89). Thus, Cx43-derived circRNAs in plasma and sera are expected to surpass this caveat. Secondly, some circRNAs are differentially expressed in cancer tissues compared to normal adjacent tissues, but not in plasma or sera of patients compared to healthy controls (27). Thus, Cx43-derived circRNAs can overcome this caveat through future studies that compare Cx43-derived circRNAs levels in plasma to Cx43 mRNA levels in tissues of patients at risk, patients with early-stages of the disease and those with more aggressive etiologies. Therefore, it is worth further investigating the proposed "initiation" Cx43-derived circRNAs and their sponged miRNAs signatures toward BC early-onset detection and prevention.

AUTHOR CONTRIBUTIONS

NN and RT assembled the relevant literature and proposed the axes. NN performed the *in silico* analysis. RT and MA mentored NN throughout the writing process and critically revised all the drafts and approved the final version for submission.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr. Sabreen Fostok and Dr. Nadine Mahfouz for their critical reading of the manuscript and Ms. Nancy Naser Al Deen for illustrating **Figure 1**. The authors would also like to acknowledge the support of the Lebanese National Council for Scientific Research (CNRS-L), the University Research Board (URB-AUB), and the International Breast Cancer and Nutrition (IBCN) project at Purdue University and the Commitment Fund Center (CFC) Research Grant University of Toronto. RT and NN are members of IBCN. NN was the recipient of the AUB-CNRS-L scholarship. Publication fees are covered by the IBCN Project.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2019.00192/full#supplementary-material

REFERENCES

1. 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
2. Musallam KM, Shamseddine AI. Cancer epidemiology in lebanon. *Middle East J Cancer.* (2010) 1:41–4. Available online at: http://mejc.sums.ac.ir/article_41921_8649e5feb8aee74e4a92ce8b6e01f817.pdf
3. Porter P. "Westernizing" women's risks? Breast cancer in lower-income countries. *N Engl J Med.* (2008) 358:213–6. doi: 10.1056/NEJMp0708307
4. Anderson LN, Cotterchio M, Boucher BA, Kreiger N. Phytoestrogen intake from foods, during adolescence and adulthood, and risk of breast cancer by estrogen and progesterone receptor tumor subgroup among Ontario women. *Int J Cancer.* (2013) 132:1683–92. doi: 10.1002/ijc.27788
5. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* (2004) 116:281–97. doi: 10.1016/S0092-8674(04)00045-5
6. Paine IS, Lewis MT. The terminal end bud: the little engine that could. *J Mammary Gland Biol Neoplasia.* (2017) 22:93–108. doi: 10.1007/s10911-017-9372-0
7. Musumeci G, Castrogiovanni P, Szychlinska MA, Aiello FC, Vecchio GM, Salvatorelli L, et al. Mammary gland: from embryogenesis to adult life. *Acta Histochem.* (2015) 117(4–5):379–85. doi: 10.1016/j.acthis.2015.02.013
8. Bazzoun D, Lelièvre S, Talhouk R. Beyond the channel: role of connexins in regulating normal and cancerous processes in the mammary gland. In: Kandouz M, editor. *Intercellular Communication in Cancer.* Dordrecht: Springer (2015). p. 1–28. doi: 10.1007/978-94-017-7380-5_1
9. Fostok SF, El-Sibai M, El-Sabban M, Talhouk RS. Gap junctions and Wnt signaling in the mammary gland: a cross-talk? *J Mammary Gland Biol Neoplasia.* (2018) 24:1–22. doi: 10.1007/s10911-018-9411-5
10. Howard BA, Gusterson BA. Human breast development. *J Mammary Gland Biol Neoplasia.* (2000) 5:119–37. doi: 10.1023/A:1026487120779
11. Dbouk HA, Mroue RM, El-Sabban ME, Talhouk RS. Connexins: a myriad of functions extending beyond assembly of gap junction channels. *Cell Commun Signal.* (2009) 7:4. doi: 10.1186/1478-811X-7-4
12. Leithe E, Mesnil M, Aasen T. The connexin 43 C-terminus: a tail of many tales. *Biochim Biophys Acta Biomembr.* (2018) 1860:48–64. doi: 10.1016/j.bbamem.2017.05.008
13. Su V, Lau AF. Connexins: mechanisms regulating protein levels and intercellular communication. *FEBS Lett.* (2014) 588:1212–20. doi: 10.1016/j.febslet.2014.01.013
14. Grek CL, Rhett JM, Bruce JS, Ghatnekar GS, Yeh ES. Connexin 43, breast cancer tumor suppressor: missed connections? *Cancer Lett.* (2016) 374:117–26. doi: 10.1016/j.canlet.2016.02.008
15. Talhouk RS, Mroue R, Mokalled M, Abi-Mosleh L, Nehme R, Ismail A, et al. Heterocellular interaction enhances recruitment of α and β-catenins and ZO-2 into functional gap-junction complexes and induces gap junction-dependant differentiation of mammary epithelial cells. *Exp Cell Res.* (2008) 314:3275–91. doi: 10.1016/j.yexcr.2008.07.030

16. Bazzoun D, Adissu H, Wang L, Urazaev A, Tenvooren I, Fostok S, et al. Connexin 43 maintains tissue polarity and regulates mitotic spindle orientation in the breast epithelium. *J Cell Sci.* (2019) 132:jcs223313. doi: 10.1242/jcs.223313
17. Fostok S, El-Sibai M, Bazzoun D, Lelièvre S, Talhouk R. Connexin 43 loss triggers cell cycle entry and invasion in non-neoplastic breast epithelium: a role for noncanonical wnt signaling. *Cancers*. (2019) 11:339. doi: 10.3390/cancers11030339
18. Giricz O, Reynolds PA, Ramnauth A, Liu C, Wang T, Stead L, et al. Hsa-miR-375 is differentially expressed during breast lobular neoplasia and promotes loss of mammary acinar polarity. *J Pathol.* (2012) 226:108–19. doi: 10.1002/path.2978
19. O'Day E, Lal A. MicroRNAs and their target gene networks in breast cancer. *Breast Cancer Res*. (2010) 12:201. doi: 10.1186/bcr2484
20. El-Saghir JA, El-Habre ET, El-Sabban ME, Talhouk RS. Connexins: a junctional crossroad to breast cancer. *Int J Dev Biol.* (2011) 55:773–80. doi: 10.1387/ijdb.113372je
21. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer*. (2004) 4:579–91. doi: 10.1038/nrc1408
22. Deng T, Lyon CJ, Bergin S, Caligiuri MA, Hsueh WA. Obesity, inflammation, and cancer. *Annu Rev Pathol.* (2016) 11:421–49. doi: 10.1146/annurev-pathol-012615-044359
23. Talhouk RS, Elble RC, Bassam R, Daher M, Sfeir A, Mosleh LA, et al. Developmental expression patterns and regulation of connexins in the mouse mammary gland: expression of connexin30 in lactogenesis. *Cell Tissue Res.* (2005) 319:49–59. doi: 10.1007/s00441-004-0915-5
24. Talhouk RS, Fares M-B, Rahme GJ, Hariri HH, Rayess T, Dbouk HA, et al. Context dependent reversion of tumor phenotype by connexin-43 expression in MDA-MB231 cells and MCF-7 cells: role of β-catenin/connexin43 association. *Exp Cell Res.* (2013) 319:3065–80. doi: 10.1016/j.yexcr.2013.10.002
25. Talhouk RS, Khalil AA, Bajjani R, Rahme GJ, El-Sabban ME. Gap junctions mediate STAT5-independent β-casein expression in CID-9 mammary epithelial cells. *Cell Commun Adhesion*. (2011) 18:104–16. doi: 10.3109/15419061.2011.639468
26. Nassar FJ, Nasr R, Talhouk R. MicroRNAs as biomarkers for early breast cancer diagnosis, prognosis and therapy prediction. *Pharmacol Therapeut*. (2017) 172:34–49. doi: 10.1016/j.pharmthera.2016.11.012
27. Zhang Z, Yang T, Xiao J. Circular RNAs: promising biomarkers for human diseases. *EBioMedicine*. (2018) 34:267–74. doi: 10.1016/j.ebiom.2018.07.036
28. Kelly JJ, Simek J, Laird DW. Mechanisms linking connexin mutations to human diseases. *Cell Tissue Res.* (2015) 360:701–21. doi: 10.1007/s00441-014-2024-4
29. Laird DW, Fistouris P, Batist G, Alpert L, Huynh HT, Carystinos GD, et al. Deficiency of connexin43 gap junctions is an independent marker for breast tumors. *Cancer Res*. (1999) 59:4104–10.
30. Naus CC, Laird DW. Implications and challenges of connexin connections to cancer. *Nat Rev Cancer*. (2010) 10:435. doi: 10.1038/nrc2841
31. Roarty K, Shore AN, Creighton CJ, Rosen JM. Ror2 regulates branching, differentiation, and actin-cytoskeletal dynamics within the mammary epithelium. *J Cell Biol.* (2015) 208:351–66. doi: 10.1083/jcb.201408058
32. Stewart MK, Gong X-Q, Barr KJ, Bai D, Fishman GI, Laird DW. The severity of mammary gland developmental defects is linked to the overall functional status of Cx43 as revealed by genetically modified mice. *Biochem J.* (2013) 449:401–13. doi: 10.1042/BJ20121070
33. Mroue R, Inman J, Mott J, Budunova I, Bissell MJ. Asymmetric expression of connexins between luminal epithelial-and myoepithelial-cells is essential for contractile function of the mammary gland. *Dev Biol.* (2015) 399:15–26. doi: 10.1016/j.ydbio.2014.11.026
34. Plum A, Hallas G, Magin T, Dombrowski F, Hagendorff A, Schumacher B, et al. Unique and shared functions of different connexins in mice. *Curr Biol.* (2000) 10:1083–91. doi: 10.1016/S0960-9822(00)00690-4
35. Kanczuga-Koda L, Sulkowska M, Koda M, Reszec J, Famulski W, Baltaziak M, et al. Expression of connexin 43 in breast cancer in comparison with mammary dysplasia and the normal mammary gland. *Folia Morphol.* (2003) 62:439–42. Available online at: https://journals.viamedica.pl/folia_morphologica/article/view/16326/12963
36. Kanczuga-Koda L, Sulkowska M, Koda M, Rutkowski R, Sulkowski S. Increased expression of gap junction protein–connexin 32 in lymph node metastases of human ductal breast cancer. *Folia Histochem Cytobiol.* (2007) 45 (Suppl 1):S175–80. Available online at: https://journals.viamedica.pl/folia_histochemica_cytobiologica/article/view/4481/3736
37. Kanczuga-Koda L, Sulkowski S, Lenczewski A, Koda M, Wincewicz A, Baltaziak M, et al. Increased expression of connexins 26 and 43 in lymph node metastases of breast cancer. *J Clin Pathol.* (2006) 59:429–33. doi: 10.1136/jcp.2005.029272
38. Kanczuga-Koda L, Sulkowski S, Tomaszewski J, Koda M, Sulkowska M, Przystupa W, et al. Connexins 26 and 43 correlate with Bak, but not with Bcl-2 protein in breast cancer. *Oncol Rep*. (2005) 14:325–9. doi: 10.3892/or.14.2.325
39. Singal R, Tu Z, Vanwert J, Ginder G, Kiang D. Modulation of the connexin26 tumor suppressor gene expression through methylation in human mammary epithelial cell lines. *Anticancer Res.* (2000) 20:59–64. Available online at: https://europepmc.org/abstract/med/10769635
40. McLachlan E, Shao Q, Wang H-l, Langlois S, Laird DW. Connexins act as tumor suppressors in three-dimensional mammary cell organoids by regulating differentiation and angiogenesis. *Cancer Res*. (2006) 66:9886–94. doi: 10.1158/0008-5472.CAN-05-4302
41. Lesko AC, Goss KH, Yang FF, Schwertner A, Hulur I, Onel K, et al. The APC tumor suppressor is required for epithelial cell polarization and three-dimensional morphogenesis. *Biochim Biophys Acta.* (2015) 1853:711–23. doi: 10.1016/j.bbamcr.2014.12.036
42. Teleki I, Krenacs T, Szasz MA, Kulka J, Wichmann B, Leo C, et al. The potential prognostic value of connexin 26 and 46 expression in neoadjuvant-treated breast cancer. *BMC Cancer.* (2013) 13:50. doi: 10.1186/1471-2407-13-50
43. Teleki I, Szasz AM, Maros ME, Gyorffy B, Kulka J, Meggyeshazi N, et al. Correlations of differentially expressed gap junction connexins Cx26, Cx30, Cx32, Cx43 and Cx46 with breast cancer progression and prognosis. *PLoS ONE*. (2014) 9:e112541. doi: 10.1371/journal.pone.0112541
44. Conklin C, Huntsman D, Yorida E, Makretsov N, Turbin D, Bechberger JF, et al. Tissue microarray analysis of connexin expression and its prognostic significance in human breast cancer. *Cancer Lett*. (2007) 255:284–94. doi: 10.1016/j.canlet.2007.05.001
45. Jamieson S, Going JJ, D'Arcy R, George WD. Expression of gap junction proteins connexin 26 and connexin 43 in normal human breast and in breast tumours. *J Pathol.* (1998) 184:37–43. doi: 10.1002/(SICI)1096-9896(199801)184:1<37::AID-PATH966>3.0.CO;2-D
46. Naoi Y, Miyoshi Y, Taguchi T, Kim SJ, Arai T, Tamaki Y, et al. Connexin26 expression is associated with lymphatic vessel invasion and poor prognosis in human breast cancer. *Breast Cancer Res Treat*. (2007) 106:11–7. doi: 10.1007/s10549-006-9465-8
47. Lin ZJ, Ming J, Yang L, Du JZ, Wang N, Luo HJ. Mechanism of regulatory effect of microRNA-206 on connexin 43 in distant metastasis of breast cancer. *Chin Med J*. (2016) 129:424–34. doi: 10.4103/0366-6999.176071
48. Chang CW, Yu JC, Hsieh YH, Yao CC, Chao JI, Chen PM, et al. MicroRNA-30a increases tight junction protein expression to suppress the epithelial-mesenchymal transition and metastasis by targeting Slug in breast cancer. *Oncotarget*. (2016) 7:16462–78. doi: 10.18632/oncotarget.7656
49. Valiunas V, Polosina Y, Miller H, Potapova I, Valiuniene L, Doronin S, et al. Connexin-specific cell-to-cell transfer of short interfering RNA by gap junctions. *J Physiol.* (2005) 568:459–68. doi: 10.1113/jphysiol.2005.090985
50. Thuringer D, Boucher J, Jego G, Pernet N, Cronier L, Hammann A, et al. Transfer of functional microRNAs between glioblastoma and microvascular endothelial cells through gap junctions. *Oncotarget*. (2016) 7:73925. doi: 10.18632/oncotarget.12136
51. Shao Y, Sun X, He Y, Liu C, Liu H. Elevated levels of serum tumor markers CEA and CA15–3 are prognostic parameters for different molecular subtypes of breast cancer. *PLoS ONE*. (2015) 10:e0133830. doi: 10.1371/journal.pone.0133830
52. Dobbe E, Gurney K, Kiekow S, Lafferty JS, Kolesar JM. Gene-expression assays: new tools to individualize treatment of early-stage breast cancer. *Am J Health Syst Pharm.* (2008) 65:23–8. doi: 10.2146/ajhp060352
53. 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

54. Checka CM, Chun JE, Schnabel FR, Lee J, Toth H. The relationship of mammographic density and age: implications for breast cancer screening. *AJR Am J Roentgenol.* (2012) 198:W292–5. doi: 10.2214/AJR.10.6049
55. Asaga S, Kuo C, Nguyen T, Terpenning M, Giuliano AE, Hoon DS. Direct serum assay for microRNA-21 concentrations in early and advanced breast cancer. *Clin Chem.* (2011) 57:84–91. doi: 10.1373/clinchem.2010.151845
56. Wu X, Somlo G, Yu Y, Palomares MR, Li AX, Zhou W, et al. *De novo* sequencing of circulating miRNAs identifies novel markers predicting clinical outcome of locally advanced breast cancer. *J Transl Med.* (2012) 10:42. doi: 10.1186/1479-5876-10-42
57. Sahlberg KK, Bottai G, Naume B, Burwinkel B, Calin GA, Børresen-Dale A-L, et al. A serum microRNA signature predicts tumor relapse and survival in triple-negative breast cancer patients. *Clin Cancer Res.* (2015) 21:1207–14. doi: 10.1158/1078-0432.CCR-14-2011
58. Hansen TB, Kjems J, Damgaard CK. Circular RNA and miR-7 in cancer. *Cancer Res.* (2013) 73:5609–12. doi: 10.1158/0008-5472.CAN-13-1568
59. Li F, Zhang L, Li W, Deng J, Zheng J, An M, et al. Circular RNA ITCH has inhibitory effect on ESCC by suppressing the Wnt/β-catenin pathway. *Oncotarget*. (2015) 6:6001. doi: 10.18632/oncotarget.3469
60. Wang X, Zhang Y, Huang L, Zhang J, Pan F, Li B, et al. Decreased expression of hsa_circ_001988 in colorectal cancer and its clinical significances. *Int J Clin Exp Pathol.* (2015) 8:16020.
61. Zhang M, Huang N, Yang X, Luo J, Yan S, Xiao F, et al. A novel protein encoded by the circular form of the SHPRH gene suppresses glioma tumorigenesis. *Oncogene*. (2018) 37:1805. doi: 10.1038/s41388-017-0019-9
62. Cocquerelle C, Mascrez B, Hetuin D, Bailleul B. Mis-splicing yields circular RNA molecules. *FASEB J.* (1993) 7:155–60. doi: 10.1096/fasebj.7.1.7678559
63. Hansen TB, Wiklund ED, Bramsen JB, Villadsen SB, Statham AL, Clark SJ, et al. miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA. *EMBO J.* (2011) 30:4414–22. doi: 10.1038/emboj.2011.359
64. Nigro J, Cho K, Fearon E, Kern S, Ruppert J, Oliner J, et al. Scrambled exons. *Cell.* (1991) 64:607–13. doi: 10.1016/0092-8674(91)90244-S
65. Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. *Nucleic Acids Res.* (2016) 44:2846–58. doi: 10.1093/nar/gkw027
66. Dudekula DB, Panda AC, Grammatikakis I, De S, Abdelmohsen K, Gorospe M. CircInteractome: a web tool for exploring circular RNAs and their interacting proteins and microRNAs. *RNA Biol.* (2016) 13:34–42. doi: 10.1080/15476286.2015.1128065
67. Armakola M, Higgins MJ, Figley MD, Barmada SJ, Scarborough EA, Diaz Z, et al. Inhibition of RNA lariat debranching enzyme suppresses TDP-43 toxicity in ALS disease models. *Nat Genet.* (2012) 44:1302. doi: 10.1038/ng.2434
68. Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, et al. Corrigendum: exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol.* (2017) 24:194. doi: 10.1038/nsmb0217-194a
69. Meng S, Zhou H, Feng Z, Xu Z, Tang Y, Li P, et al. CircRNA: functions and properties of a novel potential biomarker for cancer. *Mol Cancer*. (2017) 16:94. doi: 10.1186/s12943-017-0663-2
70. Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, et al. circRNA biogenesis competes with pre-mRNA splicing. *Mol Cell.* (2014) 56:55–66. doi: 10.1016/j.molcel.2014.08.019
71. Lu W-Y. Roles of the circular RNA circ-Foxo3 in breast cancer progression. *Cell Cycle.* (2017) 16:589. doi: 10.1080/15384101.2017.1278935
72. Lasda E, Parker R. Circular RNAs co-precipitate with extracellular vesicles: a possible mechanism for circRNA clearance. *PLoS ONE.* (2016) 11:e0148407. doi: 10.1371/journal.pone.0148407
73. Memczak S, Papavasileiou P, Peters O, Rajewsky N. Identification and characterization of circular RNAs as a new class of putative biomarkers in human blood. *PLoS ONE.* (2015) 10:e0141214. doi: 10.1371/journal.pone.0141214
74. Xie R, Tang J, Zhu X, Jiang H. Silencing of hsa_circ_0004771 inhibits proliferation and induces apoptosis in breast cancer through activation of miR-653 by targeting ZEB2 signaling pathway. *Biosci Rep.* (2019) 39:BSR20181919. doi: 10.1042/BSR20181919
75. Tang Y-Y, Zhao P, Zou T-N, Duan J-J, Zhi R, Yang S-Y, et al. Circular RNA hsa_circ_0001982 promotes breast cancer cell carcinogenesis through decreasing miR-143. *DNA Cell Biol.* (2017) 36:901–8. doi: 10.1089/dna.2017.3862
76. Xu J-Z, Shao C-C, Wang X-J, Zhao X, Chen J-Q, Ouyang Y-X, et al. circTADA2As suppress breast cancer progression and metastasis via targeting miR-203a-3p/SOCS3 axis. *Cell Death Dis.* (2019) 10:175. doi: 10.1038/s41419-019-1382-y
77. Lü L, Sun J, Shi P, Kong W, Xu K, He B, et al. Identification of circular RNAs as a promising new class of diagnostic biomarkers for human breast cancer. *Oncotarget*. (2017) 8:44096. doi: 10.18632/oncotarget.17307
78. Nair AA, Niu N, Tang X, Thompson KJ, Wang L, Kocher J-P, et al. Circular RNAs and their associations with breast cancer subtypes. *Oncotarget*. (2016) 7:80967. doi: 10.18632/oncotarget.13134
79. Du WW, Fang L, Yang W, Wu N, Awan FM, Yang Z, et al. Induction of tumor apoptosis through a circular RNA enhancing Foxo3 activity. *Cell Death Differ.* (2017) 24:357–70. doi: 10.1038/cdd.2016.133
80. Liang H-F, Zhang X-Z, Liu B-G, Jia G-T, Li W-L. Circular RNA circ-ABCB10 promotes breast cancer proliferation and progression through sponging miR-1271. *Am J Cancer Res.* (2017) 7:1566–76.
81. Kristensen L, Hansen T, Venø M, Kjems J. Circular RNAs in cancer: opportunities and challenges in the field. *Oncogene*. (2018) 37:555–65. doi: 10.1038/onc.2017.361
82. Yang Q, Du WW, Wu N, Yang W, Awan FM, Fang L, et al. A circular RNA promotes tumorigenesis by inducing c-myc nuclear translocation. *Cell Death Differ.* (2017) 24:1609–20. doi: 10.1038/cdd.2017.86
83. Zhang H, Zhu L, Bai M, Liu Y, Zhan Y, Deng T, et al. Exosomal circRNA derived from gastric tumor promotes white adipose browning by targeting the miR-133/PRDM16 pathway. *Int J Cancer*. (2019) 144:2501–15. doi: 10.1002/ijc.31977
84. Zhang H, Deng T, Ge S, Liu Y, Bai M, Zhu K, et al. Exosome circRNA secreted from adipocytes promotes the growth of hepatocellular carcinoma by targeting deubiquitination-related USP7. *Oncogene*. (2018) 1:2844–59. doi: 10.1038/s41388-018-0619-z
85. Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS ONE.* (2012) 7:e30733. doi: 10.1371/journal.pone.0030733
86. Xia S, Feng J, Chen K, Ma Y, Gong J, Cai F, et al. CSCD: a database for cancer-specific circular RNAs. *Nucl Acids Res.* (2017) 46:D925–9. doi: 10.1093/nar/gkx863
87. Lu TP, Lee CY, Tsai MH, Chiu YC, Hsiao CK, Lai LC, et al. miRSystem: an integrated system for characterizing enriched functions and pathways of microRNA targets. *PLoS ONE.* (2012) 7:e42390. doi: 10.1371/journal.pone.0042390
88. Dragomir M, Calin GA. Corrigendum: circular RNAs in cancer - lessons learned from microRNAs. *Front Oncol.* (2018) 8:307. doi: 10.3389/fonc.2018.00307
89. Brisset AC, Isakson BE, Kwak BR. Connexins in vascular physiology and pathology. *Antioxid Redox Signal.* (2009) 11:267–82. doi: 10.1089/ars.2008.2115
90. Grimson A, Farh KK-H, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol cell.* (2007) 27:91–105. doi: 10.1016/j.molcel.2007.06.017
91. Glazar P, Papavasileiou P, Rajewsky N. circBase: a database for circular RNAs. *RNA.* (2014) 20:1666–70. doi: 10.1261/rna.043687.113
92. Agarwal V, Bell GW, Nam J-W, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *Elife.* (2015) 4:e05005. doi: 10.7554/eLife.05005
93. Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO. Cell-type specific features of circular RNA expression. *PLoS Genet.* (2013) 9:e1003777. doi: 10.1371/journal.pgen.1003777

Conflict of Interest Statement: 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 in Cell and Developmental Biology

REVIEW
published: 28 August 2019
doi: 10.3389/fcell.2019.00174

Breast Tissue Biology Expands the Possibilities for Prevention of Age-Related Breast Cancers

*Tara Fresques[1], Arrianna Zirbes[2,3], Sundus Shalabi[2,3,4], Susan Samson[5], Sandy Preto[6], Martha R. Stampfer[1] and Mark A. LaBarge[1,2,3]**

[1] Department of Biological Systems and Engineering, Lawrence Berkeley National Laboratory, Berkeley, CA, United States, [2] Department of Population Sciences, Beckman Research Institute at City of Hope, Duarte, CA, United States, [3] Center for Cancer and Aging Research, Beckman Research Institute at City of Hope, Duarte, CA, United States, [4] Medical Research Center, Al-Quds University, Jerusalem, Palestine, [5] Breast Science Advocacy Core, Breast Oncology Program, University of California, San Francisco, San Francisco, CA, United States, [6] Notes4Hope, Moraga, CA, United States

Preventing breast cancer before it is able to form is an ideal way to stop breast cancer. However, there are limited existing options for prevention of breast cancer. Changes in the breast tissue resulting from the aging process contribute to breast cancer susceptibility and progression and may therefore provide promising targets for prevention. Here, we describe new potential targets, immortalization and inflammaging, that may be useful for prevention of age-related breast cancers. We also summarize existing studies of warfarin and metformin, current drugs used for non-cancerous diseases, that also may be repurposed for breast cancer prevention.

Keywords: breast cancer, prevention, chemoprevention, immortality, inflammaging, warfarin, metformin

OPEN ACCESS

Edited by:
Sophie A. Lelièvre, Purdue University, United States

Reviewed by:
Dorothy Teegarden, Purdue University, United States
Alice Hudder, Lake Erie College of Osteopathic Medicine, United States
Derek Charles Radisky, Mayo Clinic, United States

***Correspondence:**
Mark A. LaBarge
mlabarge@coh.org

Specialty section:
This article was submitted to Epigenomics and Epigenetics, a section of the journal Frontiers in Cell and Developmental Biology

Received: *03 May 2019*
Accepted: *12 August 2019*
Published: *28 August 2019*

Citation:
Fresques T, Zirbes A, Shalabi S, Samson S, Preto S, Stampfer MR and LaBarge MA (2019) Breast Tissue Biology Expands the Possibilities for Prevention of Age-Related Breast Cancers. Front. Cell Dev. Biol. 7:174. doi: 10.3389/fcell.2019.00174

INTRODUCTION

There are limited options for prevention of breast cancer. Tamoxifen, raloxifene, and aromatase inhibitors are currently used for breast cancer prevention in the recurrence setting and have been shown to be effective in large scale trials (Kinsinger et al., 2002). However, they are not used in low risk scenarios due to side effects such as deep vein thrombosis (Kinsinger et al., 2002). Epidemiological approaches to identify means to protect individuals from developing breast cancer have been heavily influenced by age and estrogen receptor status. More than 75% of breast cancers in the United States are diagnosed in women aged over 50 (Smigal et al., 2006; Jemal et al., 2007), and 80% of age-related breast cancers are hormone-receptor expressing luminal subtypes, whereas the triple negative disease is enriched among younger women (Jenkins et al., 2014). The dominant paradigm suggests that the higher incidence of age-related cancers is due to accrual of somatic mutations over time that alter regulation or activity of oncogenes and tumor suppressors (DePinho, 2000). A number of cancers show an exponential increase in incidence with age, consistent with the mutation accumulation hypothesis. However, the incidence of breast cancer decreases sometime after age 70 (Anderson et al., 2014). In addition, women from different countries, e.g., Japan versus United States, exhibit very different distributions for the age of first breast cancer diagnosis (Matsuno et al., 2007) despite both being industrialized nations with, we assume, similar mutation rates (Todhunter et al., 2018). Thus, this evidence does not support accumulation of mutations alone as an explanation of the age-related increase in breast cancer incidence. Examination of the cellular and molecular processes that underlie aging in the breast may reveal new avenues for breast cancer prevention.

A number of systemic changes occur in the breast as a result of age such as a significant decrease in estrogen production in the transition to and during menopause. These hormonal differences likely cause significant changes in the physical properties of breast tissue as many studies have found that hormone changes coincide with decreased connective tissue, increased adipose, and discontinuities in the basement membrane, which maintains normal polarity of the epithelium (Howeedy et al., 1990; Milanese et al., 2006; Well et al., 2007). Furthermore, significant changes occur as a result of age in mammary epithelial cells. For example, dysfunctional luminal-biased progenitors and luminal cells with acquired myoepithelial-like characteristics accumulate, whereas tumor-suppressing myoepithelial cells decrease in proportion (Garbe et al., 2012). These cellular changes may cause gradual functional changes at the level of tissue structure that can corrupt the tumor-suppressive activity of normal tissue architecture. These and other alterations lead to tissue-level phenotypes hypothesized to make older breast epithelia more susceptible to transformation (reviewed in LaBarge et al., 2016).

Furthermore, experiments with normal human mammary epithelial cells (HMEC) suggest that cells from older women have intrinsic qualities that pre-dispose them to develop the breast cancer subtypes that are more commonly found in older women. When normal HMEC from post-menopausal women are intentionally transformed to immortal states they exhibit gene and protein expression consistent with luminal breast cancer subtypes, whereas similarly treated cells from younger women exhibit properties consistent with a basal phenotype (Lee et al., 2015). Using heterochronus cell culture models of human mammary epithelia it was shown that the tissue microenvironment drives the age-related epigenetic and transcriptional phenotypes of the luminal epithelial lineage (Miyano et al., 2017). This suggests that age-related epigenetic states may underlie the prevalence of luminal subtype breast cancers among older women.

Aging also causes significant phenotypic changes in the putative breast cancer cells of origin, cKit-expressing luminal-biased epithelial progenitor cells (Lim et al., 2009). These cells acquire a basal differentiation bias with age (Garbe et al., 2012), due in part to gain in activity of the YAP transcription factor (Pelissier et al., 2014), which is known to provide access to epithelial-to-mesenchymal transition (EMT)-related programs (Shao et al., 2014). Intriguingly, the luminal-biased cKit-expressing epithelial progenitors that accumulate with age were shown to express a unique signature of signaling molecules (comprised of Axl, YAP, pS6, pPLCg2, pEGFR, CD44, and pGSK3), which is the same protein signature that emerges in immortal transformed luminal cells at the very earliest stages of cancer progression (Pelissier Vatter et al., 2018). Taken together, the aging process: (i) endows progenitor cells with features of early cancer, (ii) causes epigenetic changes in the epithelia that may underlie the types of breast cancers most commonly seen in older patients, and (iii) diminishes the ability of the tissue to resist malignant progression by eliminating the myoepithelial gate keepers.

We speculate that successful forms of breast cancer prevention would bolster processes that help maintain tissue integrity, such as forcing progenitors to differentiate into harmless terminal states, or decreasing the low-grade, chronic inflammation that accompanies the aging process, which is thought to precede many cancers. Alternatively, because cancer has a long preamble and aging appears to prime cells to enter early stages of malignant progression, targeting the transition states between normal and malignant may be done in the context of age-related breast cancers. In this review, we consider a number of possible biological targets that may be exploited for breast cancer prevention that span a continuum from theoretical, to drug repurposing, and even ongoing cancer prevention clinical trials. Indeed, it may be possible that common treatments for maladies that are often age-associated could be effective as chemoprevention for age-related breast cancers.

TARGETING THE TRANSITION TO IMMORTALITY

Stopping cancer before it is able to form in susceptible breast cells would be an ideal way to prevent breast cancer in general, including age-related breast cancers. Many different molecular changes can propel normal mammary epithelial cells toward cancer; therefore a good first step for developing preventive strategies is to define the processes that propel progression. Ideally, a molecular process that exhibits the following qualities would provide an excellent target for breast cancer prevention:

(1) Occurs in all precursor cancer cells.
(2) Occurs prior to the acquisition of malignant properties and is required for malignancy.
(3) Does not occur in normal finite cells.
(4) Is unique to the process of oncogenesis and has limited-to-no parallel mechanisms that can achieve the same result.

Studies to uncover processes involved in transitioning normal finite HMEC to malignancy have shown that two molecularly distinct barriers stop normal HMEC from gaining immortality, an essential step in early cancer progression (**Figure 1**) (Stampfer et al., 1997, 2003, 2013; Garbe et al., 2009, 2014; Lee et al., 2015). The first is a stress-associated senescence barrier (stasis). Cells need to inhibit the retinoblastoma pathway in order to bypass this stasis barrier and continue dividing (Garbe et al., 2009, 2014). The second barrier is replicative senescence due to critically short telomeres. Cells need to reactivate telomerase in order to overcome this barrier and become immortal (Garbe et al., 2009, 2014). The process involved in overcoming replicative senescence and becoming immortal may be an ideal target for breast cancer prevention as it meets the four criteria described above. (i) One of the defining characteristics of all cancer cells is their ability to proliferate indefinitely. Telomerase reactivation, which confers immortality, is thought to occur during the pre-malignant ductal carcinoma *in situ* (DCIS) stage of breast cancer progression (Chin et al., 2004; Meeker et al., 2004). Therefore, cancer cells achieve immortalization in their precursor population. (ii) Obtaining immortality is crucial for cells to become vulnerable to malignant transformation. This is due not just

to obtaining unlimited proliferative capacity, but also due to oncogene-induced senescence, meaning that malignancy-causing oncogenes will only cause malignancy in cells that have attained immortality (Olsen et al., 2002), but in contrast, will cause finite cells to senesce and die (Olsen et al., 2002). Therefore, therapeutics that target breast cancer precursor cells before they become immortal could stop them from becoming malignant. (iii) Normal finite cells never undergo the cancer-associated immortalization process, thus normal cells should not succumb to a therapeutic targeted toward this process. (iv) The vast majority of human carcinoma cells use reactivation of telomerase to achieve immortality. While some cancers use a homology recombination-based mechanism, termed alternative lengthening of telomeres (ALT), to become immortal, this mechanism is rarely observed in breast and most other human carcinomas (Bryan et al., 1997; Shay and Bacchetti, 1997; Subhawong et al., 2009). Thus, if telomerase reactivation is inhibited for prevention purposes, cancer precursors do not have a ready parallel bypass mechanism to compensate. For these reasons, the process of telomerase reactivation during immortalization is a promising process to target for prevention of most human carcinomas.

The molecular mechanisms that cause the immortalization process are beginning to be uncovered and include two phenomena. First, post-stasis cells acquire an error permissive for expression of the telomerase gene and become conditionally immortal (Stampfer et al., 1997, 2003; Garbe et al., 1999). However, for sufficient telomerase activity to maintain stable telomeres, these cells need to undergo a successful second event that we have termed conversion (Stampfer et al., 1997, 2003; Garbe et al., 1999). The conversion process involves a change in telomere dynamics that occurs as a result of the initial immortalization-inducing error (Stampfer et al., 1997, 2003; Garbe et al., 1999). Notably, the mean telomere restriction fragment length (TRF) of immortalized HMEC lines and most human cancers is approximately 4 kb (Stampfer et al., 1997; Listerman et al., 2013; Barthel et al., 2017). This is in stark contrast to all normal finite cells in the human body whose mean TRF does not go below ∼5 kb (Harley et al., 1990; Aubert et al., 2012). We hypothesize that the conversion process involves a restructuring of telomeres to allow regulation that supports maintaining short stable telomeres, similar to what is seen in single-celled organisms such as yeast (Shore and Bianchi, 2009).

Future research that aims to understand the molecular features of the immortalization process will be valuable to develop prevention therapeutics. Ideally, research should start with normal finite cells that have been made post-stasis following molecular perturbations that are prevalent in most breast cancers. In order to induce and follow immortalization we have previously studied cell lines that became immortal following exposure to benzo(a)pyrene (Stampfer and Bartley, 1985; Stampfer et al., 1997). More recently we have been able to induce immortalization by transduction of post-stasis HMEC with a c-Myc transgene (Garbe et al., 2009, 2014; Lee et al., 2015). Research with these and other models have revealed some molecular features that may be unique to the immortalization process, such as loss of the long non-coding RNA MORT (Nijjar et al., 1999; Stampfer et al., 2003; Garbe et al., 2014; Lee et al., 2015; Vrba et al., 2015). Another intriguing target may be proliferating cell nuclear antigen (PCNA), which is thought to undergo a post-translational modification that is detected only in cancer and cancer precursor cells, as early as the DCIS stage (Gu et al., 2018). There are pre-clinical molecules known to target and kill cancer cells harboring this unique form of PCNA and thus represent a potential prevention agent that stops recently immortalized cells in their tracks (Gu et al., 2018). Therapeutics designed to inhibit the cancer-associated immortalization process may prevent a majority of breast cancers before they have a chance to form.

TARGETING INFLAMMAGING TO REDUCE SUSCEPTIBILITY TO BREAST CANCER

The aging immune system is characterized by innate immune changes that include a type of chronic, low-grade, macrophage-centered, sterile inflammation known as inflammaging (Palmer et al., 2018). At a basic level, inflammation is an organized immune system response to infection or tissue injury in which several cell types and chemical signaling molecules are recruited to the site of injury and begin a process of wound-healing. The most common signaling molecules involved in inflammation,

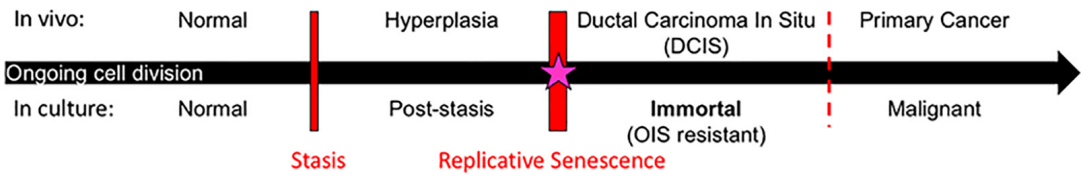


FIGURE 1 | Model of breast cancer progression and barriers for potential chemoprevention targets. Normal cells continue to divide in culture until they approach the stress-associated stasis barrier; cells can bypass stasis by functional inhibition of the retinoblastoma pathway. Post-stasis cells continue to divide until they approach the replicative senescence barrier, which results from ongoing telomere erosion producing telomere dysfunction and genomic instability. Reactivation of telomerase in post-stasis cells can confer immortality. Eroded telomeres, genomic instability, and telomerase reactivation similarly occur at the DCIS stage *in vivo*. Our research suggests that immortalization coincides with a cancer-unique re-structuring of telomere maintenance mechanisms. Immortalized cells are then resistant to oncogene induced senescence (OIS) and many oncogenes can cause them to become malignant. We propose that the immortalization barrier can be a valuable target for breast cancer prevention (starred).

which are used as characteristic markers, include: tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), and interleukins-1, 6, and 18 (IL-1, IL-6, and IL-18) (Bonafe et al., 2012; Prattichizzo et al., 2016; Xia et al., 2016). Serum levels of IL-6 and C-reactive protein (CRP), are often used to assess inflammatory levels in patients (Barbaresko et al., 2013). Regulation of expression of pro-inflammatory cytokines and proper timing of expression of opposing anti-inflammatory cytokines during an immune response is needed for homeostasis. Over-expression of pro-inflammatory cytokines can lead to chronic inflammation and autoimmunity, and conversely over-expression of anti-inflammatory cytokines can lead to immune suppression.

Immune cells in normal breast tissue primarily localize to breast lobules, where they closely associate with the epithelium, rather than stroma or fat (Degnim et al., 2014). Murine mammary gland studies revealed the importance of immune-epithelial cell interactions that cause phenotypic and compositional changes in the mammary epithelia during development (Gouon-Evans et al., 2002; Lilla and Werb, 2010; Reed and Schwertfeger, 2010; Plaks et al., 2015). The composition and function of immune cell populations are known to change in peripheral blood with age (e.g., increased macrophages and dendritic cells, decreased T cells, and reduced function of cytotoxic T cells) (Plackett et al., 2004; Weiskopf et al., 2009), and in breast tissue during breast cancer progression (e.g., increased macrophages) (Ruffell et al., 2012; Degnim et al., 2017; Linde et al., 2018). How age-related changes in immune cell populations, and their effects on aged mammary epithelia, are relevant to increased breast cancer susceptibility with age is not well-understood.

The components of inflammaging that plausibly drive breast cancer initiation and progression include age-related DNA damage, cell senescence, and obesity. Increased DNA damage accumulation with age is a contributing factor to inflammaging. When mammary stem cells and stromal fibroblasts incur DNA damage, they secrete pro-inflammatory cytokines, including IL-6 and IL-8 that can affect surrounding cells (Dieriks et al., 2010; Ivanov et al., 2010). The cytokines in turn induce further DNA damage, cause alterations in the surrounding target cells, and recruit macrophages to the area leading to more inflammation. Inflammation further enables transformation of the surrounding cells, and inflammatory lymphocytes and macrophages are thought to accelerate transformation of mammary epithelia (Lin et al., 2006; Rao et al., 2006). Increases in senescent cells are synonymous with aging which is associated with the senescence-associated secretory phenotype (SASP), a phenomenon that causes senescent cells to activate an inflammatory transcriptional program. Senescence protects cells from transformation, but paradoxically, senescent cells secrete a number of pro-inflammatory cytokines and matrix metalloproteinases that act on neighboring cells in a deleterious manner to induce changes in gene expression that are associated with transformation (Krtolica et al., 2001; Coppe et al., 2010; Borodkina et al., 2018). There is a long-established correlation between the age-associated increase in obesity and breast cancer (Picon-Ruiz et al., 2017). One plausible link between obesity and breast cancer is the pro-tumorigenic and pro-angiogenic microenvironment generated by increased secretion of pro-inflammatory cytokines, like IL-6, by macrophages in adipose tissue (Seiler et al., 2018). Thus the release of pro-inflammatory molecules and microenvironment remodeling enzymes that result from cell and tissue changes that are associated with aging comprise a similar set of mechanisms that underlie the inflammaging phenomenon.

There is an overall association between chronic low-level inflammation and aging phenotypes in multiple tissues, however, the actual impact on aging phenotypes of mammary epithelia remains to be demonstrated. If there is a relationship between inflammaging in breast with deleterious epithelial changes and increased breast cancer susceptibility, then weight loss and anti-inflammation strategies would comprise the main thrust of a prevention approach. This could also include aspirin, which has been suggested to be preventive for breast cancer through an as-yet unknown mechanism (Clarke et al., 2017). In addition, a number of foods are considered anti-inflammatory and may reduce inflammaging, such as fruits, vegetables, fish, and whole grains (Barbaresko et al., 2013; Calder et al., 2017; Kaluza et al., 2018). Continued research examining the mechanistic link between inflammaging and breast cancer susceptibility may provide more useful therapeutic targets for prevention.

WARFARIN AS A PUTATIVE PREVENTION AGENT

Warfarin is commonly prescribed in Western countries for atrial fibrillation, venous thromboembolism, and a number of other cardiac-related indications. Although use is steadily declining in favor of newer anti-coagulants that have preferable safety profiles, warfarin remains one of the most heavily prescribed anti-coagulants with as many as seven million users in the United States as of 2014 (Barnes et al., 2015). A majority of warfarin users are over 60 years of age; thus this drug is particularly intriguing in the context of age-related breast cancer prevention. Epidemiological studies have identified a putative cancer prevention effect of warfarin in this older population in multiple cancer contexts. Women who used warfarin for at least 6 months showed 10–30% reduced relative risk of breast cancer compared to non-warfarin users (Schulman and Lindmarker, 2000; Tagalakis et al., 2007; Haaland et al., 2017). Similar anti-cancer effects were reported in animal models (Ryan et al., 1968; Williamson et al., 1980; Paolino et al., 2014), which also revealed that warfarin doses with no anti-coagulation activity also could be effective in a prevention context (Kirane et al., 2015), thus potentially avoiding some of the negative safety issues associated with warfarin use.

Warfarin inhibits vitamin K oxidoreductases, resulting in depletion of vitamin K and non-carboxylated γ-carboxyglutamate domains of vitamin K-dependent proteins. Most of the ∼14 known proteins that are vitamin K-dependent are involved in coagulation of blood; however, growth arrest specific 6 (GAS6) and periostin (POSTN) also require γ-carboxylation. Haaland et al. (2017) hypothesize that

in the absence of γ-carboxylation GAS6 cannot remain anchored in the plasma membrane and thus converts GAS6 from being an Axl receptor tyrosine kinase agonist into an antagonist. Inhibiting Axl has the impact of reducing malignant traits in aggressive mammary carcinomas, as well as increasing natural killer cell activity (Gjerdrum et al., 2010; Kirane et al., 2015). Axl signaling is linked to induction of epithelial-to-mesenchymal transitions in cancer cells, and induction of stem cell-like properties, suggesting an overall role in regulation of stem-like states (Vuoriluoto et al., 2011; Jokela et al., 2018). Although speculative, inhibition of Axl with an antagonist-form of GAS6 may prevent cancer stem cells from remaining in a stem-like state and instead allow them to differentiate into terminal states (**Figure 2**). Another potential target of warfarin, periostin (POSTN), is thought to improve cancer cell survival and, in some contexts, increase proliferation by increasing microenvironment stiffness due to collagen cross-linking. GLA-domains are protein regions commonly modified by γ-carboxylation. POSTN harbors 28 vitamin K-dependent GLA-domains in its collagen-binding domain, which is an unusually large number compared to 3 to 5 GLA domains in other matricellular proteins, and the role of GLA-domain γ-carboxylation in this protein is not well understood. POSTN is expressed by myoepithelial cells in normal mammary epithelia. Although myoepithelial cells are lost during aging and breast cancer progression, POSTN is highly expressed by the carcinoma cells and cancer associated fibroblasts (Grignani et al., 1993; Grigoriadis et al., 2006). Preventing POSTN GLA-domain γ-carboxylation and stopping it from exerting its effect as a pro-survival and pro-proliferative protein may constitute a second possible mechanism for warfarin-driven breast cancer prevention.

Additional study of warfarin use in a prevention setting is merited based on the multiple human population and mouse studies showing a putative protective effect. However, contemplating the use of warfarin specifically for cancer prevention raises a number of serious safety challenges, and a better overall understanding of its effects at various doses in epithelial cells and tissue is still needed.

METFORMIN FOR PREVENTION

Metformin (1,1-dimethylbiguanide hydrochloride) belongs to the biguanide family of oral hypoglycemic agents that are used commonly to treat type II diabetes and insulin resistance. Insulin resistance occurs when peripheral tissues gradually lose their ability to uptake glucose in response to insulin. This provokes the pancreas to produce further insulin and causes elevated serum insulin levels. Metformin lowers serum glucose, increases insulin sensitivity in peripheral tissues and reduces serum insulin levels by a number of mechanisms (Rena et al., 2017). These include reducing hepatic glucose production and inhibiting mitochondrial ATP generation (Owen et al., 2000). Low ATP levels are sensed by AMP-activated protein kinase (AMPK) (Hawley et al., 2010; Rena et al., 2017), which in turn activates signaling pathways to replenish ATP supplies. Simultaneously, AMPK inhibits ATP-consuming synthetic pathways such as gluconeogenesis and lipid

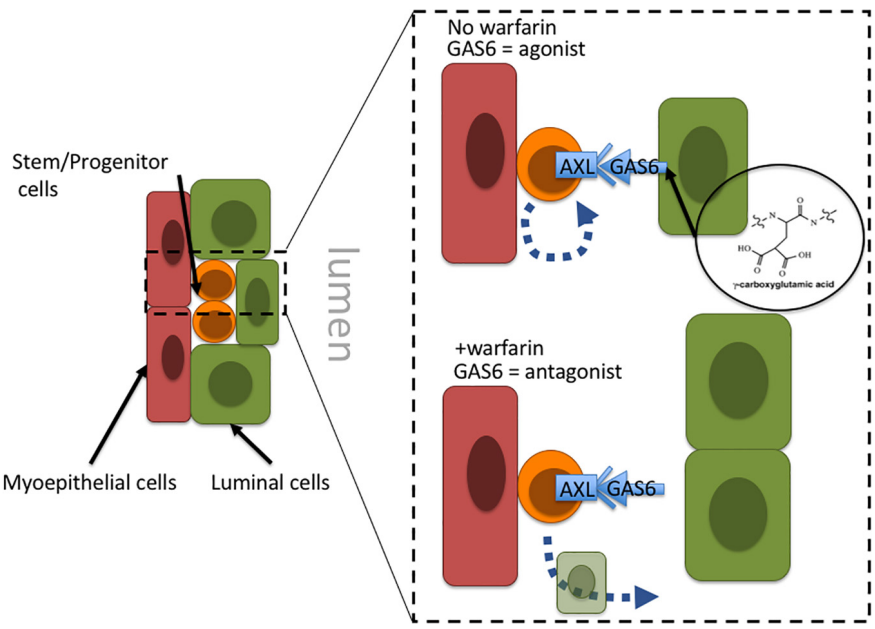


FIGURE 2 | Proposal of a tissue-level mechanism of warfarin's putative anti-breast cancer effects. In mammary epithelia Axl signaling allow cells with progenitor properties access to stem-cell gene programs; engagement with the GAS6 ligand maintains progenitors in an undifferentiated state. Warfarin inhibits gamma-carboxylation of the Axl ligand, GAS6, preventing it from remaining anchored in the plasma membrane and essentially converting GAS6 from an agonist to an Axl-antagonist. At that point the progenitors may differentiate into more terminal states. Because the epithelial progenitors are thought to comprise breast cancer cells of origin, it might be more advantageous to force them to differentiate before they become a liability.

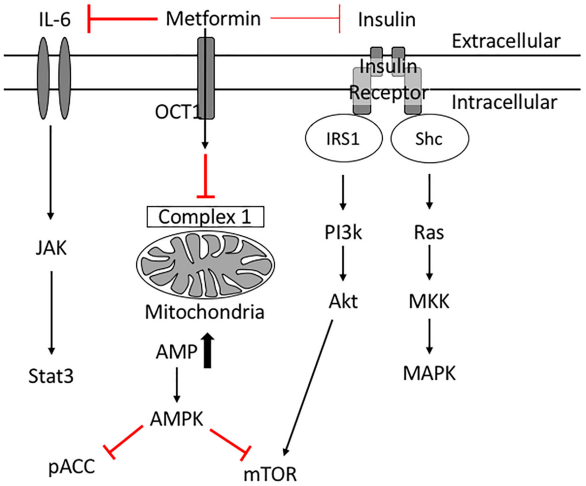


FIGURE 3 | Potential molecular mechanisms of Metformin's anti-cancer effects. Metformin inhibits complex 1 in the mitochondria thus reducing ATP production. Low levels of ATP activate AMPK which inhibits mTOR. Metformin improves peripheral tissue sensitivity to insulin and reduces insulin levels in the blood. Reduction of downstream signaling through the insulin receptor results in reduction of PI3K/Akt/mTOR signaling as well as RAS/MAPK signaling leading to reduced cellular proliferation. Metformin also induces its cancer preventative effects via inhibiting IL6 mediated activation of JAK/Stat3 signaling involved in tumorigenesis.

synthesis (Carling et al., 2011; Hardie, 2011), thus reducing insulin resistance.

Insulin resistance is a key risk factor for age-related breast cancers (Lipscombe et al., 2006; Kabat et al., 2009; Ibarra-Drendall et al., 2011; Gunter et al., 2015; Luque et al., 2017). High insulin levels are positively associated with an increased breast cancer risk in post-menopausal women (Gunter et al., 2015). In addition, women with serum insulin levels in the upper tertile are more than twice as likely to develop breast cancer (Kabat et al., 2009). High indices of insulin resistance are associated also with poor prognosis in women with early and metastatic stages of breast cancer (Gennari et al., 2014; Ferroni et al., 2016). Insulin acts as a breast cancer cell mitogen directly and indirectly via insulin-like growth factors (IGFs) (David and Linda, 2012). When insulin binds its receptor, phosphatidylinositol 3-kinase (PI3K) is activated, which in turn activates Akt/mTOR. Insulin also activates Ras and subsequently mitogen-activated protein kinase (MAPK), inducing cell proliferation and survival (David and Linda, 2012; **Figure 3**). These studies suggest therapeutics designed to treat insulin resistance may help treat breast cancer in diabetic patients.

Epidemiologic studies revealed that metformin use is associated with decreased cancer and cancer-associated mortality in diabetic patients (Bowker et al., 2006; Jiralerspong et al., 2009; Bodmer et al., 2010; Kim et al., 2018). Diabetic patients on long-term metformin were 56% less likely to develop breast cancer compared with control patients (Bodmer et al., 2010), and had reduced cancer-related mortality (Bowker et al., 2006). At a cellular level, metformin inhibits the growth of breast cancer cells *in vivo* (Zakikhani et al., 2006). Metformin is thought to be anti-neoplastic because it inhibits signaling pathways that fuel breast cancer cell proliferation and protein synthesis. For example, metformin activates AMPK (Hawley et al., 2010; Howell et al., 2017; Rena et al., 2017); activated AMPK inhibits mTOR (Howell et al., 2017) and phospho-Acetyl-CoA carboxylase (pACC) thus leading to suppression of normal and tumor cell growth (Ibarra-Drendall et al., 2011). Metformin's reduction of insulin levels reduces downstream signaling through the insulin receptor (PI3K/AKT/mTOR) (Zi et al., 2018), and simultaneously reduces signaling to the Ras/MAPK pathway (Ibarra-Drendall et al., 2011; David and Linda, 2012) collectively resulting in reduced cancer cell proliferation and survival. Through these mechanisms metformin has potential beneficial effects in diabetic breast cancer patients.

It is reasonable to speculate that metformin may help non-diabetic breast cancer patients as well by targeting different mechanisms. Indeed, metformin was shown to prevent some aging phenotypes *in vivo* and *in vitro* (Kiho et al., 2005; Diamanti-Kandarakis et al., 2007; Anisimov, 2010; Barzilai et al., 2016). For example, metformin prevents the formation of advanced glycation end products (AGEs) *in vitro*, which normally accumulate in various tissues as a result of aging and long-term diabetes (Kiho et al., 2005; Luevano-Contreras and Chapman-Novakofski, 2010; Vlassara and Uribarri, 2014). Metformin reduced AGE levels in women with polycystic ovary syndrome (characterized by insulin resistance) after a 6-month-long treatment (Diamanti-Kandarakis et al., 2007). Furthermore, metformin limited age-associated senescence in mouse myoblasts (Jadhav et al., 2013) and prevented SASP in human fetal lung fibroblasts (Moiseeva et al., 2013). While it is controversial whether itself affects glucose metabolism and insulin sensitivity (Refaie et al., 2006), especially when accounting for lean body mass, BMI and sex (Chia et al., 2018), there is enough evidence to suggest that hyperinsulinemia levels accelerate aging phenotypes, promote age-related diseases and reduces overall lifespan (Facchini et al., 2000; Johnson and Templeman, 2016). Metformin may slow these processes and improve healthspan by reducing hyperinsulinemia and improving peripheral tissue insulin sensitivity (Martin-Montalvo et al., 2013; Bannister et al., 2014).

Metformin also reduces inflammation associated with insulin resistance, diabetes and aging (Saisho, 2015). Metformin's anti-inflammatory effects include inhibition of monocyte to macrophage differentiation (Vasamsetti et al., 2015), and inhibition of multiple pro-inflammatory cytokines and related signaling such as IL-6, IL-1β, C-X-C motif ligand 1/2 (CXCL1/2) and NF-κB (Cameron et al., 2016). These effects also were observed in studies of patients with impaired fasting glucose and diabetes (Krysiak and Okopien, 2012, 2013). Reduction in IL-6 levels due to metformin administration was shown to cause a reduction of some cancer stem cells (Iliopoulos et al., 2011). Low doses of metformin selectively killed breast cancer stem cells in four different subtypes of breast cancer (Hirsch et al., 2009).

Thus, current studies suggest a beneficial role for metformin on breast cancer prevention, treatment, and outcome. Indeed, metformin is already being tested in a multicenter clinical trial for its ability to prevent breast cancer in women who exhibit atypical hyperplasia (NCT01905046). Metformin is a relatively inexpensive and safe drug with minimal side effects. The most

common side effect is minor gastrointestinal upset, whereas the most serious, yet rare, one is lactic acidosis, especially in patients with renal failure. Collectively, these factors suggest metformin is a worthy drug candidate in the context of breast cancer prevention.

PATIENT ADVOCATE PERSPECTIVES

Advocate #1

Notes4Hope.org is a non-profit organization that focuses on healthy lifestyle as a means to prevent breast cancer. There are many chemicals in our terrestrial environment, in our air, in our household and beauty products, and in our foods that have been linked, to one degree or another, to the development of breast cancer. Chemical production in the United States has increased 15-fold since the 1950s, and a number of chemicals that are used in food production and manufacturing exert unintended deleterious biological effects. More research is needed to understand whether there are negative impacts on breast tissue biology of the chemicals used in food production and product manufacturing. Furthermore, education focused on an individual's incremental and sustainable choices to reduce stress, increase wellness practices, change household and beauty products, and consume more organic and pastured foods can serve as basis for preventing breast cancer. We recognize that diet and lifestyle are intrinsic to culture, and thus conscious changes can be met with significant cultural inertia. Because the panoply of chemicals produced for medical and commercial purposes have as much potential to do harm as they have to heal, the modern pharmacopeia could be used also to augment healthy lifestyle choices. This review considers repurposing medicines like warfarin and metformin, made originally to treat heart disease and diabetes, to prevent breast cancer. While this concept is appealing, as advocates, our excitement should be counterbalanced by the same skepticism with which we view other chemicals used for medicine and manufacturing. Further research should be done to conclude whether or not these medicines do affect breast biology in a positive way, and if they can be used in a manner that does not alter an otherwise healthy aging trajectory.

Advocate #2

Rethinking the limitations of incremental progress requires new ideas and a collaborative ecosystem across sectors, disciplines, and areas of expertise. Aligning experiential and professionalized expertise and insights, advocates bring unique perspectives to the research table as they lend support, challenge assumptions, inspire change, and assist with responsibly advancing basic science and translational research agendas. Peering into the future of science to improve clinical outcomes, researchers in the LaBarge lab have collaboratively identified innovative cutting-edge scientific ideas on the frontiers of their respective disciplines. Urging cautious optimism within an understanding of cell and tissue biology, they argue that there are some opportunities that we should consider for future prevention targets. Clearly, the public needs awareness regarding emerging new scientific rationales. However, advocates caution that we must not risk fooling ourselves. There does seem to be potential benefits of repurposing anticoagulant drugs such as warfarin or diabetes drugs such as metformin, thus meriting renewed investigation as potential candidates for prevention of breast cancer. Because they act in part by inhibiting tissue-level changes associated with aging, advocates look critically at the value proposition and demand evidence of pill effectiveness and drug safety profiles. If there is insufficient evidence of safety, let us not begin giving the healthy aging population potentially toxic drugs in the name of prevention. As vital catalysts for transdisciplinary innovation, research advocates are thrilled to play a vital role in shaping this effort at study inception. They enthusiastically urge research team members to dive deeper into the scientific as well as the humanistic applications of repurposing drugs as anti-breast cancer agents for the aging population. Moreover, cooperation between researchers and advocates helps encourage team members to speak up about the landscape of uncertainties encountered as they jointly tackle what accounts for the uniqueness of breast cancer prevention in the aging population.

DISCUSSION

Our intention with this review is to stimulate thinking around how breast cancer prevention might be approached differently by considering the mechanisms driving change in breast tissue that are consequences of aging – the single greatest risk factor for breast cancer. Herein, we examined a continuum from highly theoretical aspects of breast tissue biology that represent potential prevention targets, such as the transition between normal and immortal states, to treatment modalities that are already in some form of clinical deployment. We hypothesized that age-related changes in the tissue may create a susceptible microenvironment for breast cancer progression that can be targeted with drugs for preventing breast cancer. Epidemiological evidence suggests that two existing drugs, warfarin and metformin, typically used for non-cancer diseases, merit renewed investigation as potential candidates for prevention of breast cancer and that they act in part by inhibiting tissue-level changes associated with aging. However, even in our optimism toward the repurposing of these drugs, it must be respected that these drugs (warfarin in particular) can have dangerous side effects. Thus, it will be crucial to understand whether the animal experiments, showing that sub-therapeutic doses of warfarin can exert anti-cancer effects, are safely translatable to humans. If the negative impacts of these decades-old medications cannot be sufficiently mitigated to warrant testing in a normal risk population, then use in high-risk populations could be considered, as is currently the case for metformin. The aging immune system also likely contributes to aging phenotypes that, at the tissue level, contribute to breast cancer and is therefore an important area of research that may provide novel targets for prevention of age-associated breast cancer. The consequences of age-related shifts in the immune system and epithelial-immune cell interactions over a lifetime need to be better understood. Research examining the

immortalization barrier to breast cancer progression is in its infancy, but may identify new targets of this rate-limiting step in cancer progression.

AUTHOR CONTRIBUTIONS

TF, ML, AZ, SSh, SSa, and SP wrote the different sections of the first draft of the review. TF, ML, AZ, and SSh composed the figures. MS revised the sections of the manuscript and figures. TF and ML composed, revised, and approved the final submission.

FUNDING

ML is grateful to the Congressionally Directed Medical Research Programs – Breast Cancer Research Program (BC141351 and BC181737), the NIA (R01AG040081), NIBIB (R01EB024989), U54HG008100 (to Joe Gray), the City of Hope Center for Cancer and Aging, City of Hope Program in Molecular and Cellular Biology of Cancer, the Conrad N. Hilton Foundation, and the Circle 1500 Foundation. MS and TF were supported by the United States Department of Energy under the Contract No. DE-AC02-05CH11231.

REFERENCES

Anderson, W. F., Rosenberg, P. S., Prat, A., Perou, C. M., and Sherman, M. E. (2014). How many etiological subtypes of breast cancer: two, three, four, or more? *J. Natl. Cancer Inst.* 106:dju165. doi: 10.1093/jnci/dju165

Anisimov, V. N. (2010). Metformin for aging and cancer prevention. *Aging* 2, 760–774. doi: 10.18632/aging.100230

Aubert, G., Baerlocher, G. M., Vulto, I., Poon, S. S., and Lansdorp, P. M. (2012). Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. *PLoS Genet.* 8:e1002696. doi: 10.1371/journal.pgen.1002696

Bannister, C. A., Holden, S. E., Jenkins-Jones, S., Morgan, C. L., Halcox, J. P., Schernthaner, G., et al. (2014). Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls. *Diabetes Obes. Metab.* 16, 1165–1173. doi: 10.1111/dom.12354

Barbaresko, J., Koch, M., Schulze, M. B., and Nothlings, U. (2013). Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. *Nutr. Rev.* 71, 511–527. doi: 10.1111/nure.12035

Barnes, G. D., Lucas, E., Alexander, G. C., and Goldberger, Z. D. (2015). National trends in ambulatory oral anticoagulant use. *Am. J. Med.* 128, 1300.e2–1305.e2. doi: 10.1016/j.amjmed.2015.05.044

Barthel, F. P., Wei, W., Tang, M., Martinez-Ledesma, E., Hu, X., Amin, S. B., et al. (2017). Systematic analysis of telomere length and somatic alterations in 31 cancer types. *Nat. Genet.* 49, 349–357. doi: 10.1038/ng.3781

Barzilai, N., Crandall, J. P., Kritchevsky, S. B., and Espeland, M. A. (2016). Metformin as a tool to target aging. *Cell Metab.* 23, 1060–1065. doi: 10.1016/j.cmet.2016.05.011

Bodmer, M., Meier, C., Krahenbuhl, S., Jick, S. S., and Meier, C. R. (2010). Long-term metformin use is associated with decreased risk of breast cancer. *Diabetes Care* 33, 1304–1308. doi: 10.2337/dc09-1791

Bonafe, M., Storci, G., and Franceschi, C. (2012). Inflamm-aging of the stem cell niche: breast cancer as a paradigmatic example: breakdown of the multi-shell cytokine network fuels cancer in aged people. *Bioessays* 34, 40–49. doi: 10.1002/bies.201100104

Borodkina, A. V., Deryabin, P. I., Giukova, A. A., and Nikolsky, N. N. (2018). "Social Life" of senescent cells: what is SASP and why study it? *Acta Nat.* 10, 4–14.

Bowker, S. L., Majumdar, S. R., Veugelers, P., and Johnson, J. A. (2006). Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. *Diabetes Care* 29, 254–258. doi: 10.2337/diacare.29.02.06.dc05-1558

Bryan, T. M., Englezou, A., Dalla-Pozza, L., Dunham, M. A., and Reddel, R. R. (1997). Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nat. Med.* 3, 1271–1274. doi: 10.1038/nm1197-1271

Calder, P. C., Bosco, N., Bourdet-Sicard, R., Capuron, L., Delzenne, N., Dore, J., et al. (2017). Health relevance of the modification of low grade inflammation in ageing (inflammageing) and the role of nutrition. *Ageing Res. Rev.* 40, 95–119. doi: 10.1016/j.arr.2017.09.001

Cameron, A. R., Morrison, V. L., Levin, D., Mohan, M., Forteath, C., Beall, C., et al. (2016). Anti-inflammatory effects of metformin irrespective of diabetes status. *Circ. Res.* 119, 652–665. doi: 10.1161/CIRCRESAHA.116.308445

Carling, D., Mayer, F. V., Sanders, M. J., and Gamblin, S. J. (2011). AMP-activated protein kinase: nature's energy sensor. *Nat. Chem. Biol.* 7, 512–518. doi: 10.1038/nchembio.610

Chia, C. W., Egan, J. M., and Ferrucci, L. (2018). Age-related changes in glucose metabolism, hyperglycemia, and cardiovascular risk. *Circ. Res.* 123, 886–904. doi: 10.1161/CIRCRESAHA.118.312806

Chin, K., de Solorzano, C. O., Knowles, D., Jones, A., Chou, W., Rodriguez, E. G., et al. (2004). In situ analyses of genome instability in breast cancer. *Nat. Genet.* 36, 984–988. doi: 10.1038/ng1409

Clarke, C. A., Canchola, A. J., Moy, L. M., Neuhausen, S. L., Chung, N. T., Lacey, J. V., et al. (2017). Regular and low-dose aspirin, other non-steroidal anti-inflammatory medications and prospective risk of HER2-defined breast cancer: the California teachers study. *Breast Cancer Res.* 19:52. doi: 10.1186/s13058-017-0840-7

Coppe, J. P., Desprez, P. Y., Krtolica, A., and Campisi, J. (2010). The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu. Rev. Pathol.* 5, 99–118. doi: 10.1146/annurev-pathol-121808-102144

David, P. R., and Linda, V.-D. (2012). The cellular and molecular mechanisms by which insulin influences breast cancer risk and progression. *Endocr. Relat. Cancer* 19, R225–R241. doi: 10.1530/ERC-12-0203

Degnim, A. C., Brahmbhatt, R. D., Radisky, D. C., Hoskin, T. L., Stallings-Mann, M., Laudenschlager, M., et al. (2014). Immune cell quantitation in normal breast tissue lobules with and without lobulitis. *Breast Cancer Res. Treat.* 144, 539–549. doi: 10.1007/s10549-014-2896-8

Degnim, A. C., Hoskin, T. L., Arshad, M., Frost, M. H., Winham, S. J., Brahmbhatt, R. A., et al. (2017). Alterations in the immune cell composition in premalignant breast tissue that precede breast cancer development. *Clin. Cancer Res.* 23, 3945–3952. doi: 10.1158/1078-0432.CCR-16-2026

DePinho, R. A. (2000). The age of cancer. *Nature* 408, 248–254.

Diamanti-Kandarakis, E., Alexandraki, K., Piperi, C., Aessopos, A., Paterakis, T., Katsikis, I., et al. (2007). Effect of metformin administration on plasma advanced glycation end product levels in women with polycystic ovary syndrome. *Metab. Clin. Exp.* 56, 129–134. doi: 10.1016/j.metabol.2006.09.006

Dieriks, B., De Vos, W. H., Derradji, H., Baatout, S., and Van Oostveldt, P. (2010). Medium-mediated DNA repair response after ionizing radiation is correlated with the increase of specific cytokines in human fibroblasts. *Mutat. Res.* 687, 40–48. doi: 10.1016/j.mrfmmm.2010.01.011

Facchini, F. S., Hua, N. W., Reaven, G. M., and Stoohs, R. A. (2000). Hyperinsulinemia: the missing link among oxidative stress and age-related diseases? *Free Radic. Biol. Med.* 29, 1302–1306. doi: 10.1016/s0891-5849(00)00438-x

Ferroni, P., Riondino, S., Laudisi, A., Portarena, I., Formica, V., Alessandroni, J., et al. (2016). Pretreatment insulin levels as a prognostic factor for breast cancer progression. *Oncologist* 21, 1041–1049. doi: 10.1634/theoncologist.2015-0462

Garbe, J., Wong, M., Wigington, D., Yaswen, P., and Stampfer, M. R. (1999). Viral oncogenes accelerate conversion to immortality of cultured conditionally immortal human mammary

epithelial cells. *Oncogene* 18, 2169–2180. doi: 10.1038/sj.onc.1202523

Garbe, J. C., Bhattacharya, S., Merchant, B., Bassett, E., Swisshelm, K., Feiler, H. S., et al. (2009). Molecular distinctions between stasis and telomere attrition senescence barriers shown by long-term culture of normal human mammary epithelial cells. *Cancer Res.* 69, 7557–7568. doi: 10.1158/0008-5472.CAN-09-0270

Garbe, J. C., Pepin, F., Pelissier, F. A., Sputova, K., Fridriksdottir, A. J., Guo, D. E., et al. (2012). Accumulation of multipotent progenitors with a basal differentiation bias during aging of human mammary epithelia. *Cancer Res.* 72, 3687–3701. doi: 10.1158/0008-5472.CAN-12-0157

Garbe, J. C., Vrba, L., Sputova, K., Fuchs, L., Novak, P., Brothman, A. R., et al. (2014). Immortalization of normal human mammary epithelial cells in two steps by direct targeting of senescence barriers does not require gross genomic alterations. *Cell Cycle* 13, 3423–3435. doi: 10.4161/15384101.2014.954456

Gennari, A., Puntoni, M., Nanni, O., De Censi, A., Bruzzi, P., Paleari, L., et al. (2014). Impact of insulin resistance (IR) on the prognosis of metastatic breast cancer (MBC) patients treated with first-line chemotherapy (CT). *J. Clin. Oncol.* 32:514. doi: 10.1200/jco.2014.32.15_suppl.514

Gjerdrum, C., Tiron, C., Hoiby, T., Stefansson, I., Haugen, H., Sandal, T., et al. (2010). Axl is an essential epithelial-to-mesenchymal transition-induced regulator of breast cancer metastasis and patient survival. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1124–1129. doi: 10.1073/pnas.0909333107

Gouon-Evans, V., Lin, E. Y., and Pollard, J. W. (2002). Requirement of macrophages and eosinophils and their cytokines/chemokines for mammary gland development. *Breast Cancer Res.* 4, 155–164.

Grignani, F., Ferrucci, P. F., Testa, U., Talamo, G., Fagioli, M., Alcalay, M., et al. (1993). The acute promyelocytic leukemia-specific PML-RAR alpha fusion protein inhibits differentiation and promotes survival of myeloid precursor cells. *Cell* 74, 423–431. doi: 10.1016/0092-8674(93)80044-f

Grigoriadis, A., Mackay, A., Reis-Filho, J. S., Steele, D., Iseli, C., Stevenson, B. J., et al. (2006). Establishment of the epithelial-specific transcriptome of normal and malignant human breast cells based on MPSS and array expression data. *Breast Cancer Res.* 8:R56.

Gu, L., Lingeman, R., Yakushijin, F., Sun, E., Cui, Q., Chao, J., et al. (2018). The anticancer activity of a first-in-class small-molecule targeting PCNA. *Clin. Cancer Res.* 24, 6053–6065. doi: 10.1158/1078-0432.CCR-18-0592

Gunter, M. J., Xie, X., Xue, X., Kabat, G. C., Rohan, T. E., Wassertheil-Smoller, S., et al. (2015). Breast cancer risk in metabolically healthy but overweight postmenopausal women. *Cancer Res.* 75, 270–274. doi: 10.1158/0008-5472.CAN-14-2317

Haaland, G. S., Falk, R. S., Straume, O., and Lorens, J. B. (2017). Association of warfarin use with lower overall cancer incidence among patients older than 50 years. *JAMA Intern. Med.* 177, 1774–1780. doi: 10.1001/jamainternmed.2017.5512

Hardie, D. G. (2011). AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. *Genes Dev.* 25, 1895–1908. doi: 10.1101/gad.17420111

Harley, C. B., Futcher, A. B., and Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. *Nature* 345, 458–460. doi: 10.1038/345458a0

Hawley, S. A., Ross, F. A., Chevtzoff, C., Green, K. A., Evans, A., Fogarty, S., et al. (2010). Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metab.* 11, 554–565. doi: 10.1016/j.cmet.2010.04.001

Hirsch, H. A., Iliopoulos, D., Tsichlis, P. N., and Struhl, K. (2009). Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res.* 69, 7507–7511. doi: 10.1158/0008-5472.CAN-09-2994

Howeedy, A. A., Virtanen, I., Laitinen, L., Gould, N. S., Koukoulis, G. K., and Gould, V. E. (1990). Differential distribution of tenascin in the normal, hyperplastic, and neoplastic breast. *Lab. Invest.* 63, 798–806.

Howell, J. J., Hellberg, K., Turner, M., Talbott, G., Kolar, M. J., Ross, D. S., et al. (2017). Metformin inhibits hepatic mTORC1 signaling via dose-dependent mechanisms involving AMPK and the TSC complex. *Cell Metab.* 25, 463–471. doi: 10.1016/j.cmet.2016.12.009

Ibarra-Drendall, C., Dietze, E. C., and Seewaldt, V. L. (2011). Metabolic syndrome and breast cancer risk: is there a role for metformin? *Curr. Breast Cancer Rep.* 3, 142–150. doi: 10.1007/s12609-011-0050-8

Iliopoulos, D., Hirsch, H. A., Wang, G., and Struhl, K. (2011). Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1397–1402. doi: 10.1073/pnas.1018898108

Ivanov, V. N., Zhou, H., Ghandhi, S. A., Karasic, T. B., Yaghoubian, B., Amundson, S. A., et al. (2010). Radiation-induced bystander signaling pathways in human fibroblasts: a role for interleukin-33 in the signal transmission. *Cell. Signal.* 22, 1076–1087. doi: 10.1016/j.cellsig.2010.02.010

Jadhav, K. S., Dungan, C. M., and Williamson, D. L. (2013). Metformin limits ceramide-induced senescence in C2C12 myoblasts. *Mech. Ageing Dev.* 134, 548–559. doi: 10.1016/j.mad.2013.11.002

Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J., and Thun, M. J. (2007). Cancer statistics, 2007. *CA Cancer J. Clin.* 57, 43–66. doi: 10.3322/canjclin.57.1.43

Jenkins, E. O., Deal, A. M., Anders, C. K., Prat, A., Perou, C. M., Carey, L. A., et al. (2014). Age-specific changes in intrinsic breast cancer subtypes: a focus on older women. *oncologist* 19, 1076–1083. doi: 10.1634/theoncologist.2014-0184

Jiralerspong, S., Palla, S. L., Giordano, S. H., Meric-Bernstam, F., Liedtke, C., Barnett, C. M., et al. (2009). Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *J. Clin. Oncol.* 27, 3297–3302. doi: 10.1200/JCO.2009.19.6410

Johnson, J. D., and Templeman, N. M. (2016). Hyperinsulinemia causes age-dependent insulin resistance and reduces lifespan image 10. *Can. J. Diabetes* 40, S59–S60.

Jokela, T. A., Engelsen, A. S. T., Rybicka, A., Pelissier Vatter, F. A., Garbe, J. C., Miyano, M., et al. (2018). Microenvironment-induced non-sporadic expression of the AXL and cKIT receptors are related to epithelial plasticity and drug resistance. *Front. Cell. Dev. Biol.* 6:41. doi: 10.3389/fcell.2018.00041

Kabat, G. C., Kim, M., Caan, B. J., Chlebowski, R. T., Gunter, M. J., Ho, G. Y. F., et al. (2009). Repeated measures of serum glucose and insulin in relation to postmenopausal breast cancer. *Int. J. Cancer* 125, 2704–2710. doi: 10.1002/ijc.24609

Kaluza, J., Harris, H., Melhus, H., Michaelsson, K., and Wolk, A. (2018). Questionnaire-based anti-inflammatory diet index as a predictor of low-grade systemic inflammation. *Antioxid. Redox Signal.* 28, 78–84. doi: 10.1089/ars.2017.7330

Kiho, T., Kato, M., Usui, S., and Hirano, K. (2005). Effect of buformin and metformin on formation of advanced glycation end products by methylglyoxal. *Clin. Chim. Acta* 358, 139–145. doi: 10.1016/j.cccn.2005.02.012

Kim, H. J., Lee, S., Chun, K. H., Jeon, J. Y., Han, S. J., Kim, D. J., et al. (2018). Metformin reduces the risk of cancer in patients with type 2 diabetes: an analysis based on the Korean National Diabetes Program Cohort. *Medicine* 97:e0036. doi: 10.1097/MD.0000000000010036

Kinsinger, L. S., Harris, R., Woolf, S. H., Sox, H. C., and Lohr, K. N. (2002). Chemoprevention of breast cancer: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* 137, 59–69.

Kirane, A., Ludwig, K. F., Sorrelle, N., Haaland, G., Sandal, T., Ranaweera, R., et al. (2015). Warfarin blocks Gas6-mediated Axl activation required for pancreatic cancer epithelial plasticity and metastasis. *Cancer Res.* 75, 3699–3705. doi: 10.1158/0008-5472.CAN-14-2887-T

Krtolica, A., Parrinello, S., Lockett, S., Desprez, P. Y., and Campisi, J. (2001). Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12072–12077. doi: 10.1073/pnas.211053698

Krysiak, R., and Okopien, B. (2012). Lymphocyte-suppressing and systemic anti-inflammatory effects of high-dose metformin in simvastatin-treated patients with impaired fasting glucose. *Atherosclerosis* 225, 403–407. doi: 10.1016/j.atherosclerosis.2012.09.034

Krysiak, R., and Okopien, B. (2013). The effect of metformin on monocyte secretory function in simvastatin-treated patients with impaired fasting glucose. *Metab. Clin. Exp.* 62, 39–43. doi: 10.1016/j.metabol.2012.06.009

LaBarge, M. A., Mora-Blanco, E. L., Samson, S., and Miyano, M. (2016). Breast cancer beyond the age of mutation. *Gerontology* 62, 434–442. doi: 10.1159/000441030

Lee, J. K., Garbe, J. C., Vrba, L., Miyano, M., Futscher, B. W., Stampfer, M. R., et al. (2015). Age and the means of bypassing stasis influence the intrinsic subtype of immortalized human mammary epithelial cells. *Front. Cell. Dev. Biol.* 3:13. doi: 10.3389/fcell.2015.00013

Lilla, J. N., and Werb, Z. (2010). Mast cells contribute to the stromal microenvironment in mammary gland branching morphogenesis. *Dev. Biol.* 337, 124–133. doi: 10.1016/j.ydbio.2009.10.021

Lim, E., Vaillant, F., Wu, D., Forrest, N. C., Pal, B., Hart, A. H., et al. (2009). Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. *Nat. Med.* 15, 907–913. doi: 10.1038/nm.2000

Lin, E. Y., Li, J. F., Gnatovskiy, L., Deng, Y., Zhu, L., Grzesik, D. A., et al. (2006). Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res.* 66, 11238–11246. doi: 10.1158/0008-5472.can-06-1278

Linde, N., Casanova-Acebes, M., Sosa, M. S., Mortha, A., Rahman, A., Farias, E., et al. (2018). Macrophages orchestrate breast cancer early dissemination and metastasis. *Nat. Commun.* 9:21. doi: 10.1038/s41467-017-02481-5

Lipscombe, L. L., Goodwin, P. J., Zinman, B., McLaughlin, J. R., and Hux, J. E. (2006). Increased prevalence of prior breast cancer in women with newly diagnosed diabetes. *Breast Cancer Res. Treat.* 98, 303–309. doi: 10.1007/s10549-006-9166-3

Listerman, I., Sun, J., Gazzaniga, F. S., Lukas, J. L., and Blackburn, E. H. (2013). The major reverse transcriptase-incompetent splice variant of the human telomerase protein inhibits telomerase activity but protects from apoptosis. *Cancer Res.* 73, 2817–2828. doi: 10.1158/0008-5472.CAN-12-3082

Luevano-Contreras, C., and Chapman-Novakofski, K. (2010). Dietary advanced glycation end products and aging. *Nutrients* 2, 1247–1265. doi: 10.3390/nu2121247

Luque, R. M., Lopez-Sanchez, L. M., Villa-Osaba, A., Luque, I. M., Santos-Romero, A. L., Yubero-Serrano, E. M., et al. (2017). Breast cancer is associated to impaired glucose/insulin homeostasis in premenopausal obese/overweight patients. *Oncotarget* 8, 81462–81474. doi: 10.18632/oncotarget.20399

Martin-Montalvo, A., Mercken, E. M., Mitchell, S. J., Palacios, H. H., Mote, P. L., Scheibye-Knudsen, M., et al. (2013). Metformin improves healthspan and lifespan in mice. *Nat. Commun.* 4:2192. doi: 10.1038/ncomms3192

Matsuno, R. K., Anderson, W. F., Yamamoto, S., Tsukuma, H., Pfeiffer, R. M., Kobayashi, K., et al. (2007). Early- and late-onset breast cancer types among women in the United States and Japan. *Cancer Epidemiol. Biomark. Prevent.* 16, 1437–1442. doi: 10.1158/1055-9965.epi-07-0108

Meeker, A. K., Hicks, J. L., Gabrielson, E., Strauss, W. M., De Marzo, A. M., and Argani, P. (2004). Telomere shortening occurs in subsets of normal breast epithelium as well as in situ and invasive carcinoma. *Am. J. Pathol.* 164, 925–935. doi: 10.1016/s0002-9440(10)63180-x

Milanese, T. R., Hartmann, L. C., Sellers, T. A., Frost, M. H., Vierkant, R. A., Maloney, S. D., et al. (2006). Age-related lobular involution and risk of breast cancer. *J. Natl. Cancer Inst.* 98, 1600–1607.

Miyano, M., Sayaman, R. W., Stoiber, M. H., Lin, C. H., Stampfer, M. R., Brown, J. B., et al. (2017). Age-related gene expression in luminal epithelial cells is driven by a microenvironment made from myoepithelial cells. *Aging* 9, 2026–2051. doi: 10.18632/aging.101298

Moiseeva, O., Deschenes-Simard, X., St-Germain, E., Igelmann, S., Huot, G., Cadar, A. E., et al. (2013). Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF-kappaB activation. *Aging Cell* 12, 489–498. doi: 10.1111/acel.12075

Nijjar, T., Wigington, D., Garbe, J. C., Waha, A., Stampfer, M. R., and Yaswen, P. (1999). p57KIP2 expression and loss of heterozygosity during immortal conversion of cultured human mammary epithelial cells. *Cancer Res.* 59, 5112–5118.

Olsen, C. L., Gardie, B., Yaswen, P., and Stampfer, M. R. (2002). Raf-1-induced growth arrest in human mammary epithelial cells is p16-independent and is overcome in immortal cells during conversion. *Oncogene* 21, 6328–6339. doi: 10.1038/sj.onc.1205780

Owen, M. R., Doran, E., and Halestrap, A. P. (2000). Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem. J.* 348(Pt 3), 607–614. doi: 10.1042/bj3480607

Palmer, C. S., Palchaudhuri, R., Albargy, H., Abdel-Mohsen, M., and Crowe, S. M. (2018). Exploiting immune cell metabolic machinery for functional HIV cure and the prevention of inflammaging. *F1000Res.* 7:125. doi: 10.12688/f1000research.11881.1

Paolino, M., Choidas, A., Wallner, S., Pranjic, B., Uribesalgo, I., Loeser, S., et al. (2014). The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. *Nature* 507, 508–512. doi: 10.1038/nature12998

Pelissier, F. A., Garbe, J. C., Ananthanarayanan, B., Miyano, M., Lin, C., Jokela, T., et al. (2014). Age-related dysfunction in mechanotransduction impairs differentiation of human mammary epithelial progenitors. *Cell Rep.* 7, 1926–1939. doi: 10.1016/j.celrep.2014.05.021

Pelissier Vatter, F. A., Schapiro, D., Chang, H., Borowsky, A. D., Lee, J. K., Parvin, B., et al. (2018). High-dimensional phenotyping identifies age-emergent cells in human mammary epithelia. *Cell Rep.* 23, 1205–1219. doi: 10.1016/j.celrep.2018.03.114

Picon-Ruiz, M., Morata-Tarifa, C., Valle-Goffin, J. J., Friedman, E. R., and Slingerland, J. M. (2017). Obesity and adverse breast cancer risk and outcome: mechanistic insights and strategies for intervention. *CA Cancer J. Clin.* 67, 378–397. doi: 10.3322/caac.21405

Plackett, T. P., Boehmer, E. D., Faunce, D. E., and Kovacs, E. J. (2004). Aging and innate immune cells. *J. Leukoc. Biol.* 76, 291–299.

Plaks, V., Boldajipour, B., Linnemann, J. R., Nguyen, N. H., Kersten, K., Wolf, Y., et al. (2015). Adaptive immune regulation of mammary postnatal organogenesis. *Dev. Cell* 34, 493–504. doi: 10.1016/j.devcel.2015.07.015

Prattichizzo, F., Giuliani, A., Recchioni, R., Bonafe, M., Marcheselli, F., De Carolis, S., et al. (2016). Anti-TNF-alpha treatment modulates SASP and SASP-related microRNAs in endothelial cells and in circulating angiogenic cells. *Oncotarget* 7, 11945–11958. doi: 10.18632/oncotarget.7858

Rao, V. P., Poutahidis, T., Ge, Z., Nambiar, P. R., Horwitz, B. H., Fox, J. G., et al. (2006). Proinflammatory CD4(CD45RB(hi) lymphocytes promote mammary and intestinal carcinogenesis in Apc(Min/() mice. *Cancer Res.* 66, 57–61. doi: 10.1158/0008-5472.can-05-3445

Reed, J. R., and Schwertfeger, K. L. (2010). Immune cell location and function during post-natal mammary gland development. *J. Mammary Gland Biol. Neoplasia* 15, 329–339. doi: 10.1007/s10911-010-9188-7

Refaie, M. R., Sayed-Ahmed, N. A., Bakr, A. M., Abdel Aziz, M. Y., El Kannishi, M. H., and Abdel-Gawad, S. S. (2006). Aging is an inevitable risk factor for insulin resistance. *J. Taibah Univ. Med. Sci.* 1, 30–41. doi: 10.1016/s1658-3612(06)70005-1

Rena, G., Hardie, D. G., and Pearson, E. R. (2017). The mechanisms of action of metformin. *Diabetologia* 60, 1577–1585. doi: 10.1007/s00125-017-4342-z

Ruffell, B., Au, A., Rugo, H. S., Esserman, L. J., Hwang, E. S., and Coussens, L. M. (2012). Leukocyte composition of human breast cancer. *Proc. Natl. Acad. Sci. U.S.A.* 109, 2796–2801.

Ryan, J. J., Ketcham, A. S., and Wexler, H. (1968). Reduced incidence of spontaneous metastases with long-term Coumadin therapy. *Ann. Surg.* 168, 163–168.

Saisho, Y. (2015). Metformin and inflammation: its potential beyond glucose-lowering effect. *Endocr. Metab. Immune Disord. Drug Targets* 15, 196–205. doi: 10.2174/1871530315666150316124019

Schulman, S., and Lindmarker, P. (2000). Incidence of cancer after prophylaxis with warfarin against recurrent venous thromboembolism. Duration of Anticoagulation Trial. *N. Engl. J. Med.* 342, 1953–1958. doi: 10.1056/nejm200006293422604

Seiler, A., Chen, M. A., Brown, R. L., and Fagundes, C. P. (2018). Obesity, dietary factors, nutrition, and breast cancer risk. *Curr. Breast Cancer Rep.* 10, 14–27. doi: 10.1007/s12609-018-0264-0

Shao, D. D., Xue, W., Krall, E. B., Bhutkar, A., Piccioni, F., Wang, X., et al. (2014). KRAS and YAP1 converge to regulate EMT and tumor survival. *Cell* 158, 171–184. doi: 10.1016/j.cell.2014.06.004

Shay, J. W., and Bacchetti, S. (1997). A survey of telomerase activity in human cancer. *Eur. J. Cancer* 33, 787–791. doi: 10.1016/s0959-8049(97)00062-2

Shore, D., and Bianchi, A. (2009). Telomere length regulation: coupling DNA end processing to feedback regulation of telomerase. *EMBO J.* 28, 2309–2322. doi: 10.1038/emboj.2009.195

Smigal, C., Jemal, A., Ward, E., Cokkinides, V., Smith, R., Howe, H. L., et al. (2006). Trends in breast cancer by race and ethnicity: update 2006. *CA Cancer J. Clin.* 56, 168–183. doi: 10.3322/canjclin.56.3.168

Stampfer, M. R., and Bartley, J. C. (1985). Induction of transformation and continuous cell lines from normal human mammary epithelial cells after

exposure to benzo[a]pyrene. *Proc. Natl. Acad. Sci. U.S.A.* 82, 2394–2398. doi: 10.1073/pnas.82.8.2394

Stampfer, M. R., Bodnar, A., Garbe, J., Wong, M., Pan, A., Villeponteau, B., et al. (1997). Gradual phenotypic conversion associated with immortalization of cultured human mammary epithelial cells. *Mol. Biol. Cell* 8, 2391–2405. doi: 10.1091/mbc.8.12.2391

Stampfer, M. R., Garbe, J., Nijjar, T., Wigington, D., Swisshelm, K., and Yaswen, P. (2003). Loss of p53 function accelerates acquisition of telomerase activity in indefinite lifespan human mammary epithelial cell lines. *Oncogene* 22, 5238–5251. doi: 10.1038/sj.onc.1206667

Stampfer, M. R., LaBarge, M. A., and Garbe, J. C. (2013). "An integrated human mammary epithelial cell cuture system for studying carginogenesis and aging," in *Cell and Molecular Biology of Breast Cancer*, ed. H. Schatten, (Totowa, NJ: Humana Press), 323–361. doi: 10.1007/978-1-62703-634-4_15

Subhawong, A. P., Heaphy, C. M., Argani, P., Konishi, Y., Kouprina, N., Nassar, H., et al. (2009). The alternative lengthening of telomeres phenotype in breast carcinoma is associated with HER-2 overexpression. *Mod. Pathol.* 22, 1423–1431. doi: 10.1038/modpathol.2009.125

Tagalakis, V., Tamim, H., Blostein, M., Collet, J. P., Hanley, J. A., and Kahn, S. R. (2007). Use of warfarin and risk of urogenital cancer: a population-based, nested case-control study. *Lancet Oncol.* 8, 395–402. doi: 10.1016/s1470-2045(07)70046-3

Todhunter, M. E., Sayaman, R. W., Miyano, M., and LaBarge, M. A. (2018). Tissue aging: the integration of collective and variant responses of cells to entropic forces over time. *Curr. Opin. Cell Biol.* 54, 121–129. doi: 10.1016/j.ceb.2018.05.016

Vasamsetti, S. B., Karnewar, S., Kanugula, A. K., Thatipalli, A. R., Kumar, J. M., and Kotamraju, S. (2015). Metformin inhibits monocyte-to-macrophage differentiation via AMPK-mediated inhibition of STAT3 activation: potential role in atherosclerosis. *Diabetes Metab. Res. Rev.* 64, 2028–2041. doi: 10.2337/db14-1225

Vlassara, H., and Uribarri, J. (2014). Advanced glycation end products (AGE) and diabetes: cause, effect, or both? *Curr. Diab. Rep.* 14:453. doi: 10.1007/s11892-013-0453-1

Vrba, L., Garbe, J. C., Stampfer, M. R., and Futscher, B. W. (2015). A lincRNA connected to cell mortality and epigenetically-silenced in most common human cancers. *Epigenetics* 10, 1074–1083. doi: 10.1080/15592294.2015.1106673

Vuoriluoto, K., Haugen, H., Kiviluoto, S., Mpindi, J. P., Nevo, J., Gjerdrum, C., et al. (2011). Vimentin regulates EMT induction by Slug and oncogenic H-Ras and migration by governing Axl expression in breast cancer. *Oncogene* 30, 1436–1448. doi: 10.1038/onc.2010.509

Weiskopf, D., Weinberger, B., and Grubeck-Loebenstein, B. (2009). The aging of the immune system. *Transplant. Int.* 22, 1041–1050.

Well, D., Yang, H., Houseni, M., Iruvuri, S., Alzeair, S., Sansovini, M., et al. (2007). Age-related structural and metabolic changes in the pelvic reproductive end organs. *Semin. Nucl. Med.* 37, 173–184. doi: 10.1053/j.semnuclmed.2007.01.004

Williamson, R. C., Lyndon, P. J., and Tudway, A. J. (1980). Effects of anticoagulation and ileal resection on the development and spread of experimental intestinal carcinomas. *Br. J. Cancer* 42, 85–94. doi: 10.1038/bjc.1980.206

Xia, S., Zhang, X., Zheng, S., Khanabdali, R., Kalionis, B., Wu, J., et al. (2016). An update on inflamm-aging: mechanisms, prevention, and treatment. *J. Immunol. Res.* 2016:8426874. doi: 10.1155/2016/8426874

Zakikhani, M., Dowling, R., Fantus, I. G., Sonenberg, N., and Pollak, M. (2006). Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. *Cancer Res.* 66, 10269–10273. doi: 10.1158/0008-5472.can-06-1500

Zi, F., Zi, H., Li, Y., He, J., Shi, Q., and Cai, Z. (2018). Metformin and cancer: an existing drug for cancer prevention and therapy. *Oncol. Lett.* 15, 683–690. doi: 10.3892/ol.2017.7412

Conflict of Interest Statement: ML and MS have patents that relate to their research in breast cancer prevention.

The remaining 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 in Oncology

REVIEW
published: 04 September 2019
doi: 10.3389/fonc.2019.00765

The Many Faces of Obesity and Its Influence on Breast Cancer Risk

Tanya Agurs-Collins[1], *Sharon A. Ross*[2]* *and Barbara K. Dunn*[2]

[1] Division of Cancer Control and Population Sciences, National Cancer Institute, Rockville, MD, United States, [2] Division of Cancer Prevention, National Cancer Institute, Rockville, MD, United States

Obesity is associated with increased risk of breast and other cancers. However, the complexity of the underlying mechanisms, together with the interplay of diet and physical activity—contributing to energy balance—and the role of adipose tissue, pose challenges to our understanding of the basis of this increased risk. Epidemiologic studies have documented a higher obesity prevalence in US black women compared to white women. Elucidation of the contribution of potential biological differences among racially distinct groups to their differences in breast cancer (BC) risk and mortality have been topics of considerable interest in recent years. The racial and ethnic variation in body fat distribution may account for at least part of the differences in breast cancer rates in these populations. Yet, while black women exhibit higher rates of obesity compared to white women, this does not translate directly into higher rates of BC. In fact, overall, BC in black women occurs with a lower incidence than BC in white women. Obesity is a known risk factor for postmenopausal breast cancer, and growing evidence suggests that abdominal obesity, also known as central obesity, may increase risk for triple negative breast cancer, which is more common in premenopausal women. The positive association of postmenopausal BC risk and specifically estrogen receptor (ER)-positive BC, is presumably due largely to accumulation of estrogen in the adipose tissue of the breast and other tissues. Of the two main types of adipose tissue—subcutaneous and visceral—visceral adipocytes are more active metabolically. Such adipose tissue harbors multiple molecular entities that promote carcinogenesis: endocrine molecules/hormones, immunologic factors, inflammatory cytokines, metabolic alterations, and other components of the microenvironment. Expression of these culpable entities is largely regulated by epigenetic mechanisms. The interrelationship between these entities and drivers of epigenetic alteration are critical to the regulation of pathways connecting obesity and cancer risk. Initiatives to counteract the carcinogenic effects of obesity have primarily involved modulation of energy balance by diet. However, targeting of specific molecular abnormalities characterizing adiposity offers an alternative approach to preventing cancer. Our goal in this review is to first discuss the major mechanisms contributing to the obesity-breast cancer link. We will also consider race, specifically black/white differences, as they relate to the association of obesity with breast cancer risk. Then we will enumerate strategies targeting these mechanisms to reduce BC risk, in large part by way of dietary interventions with potential to mitigate the cancer-promoting components of adiposity.

Keywords: adiposity, breast cancer risk, endocrine function, epigenetics, obesity, weight loss

OPEN ACCESS

Edited by:
Martine Marie Bellanger, École des Hautes Etudes en Santé Publique, France

Reviewed by:
Wagner Ricardo Montor, Santa Casa of São Paulo, Brazil
Parisa Tehranifar, Columbia University, United States

***Correspondence:**
Sharon A. Ross
rosssha@mail.nih.gov

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

Received: *09 May 2019*
Accepted: *29 July 2019*
Published: *04 September 2019*

Citation:
Agurs-Collins T, Ross SA and Dunn BK (2019) The Many Faces of Obesity and Its Influence on Breast Cancer Risk. Front. Oncol. 9:765. doi: 10.3389/fonc.2019.00765

INTRODUCTION

Obesity, a state of increased adiposity, is categorized according to body mass index (BMI) as having a BMI >30 kg/m^2 (1, 2) and is now considered a chronic disease (3). The weight gain, along with associated metabolic disturbances, that characterizes obesity results from disruption of energy balance, causing tissue stress and dysfunction (4, 5). The serious consequences of these physiological effects of obesity have evolved into major health concerns in recent years. Obesity is increasingly becoming a worldwide epidemic, with global obesity rates nearly tripling since 1975 (3). In 2015, the worldwide prevalence of obesity among adults reached 12%, with higher rates among women (2, 6).

EPIDEMIOLOGY OF OBESITY AND BREAST CANCER RISK ACCORDING TO LIFE STAGE AND RACE

High adiposity (BMI, adult weight gain, and abdominal obesity) is a risk factor for several types of cancer, including breast cancer (7). The association between overweight/obesity and breast cancer risk varies in relation to several factors including menopausal status and specific life stages. For postmenopausal women, several meta-analyses have consistently shown positive associations among high adiposity, adult weight gain, and risk of hormone receptor-positive (estrogen receptor-positive/ER+ and progesterone receptor-positive/PR+) breast cancer (6, 8–12). Conversely, the epidemiologic literature supports an inverse association or no association between high BMI and premenopausal hormone receptor-positive breast cancer risk (13–15). Additionally, high BMI during childhood, adolescence, and early adulthood is associated with decreased risk of premenopausal breast cancer (12, 16, 17). However, the association between measures of adiposity and premenopausal breast cancer risk may vary by ethnicity. For example, a few studies suggest that high adiposity may confer greater risk for premenopausal breast cancer among Asian women (18, 19). Other studies assessed abdominal, i.e., central, adiposity, and found a significantly positive association with both pre-and postmenopausal breast cancer risk (20, 21). The association appears to be strongest with triple negative breast cancer (TNBC), which occurs most often in women under 40 years of age (22). Harris et al. (23) revealed that measures of abdominal obesity (e.g., waist circumference, waist-to-hip ratio) were associated with increased risk for premenopausal ER- breast cancer when examining the highest vs. the lowest quintile for each measurement. Similarly, Pierobon and Frankenfeld (24) demonstrated in a systematic review and meta-analysis that a significant association existed between TNBC and obesity, but when stratified by menopausal status the results were significant only among premenopausal women.

These obesity-breast cancer associations can also be addressed in relation to race or ethnicity. This approach is especially relevant given that the prevalence of obesity in the U.S. is higher among blacks than whites. In 2015–2016, the highest rates of obesity in the U.S. population was among black women (54.8%) (10). This contrasts with an overall rate of 39.8% in the general population. Furthermore, variation in body fat distribution among racial and ethnic groups may account for differences in breast cancer rates by menopausal status and breast cancer subtypes (25–27). However, clear patterns have not been identified. The AMBER Consortium, a collaboration of four studies, examined obesity and body fat distribution among black women (26). In this study, breast cancer subtypes were examined by menopausal status, BMI, and abdominal obesity. For postmenopausal black women, higher recent BMI (> 35 kg/m^2) was associated with ER+ breast cancer and decreased risk of TNBC. Among premenopausal black women, higher BMI (> 30 kg/m^2) was associated with decreased risk of ER+ breast cancer. When examining abdominal obesity, breast cancer risk also differed by menopausal status. For postmenopausal black women, a high waist-to-hip ratio (WHR) (>0.88 vs. ≤0.64 cm) was associated with increased risk for each tumor subtype (ER-, ER+, PR-, PR+), and a higher risk for TNBC tumors. In contrast, among premenopausal black women, high WHR (>0.88 vs. ≤0.64 cm) was only associated with increased risk of ER+ breast cancer (26). Other studies have also shown that regardless of menopausal status, abdominal obesity increases the risk for TNBC among black women; TNBC is a particularly aggressive phenotype (22, 27); however, inconsistent results have been reported (28).

The Carolina Breast Cancer Study, which is contained within the AMBER Consortium, demonstrated an increased incidence of TNBC in premenopausal women. An association with obesity is suggested by the observation that women with a high compared to low WHR had a significantly higher risk of developing basal-type TNBC. This increased risk of TNBC in association with obesity applies to both pre- and postmenopausal black women (29), although the risk is highest in premenopausal women (22, 29).

To summarize, the relationship between adiposity and breast cancer risk is complex and varies depending upon several factors. Increased breast cancer risk in postmenopausal women is especially notable among those who are obese (2), as demonstrated in large studies using different study designs (20, 21, 24).

On the one hand, early life obesity is protective against premenopausal breast cancer, whereas the scientific literature provides clear and consistent evidence linking high adult adiposity as a risk factor with postmenopausal breast cancer. Although the incidence of overall breast cancer is lower among black women compared to white women, black women have a higher incidence of ER- and TNBC tumors and their tumors tend to be of a higher grade than tumors in women from other racial and ethnic groups (30). The increased frequency of these tumors may be partially attributable to the higher abdominal adiposity rates in black populations.

Obesity, Socioeconomic Status, and Breast Cancer Risk

Obesity is associated with socioeconomic status (SES) in high-and-middle income countries (6). In high-income countries,

the shift in the food supply created opportunities to consume inexpensive, energy-dense foods with low nutritional value, which is a major driver of the obesity epidemic, especially among low SES individuals (31). For example, a systematic review revealed that lower life course SES was associated with obesity risk (summary OR: 1.35; 95% CI: 1.04, 1.76) and higher waist circumference (summary OR: 4.67; 95% CI: 4.15, 5.20) (32). In women, the overall obesity prevalence was shown to decrease with increased income and educational attainment (33). SES is linked not only to obesity risk, but also to breast cancer incidence and mortality (34). Evidence also exists for a relationship between SES and breast cancer outcomes, with low SES being associated with advanced disease stage at the time of diagnosis, greater disease recurrence, and poorer survival in multiple studies (34). However, other studies suggest that the contribution of SES to racial and ethnic disparities in breast cancer is modest and varies by hormone receptor subtypes and stage at diagnosis (35). Thus, the relationship between SES and obesity may affect breast cancer risk and prognosis differently according to race and ethnicity. Limited research has been conducted to identify a direct association between SES and breast cancer risk (36, 37). However, the indirect link via their mutual association with obesity emphasizes the importance of such investigations, especially in light of the current epidemic of obesity (31).

Obesity Prevention and Breast Cancer Risk

Intervention studies aimed at reducing the incidence of obesity can provide opportunities to decrease breast cancer risk, specifically post-menopausal breast cancer. The increase in obesity rates is associated with changes in the food and built environments which contribute to increased consumption of energy-dense foods and less physical activity. These changes result in a positive energy balance—the state in which energy intake exceeds energy expenditure—which, over time, can lead to obesity. Several studies have shown that reducing caloric intake and increasing physical activity may be protective against both pre- and post-menopausal breast cancer (38, 39). As such, targeting modifiable risk factors of obesity such as diet and physical activity is one strategy to reduce breast cancer risk and improve survival.

The complex interplay of diet and physical activity, together with the role of adipose tissue, pose challenges to our understanding of the mechanisms by which obesity confers increased breast cancer risk. Furthermore, obesity is intertwined with social deprivation, environmental conditions, genetics, hormones, and epigenetic factors, all of which can impact breast cancer risk and the aggressiveness of breast cancer phenotypes. In this review we discuss obesity and diet-related biological mechanisms with the aim of identifying molecular and behavioral targets that can inform research into novel interventions to reduce breast cancer incidence and mortality. The focus of this review is on the relationship between obesity and postmenopausal breast cancer risk. Although it is an important topic, the interplay between adiposity and breast cancer survival is not addressed here.

MECHANISTIC BASIS OF OBESITY AND ITS IMPACT ON BREAST CANCER RISK

Adipose Tissue as an Endocrine Organ, Regulating Metabolism and Immune Responses

The increased adipose tissue that characterizes the state of obesity is not merely a passive reservoir to store lipids and energy, as once thought. Adipose tissue is biologically active, and is now considered to be an "endocrine organ," given the multiple factors it produces that impact systemic energy metabolism, neuroendocrine function, and immune responses (40). These areas of adipose function can be broadly classified as protein products that affect the metabolism of distant cells/tissues and enzymes that are involved in steroid hormone metabolism.

Metabolic Dysregulation in Obesity

In obesity multiple metabolic changes are observed, including alterations in lipids, hyperglycemia and glucose intolerance, and insulin resistance/hyperinsulinemia (1, 5, 41–43). Dysregulated secretion of adipocyte-derived proteins (adipokines) which act both locally and systemically is also observed. These changes in secreted hormones and other factors include increased leptin, decreased adiponectin and resistin, retinol binding protein-4 (RBP4), tumor necrosis factor-α (TNF-α), interleukin-1β (Il-1β), and IL-6 (5, 40, 44, 45). Leptin has been a focus of much early work on obesity. Although the primary function of the protein leptin has generally been viewed as promoting leanness, by signaling back to the CNS to decrease intake of food and increase energy expenditure to limit obesity, the overall role of leptin is far more complex and to date remains somewhat elusive (46). From an oncology perspective, high leptin levels appear to correlate with increased risk of certain cancers, including breast cancer (1).

Of note, all accumulations of adipose tissue, i.e., adipose depots, are not the same. The adipose depots that characterize obesity are complex and must be analyzed at a granular level in order to understand their effect on cancer risk. Excessive visceral deposits of adipose tissue, primarily in the abdomen, are considered to be the main culprits involved in disease causation (47, 48). Specific abdominal organs such as the greater omentum (referred to as the "abdominal policeman") are preferred sites of this visceral adiposity tissue (VAT). In contrast, subcutaneous adipose tissue (SAT) is generally less active in the mechanisms implicated in these disruptions of biologic homeostasis. Excessive adipose tissue, especially VAT, is associated with the "metabolic syndrome," involving insulin resistance, hyperglycemia, dyslipidemia, and hypertension. Prothrombotic and proinflammatory states are also characteristic of VAT. Besides the adipocytes, which secrete endocrine hormones such as leptin and adiponectin, adipose tissue contains other types of cells that also secrete proteins. Examples include leukocytes and stromovascular cells which, along with adipocytes, express TNF-α, particularly in SAT (40, 49). These dissimilar cell types function in an integrated manner, consistent with the view that adipose tissue is actually an entire

endocrine organ (40). White adipose tissue (WAT), the subtype of adipose tissue whose main function is to store energy in the form of lipids and maintain energy homeostasis (50–52), functions as a complex secretory and endocrine organ. In the obese state adipocytes in WAT secrete a number of inflammatory cytokines, including TNF-α and IL-6 (51).

Immune Function of Adipose Tissue

These metabolic functions are intimately connected to the immune activities of adipose tissue (4). In addition to adipocytes and stromovascular cells, leukocytes, which include a variety of immune cells—macrophages, neutrophils, T cells, B cells and mast cells—are found in increased numbers in adipose tissue of obese individuals. In particular, macrophages, which make up 5-10% of cells in healthy adipose tissue, constitute 50% of all cell types in hypertrophic adipose tissue (4, 49). The macrophages located within adipose deposits skew toward the M1 type, which secretes inflammatory cytokines, including TNF-α, IL-6, and IL-1β; this contrasts with M2 macrophages which have the antithetical effect of improving metabolic function and reducing adipose inflammation. The inflammatory macrophages are the primary cell type responsible for inflammation associated with obesity. As a result, in obesity the circulating levels of these macrophage-secreted factors are elevated, resulting in a chronic inflammatory state (52). Although self-limited inflammation in response to pathogens is a normal function of the innate immune system, including macrophages, individuals with obesity and metabolic syndrome experience chronic low-grade inflammation, which is associated with higher levels of inflammatory cytokines in both plasma and subcutaneous adipose tissue (4, 53). Such impaired resolution of acute inflammation leads to metabolic tissue stress with tissue destruction and dysfunction (53), including insulin resistance and diabetes (5, 45, 54). Thus, the connection between obesity and metabolic dysfunction/insulin resistance is dependent at least in part on inflammation which is initiated by the innate immune system (54).

The dysfunctional milieu of obesity-associated adipose tissue has additional adverse immune effects, such as ectopic accumulation of lipids in non-adipose tissue, including tissues of the immune system: bone marrow and thymus (49). Obesity results in altered lymphocyte tissue architecture and integrity with shifts in populations of immune cells that lead to inflammatory phenotypes (4). Among these changes are increases in T helper type 1 (Th1) cells and cytotoxic CD8+ T cells, which produce cytokines [interferon-ɣ (IFN-ɣ), TNF, and IL-6] that induce M1 macrophages, which, in turn, secrete proinflammatory cytokines (TNF, IL-6, IL-1β, and others) (49). B cells are also increased in VAT, as shown in mice fed a high-fat diet (48). Total B cells, B-1a cells and B2 cells are all elevated in this setting. Increased abundance of mature B cells which had undergone class switching, including IgG+ cells which are involved in progressive immune activity, is observed. These mice exhibit increased serum concentrations of IgG2c, a pro-inflammatory isotype. B lymphocytes are therefore involved in the development of VAT inflammation, to which they contribute by activating CD8+ and Th1 cells as well as releasing pathogenic antibodies. The downstream metabolic effects of pro-inflammatory cytokine produced by the CD8+ and Th1 cells include insulin resistance and glucose intolerance, which ultimately are attributable to B cell activity.

ENDOCRINE FUNCTION OF ADIPOSE TISSUE IN OBESITY INCREASES BREAST CANCER RISK

Immune System: Role in Breast Cancer Risk

The alterations in the immune system that are associated with obesity can predispose to development of 13 cancer types via a variety of mechanisms (2, 53, 55). The mechanistic underpinnings of the observed causal relationship of obesity with breast cancer exemplify the intertwining of the various adipose mechanisms described above. In one prospective population-based cohort of postmenopausal women followed from 1990 through 2005, 272 women were diagnosed with incident breast cancer. Among three markers altered by obesity [leptin, adiponectin and soluble TNF receptor 2 (sTNF-R2)], plasma levels of sTNF-R2 and leptin showed independent positive association with breast cancer risk (56). Given the known carcinogenic nature of the inflammatory cytokine TNF, derived from macrophages that infiltrate adipose tissue, these data are consistent with an immunologic mechanism linking obesity and breast cancer. In the setting of obesity, WAT becomes altered, manifesting changes in production of steroid hormones and adipokines as well as chronic subclinical inflammation, activities which predispose to cancer (50). M1 macrophages, the CD68 staining immune cells that secrete inflammatory cytokines—TNF-α, IL-6, and IL-1β–that are implicated in promoting obesity-associated inflammation (49), are abundant in breast WAT (50, 52). These macrophages aggregate in histologically defined crown-like structures (CLS) in which they surround necrotic adipocytes, a histopathologic feature that is observed in mice and humans (41, 47, 57). Macrophage-based CLS formations are found in normal breast tissue, at a higher frequency in obese women (58, 59). These breast CLS (CLS-B) serve as measures of breast inflammation, quantified as the CLS-B index (60).

Steroid Hormones: Role in Breast Cancer Risk

The increased incidence of estrogen-receptor-positive (ER+) breast cancer in obesity supports the role for estrogen, a steroid hormone, in breast carcinogenesis (61), bringing the endocrine function of adipose tissue into play. Key factors that are increased in breast tissue of obese women have been shown to play a role in stimulating expression of aromatase, the enzyme that carries out the rate-limiting step of estrogen biosynthesis (56, 61). The mechanisms responsible for production of these factors rely on activation of the immune system, bridging the previously described immune and hormonal effects of obesity. For example, TNF produced by adipose-infiltrating macrophages stimulates expression of aromatase in adipose fibroblasts (56, 61).

Prostaglandin E_2 (PGE_2), an inflammatory factor, and hypoxia-inducible factor 1α (HIF-1 α) both participate in inducing aromatase production by adipose stromal cells (ASCs) (62). Elevated levels of aromatase are found in VAT and SAT as well as adipose tissue in the breast of obese postmenopausal women (63), including inflamed breast adipose tissue of obese women with breast cancer (64). This "obesity-inflammation-aromatase axis" has been proposed to play an important role in increased risk of ER+ breast cancer in postmenopausal women, by elevating estrogen levels in the breasts of women in whom levels of estrogen in the general circulation are reduced (60, 64, 65).

MOLECULAR MECHANISMS CONTRIBUTING TO OBESITY AND BREAST CANCER: GENETICS, EPIGENETICS, AND MICROBIOMICS

At the molecular, mechanistic level, genetics, epigenetics, and microbiomics are likely involved in susceptibility to weight gain and obesity (66). These molecular factors may also interact to give rise to obese phenotypes. Furthermore, the interaction between these molecular factors with behavior and environmental factors likely add to the etiologic complexity and biological variation that is observed with weight gain and the obese state. Moreover, dysregulation of these molecular mechanisms may explain not only the link between obesity and breast cancer, but also the comorbid conditions associated with obesity.

Genetics

Many gene variants have been found to be associated with obesity. Recent reviews highlight both the candidate gene approach utility for identifying monogenic obesity genes as well as genetic variants identified through Genome Wide Association Studies (GWAS), which implicate genes from several biological pathways in polygenic obesity (66–68). These GWAS approaches have revealed that loci associated with obesity carry genes involved in pathways influencing neuro-circuits of appetite and satiety regulation (*BDNF, MC4R, NEGR, POMC*) (69–73), insulin secretion and action (*TCF7L2, IRS1*) (69, 74), adipogenesis (75) and energy and lipid metabolism [*FTO, RPTOR, MAP2K5* (69, 74, 76)]. Using well-powered GWAS studies, more than 870 SNPs have been found to be associated with BMI (68). However, the findings also indicate that these loci only explain 5% of the variance of BMI (77). Although challenging, attempting to explain the remaining variability is a focus of obesity research. In this regard, the utilization of other omics, such as transcriptomics, proteomics, epigenomics, microbiomics, and metabolomics, may increase the phenotypic prediction of weight gain (66, 78). Associations between obesity, genetics and breast cancer have been documented and more are emerging. One example concerns the fat mass and obesity associated (*FTO*) gene, which was the topic of a recent systematic review that promulgated *FTO* gene as a possible mediator for the association between obesity and breast cancer (79). The *FTO* gene encodes a dependent oxygenase related to 2-oxoglutarate that has a role in DNA demethylation but its molecular mechanism in obesity and metabolism has not been elucidated (80). In their systematic review, Akbari et al. (79) suggested that polymorphisms in the *FTO* gene may influence the risk of breast cancer as well as obesity through expression of the homeobox transcription factor iriquois 3 (*IRX3*) gene. *IRX3* is a developmental transcription factor that more recently has been implicated in regulating energy expenditure (81).

Epigenetics

With a great degree of complexity and flexibility, epigenetic mechanisms influence how genetic information is transcribed and translated into proteins, ultimately affecting health and disease, including the conditions of weight gain and obesity. In contrast to genetic modifications, which lead to a change in the base sequence of DNA, epigenetic changes are thought to be reversible and consist of chemical modifications to DNA (or DNA-associated chromosomal proteins called histones) that occur in the absence of a change in the DNA sequence (82). Epigenetic mechanisms include DNA methylation, histone modifications, and microRNA-mediated regulation, which can be passed on mitotically (through cell division) or meiotically (through generational inheritance) (83). Epigenetics has emerged as a significant link between genes and the environment, serving as a molecular mechanism to explain individual variation in biological response to environmental factors. Interestingly, recent evidence suggests an association between obesity and DNA methylation; but whether this is a cause or a consequence of the obese phenotype requires mechanistic examination (84). A brief discussion of the relationship between DNA methylation, obesity and breast cancer follows. The role of microRNA and histones in influencing obesity and their relationships to breast cancer are discussed elsewhere (83, 85–87).

DNA Methylation

In mammals, the addition of methyl groups to DNA (methylation) occurs predominantly at cytosines adjacent to guanines ("CpG" sites) through DNA methyltransferases. Promoter DNA methylation disrupts the binding of transcription factors and attracts methyl-binding proteins that typically initiate chromatin compaction and gene silencing (88). Promoter hypomethylation, on the other hand, is associated with activation of transcription. DNA methylation is the best studied and most stable epigenetic mechanism, and both candidate gene methylation and epigenome-wide methylation studies have been performed to understand connections with obesity (68, 83, 87). These have led to discovery of DNA methylation changes that are associated with many genes and pathways related to obesity and its comorbidities, including appetite control, insulin signaling, immunity, and inflammation. Interestingly, candidate genes implicated in monogenic obesity (e.g., *POMC*) have also been found to be influenced by DNA methylation changes contributing to common obesity (89). With the use of genetic association analyses along with epigenome-wide association analyses, alterations in DNA methylation have been shown to be the result of obesity rather than the cause of obesity (90). This study suggested epigenetics as a mechanism by which some individuals with excess BMI move to the next step in the

causal pathway to metabolic disease. Other evidence, however, is suggestive of a putative causal relationship for DNA methylation alterations in the onset of obesity and metabolic disease. Such is the case for evidence from the Dutch Winter Hunger cohort with inclusion of subjects that experienced famine early in life (91). Investigators recently performed a genome-wide analysis of differential DNA methylation in whole blood from this cohort (92). They show that the associations between exposure to an adverse environment during early development and health outcomes in adulthood are mediated by alterations in DNA methylation; interestingly, *PIM3* methylation (cg09349128), a gene involved in energy metabolism, mediated approximately 13% of the association between famine exposure and BMI.

Obesity, Epigenetics, Breast Cancer

There is an emerging interest in interrogating DNA methylation as a possible mechanistic link between obesity and breast cancer. An example concerns estrogen receptor 1 (*ESR1*) gene hypermethylation, which may be involved in the development of breast cancer. Investigators hypothesized that BMI and estrogen-related reproductive risk factors may influence the methylation status of the *ESR1* CpG loci in the normal breasts of healthy women. They found that *ESR1* promoter methylation in women with a BMI $\geq$30 kg/m^2 was higher than in the subgroups of women with BMI $<$ 25 kg/m^2 or BMI 25–29 kg/m^2 and was also higher in postmenopausal women compared with that in premenopausal women (93). The finding provides possible clues to the relationship between epigenetic changes within the *ESR1* gene CpG island and postmenopausal obesity and aging in cancer-free women, and merits additional study. In another example, investigators explored the association of adiposity-related CpG loci and subsequent risk of postmenopausal breast cancer, colorectal cancer and myocardial infarction (94). Using peripheral blood leucocytes from over 1900 individuals from four prospective European cohorts, these investigators measured the relationship between DNA methylation profiles and body mass index, waist circumference, waist-hip and waist-height ratio within a meta-analytical framework that also assessed the relationship of adiposity-related CpG to comorbidities. Among the 40 adiposity-related CpG loci identified, two loci in *IL2RB* and *FGF18* and one CpG locus in an intergenic region of chromosome 1 were associated with colorectal cancer and myocardial infarction development (94). However, none of the adiposity-related CpG loci were associated with post-menopausal breast cancer following Bonferroni correction; the authors also noted that the number of post-menopausal breast cancer cases included in the study was relatively small.

DNA methylation has been suggested as a mechanism that could explain inter-individual variability in terms of weight loss response as well as the metabolic response to weight loss (95). In this regard, there is interest in examining whether weight loss might reverse abnormal DNA methylation changes observed in obesity and thereby reduce comorbidities. Rossi et al. identified several hypermethylated gene promoters in mice that were obese, compared to leaner controls (96). Interestingly, many of these genes showed intermediate methylation in formerly obese mice, suggesting that some obesity-associated epigenetic changes may be resistant to reprogramming after weight loss. These authors also found that weight loss in the formerly obese mice did not reduce proinflammatory cytokine gene expression nor the basal-like mammary tumor burden (96). The authors mention that weight loss in combination with epigenetic or anti-inflammatory interventions may be needed to disrupt the obesity–breast cancer link. Furthermore, examination of DNA methylation, in combination with genetic variants, gut microbiota and other molecular mechanisms, might be useful in understanding the relationship between obesity, weight loss and breast cancer.

Microbiomics

The collective genomes of the microbes (composed of bacteria, bacteriophage, fungi, protozoa, and viruses) that live inside and on the human body are referred to as the microbiome (97). Alterations of gut microbiota and its microbiome are associated with obesity and are responsive to weight loss (98). For example, transferring the luminal contents from the ceca of obese and lean mice to germ-free animal recipients resulted in more weight gain over a 2-week period in recipients receiving the microbes from obese animals compared to the recipients inoculated with the lean mouse microbes, despite equivalent food intake (99). Hints are also found from human studies, including a study in twins which found that obese individuals displayed reduced bacterial diversity, a depletion of *Bacteroidetes* as well as greater abundance of carbohydrate and lipid-utilizing microbial genes compared to lean individuals (100). Many mechanisms have been implicated in these associations such as increased dietary energy harvest, microbe-induced changes in host glucose and lipid metabolism, microbial signaling through host endocrine systems, and chronic low-grade inflammation leading to insulin resistance (98). Backhed et al. observed a direct link between the intestinal microbiome and increased adiposity when they inoculated germ-free mice with the cecal contents from conventional mice (101). These recipient mice gained weight despite calorie restriction; experiments revealed that weight gain was in part due to increased intestinal monosaccharide absorption and increased hepatic lipogenesis. Furthermore, the microbiome in these mice suppressed a host gene (*Fiaf* or fasting-induced adipose factor) coding a circulating lipoprotein lipase inhibitor (Angptl4), which resulted in an increase in triglyceride deposition in adipose tissue (102). The magnitude of the contribution of the gut microbiota and its gene content to obesity and its related comorbidities is still uncertain (66). Perhaps a better understanding of host-microbe and microbe-microbe interactions may lead to the development of novel strategies for reversing obesity (103).

Microbial perturbations (dysbiosis) have been observed in breast cancer patients compared to healthy individuals (104, 105). Here it is interesting to note that the gut microbiota may influence the production of estrogen metabolites and it has been hypothesized that alterations in the microbiota might lead to elevated levels of circulating estrogens and its metabolites, thus increasing the risk of breast cancer (105). Although an altered intestinal microbiome has been implicated in obesity and alterations of the microbiome (both distal and local) may influence breast cancer risk, little to no research has examined the

mechanisms that may explain the association between obesity, microbiome and breast cancer.

TACKLING OBESITY: THE MANY FACETS OF WEIGHT LOSS

Obesity-Targeting Weight Loss Interventions—Addressing Above Mechanisms

Several observational studies found that adult weight loss was associated with decreased risk for postmenopausal breast cancer (106–109), although others did not find an association (110, 111). A meta-analysis assessing the effect of weight loss on breast cancer incidence found that weight loss significantly reduces breast cancer risk in both pre- and post- menopausal women (112). In a recent study, investigators examined the effect of weight change on breast cancer incidence in 61,335 postmenopausal women enrolled in the Women's Health Initiative Observational Study (109). This study reported that women who lost weight (> 5% of body weight) compared to women with stable weight had a significantly lower breast cancer risk (HR, 0.88, 95% CI, 0.78–0.98). Similar findings were found in the Nurses' Health Study for weight loss and reduced breast cancer risk (HR, 0.77, 95% CI = 0.65–0.91) (108). These results are also supported by bariatric surgery research revealing a reduction in the risk of breast cancer (113). Although the presented evidence that weight loss is associated with decreased breast cancer risk appears to be convincing, more rigorous data involving clinical trials and timing of weight loss are needed.

Weight loss, a state of negative energy balance, is believed to significantly influence postmenopausal breast cancer risk through alterations in several pathways including sex-steroid hormones, endocrine hormones, and inflammatory markers. Obesity-targeting weight loss interventions that include hypocaloric diets and/or exercise have been shown to significantly reduce total body weight, adipose tissue (visceral and subcutaneous) and biomarkers associated with breast cancer risk (114). Here we review how weight loss can modulate obesity-related mechanisms that favor decreased breast cancer risk. Randomized trials of weight loss as an intervention in cancer survivors has been reviewed elsewhere (115, 116).

Weight-Loss and Sex-Steroid Hormones

As described above, excess adipose tissue modulates steroid aromatization, resulting in elevated levels of estrogen and, therefore, increased breast cancer risk. Weight loss interventions have been shown to have beneficial effects on estradiol, free estradiol, sex hormone binding globin (SHBG) and free testosterone concentrations (117, 118). For example, the Nutrition and Exercise in Women (NEW) study revealed that participants in the diet plus exercise group had greater reductions in total body weight and waist circumference compared to diet-only and exercise-only groups (mean 8.9, 7.2, 2.0 kg, respectively) (119). A dose-response relationship was also found, such that greater weight loss was associated with greater decreases in estrone, estradiol, free estradiol, and free testosterone, as well as a greater increase in SHBG (120). Another study found that overweight and obese postmenopausal women with >10 vs. <10% weight loss, had significant changes in bioavailable estradiol ($p < 0.001$), testosterone ($p = 0.033$), and SHBG ($p < 0.001$) (121). Research studies and meta-analyses provide sufficient evidence that weight loss interventions, in the form of reduced caloric intake and exercise, are associated with significant reductions in sex-steroid hormones (39, 118).

Weight-Loss and Endocrine Hormones (Insulin and IGF-1)

Abdominal obesity, specifically visceral fat, is associated with metabolic abnormalities such as hyperinsulinemia, insulin resistance and elevated IGF-1 concentrations, all of which are risk factors for breast cancer (122, 123). Obesity-targeting weight loss interventions have produced favorable changes in fasting insulin, glucose and HOMA-IR concentrations (121, 124–126). For example, weight losses > 10% were associated with a median absolute change in insulin concentrations (−3.4 μIU/ml; $p = 0.018$) among women at increased risk for breast cancer (121). Another study revealed that weight loss (subcutaneous and visceral fat) at 6 months was significantly associated with reductions in fasting insulin and HOMA concentrations, which remained significantly lower than baseline at 12 months, even after weight regain for women assigned to the diet group (124). However, the literature is somewhat contradictory as it relates to insulin-like growth factor-1 (IGF-1) concentrations. Several weight loss interventions have shown that weight loss is positively associated with IGF-1 concentrations and decreased IGFBP-1 & 3 (114, 121, 124, 127). Mason et al. (128) found no significant changes in IGF-1 or IGFBP-3 by intervention arm, but did find that greater weight loss was associated with elevated IGF-1 and molar ratio of IGF-1: IGFBP-1 concentrations in obese postmenopausal women. However, a few interventions found either no significant change (125) or slight decreased serum IGF-1 and increased IGFBP-3 concentrations after the adoption of a very low-calorie diet (129). A multicenter trial examining caloric restriction of 25% over 2 years suggests that insignificant changes in IGF-1 and IGF-1:IGFBP-3 molar ratio concentrations may be related to chronic high protein intake (130). It is well-established that weight loss can reduce insulin, glucose, and measures of insulin resistance. However, large intervention studies are needed to better understand the effects of weight loss on IGF-1 concentrations.

Weight Loss and Inflammatory Markers

White adipose tissue is metabolically active and is a major contributor to the release of cytokines and adipokines in the bloodstream (131). Weight loss interventions have shown reductions in systemic markers of chronic inflammation (121, 124, 132–134). A study in obese postmenopausal women found that those randomized to the diet plus exercise group and the diet only group experienced the greatest amount of weight loss, which, in turn, was associated with significant increases in adiponectin (+11.7 % and 18.5% in each group, respectively) as well as reductions in leptin (p -trend <0.001), compared to the control group (135). Another study found that obese

postmenopausal women assigned to a hypocaloric diet plus aerobic exercise condition vs. a diet-only condition lost more weight, particularly abdominal fat, and had significantly greater reductions in C-reactive protein (CRP), IL-6, sIL-6R, and TNFR1 concentrations (136). In this study, reductions in abdominal fat stimulated lipolysis, which correlated with reductions in plasma IL-6 and TNFR1 (136). Other studies in obese postmenopausal women reported that > 10% total weight loss and reductions in waist circumference produced favorable changes in CRP, adiponectin, leptin, and the molar ratio of adiponectin: leptin concentrations at 12 weeks and at 1- year follow-up (121). A systematic review and meta-analysis found that diet-induced weight loss was associated with reductions in adiponectin concentrations (137). Similar findings have shown reductions in several systemic concentrations of acute phase reactants and pro-inflammatory cytokines after weight loss intervention (124, 138, 139). Nicklas et al. (140) observed that the strongest correlations with change in CRP was a change in weight, waist circumference, insulin and HOMA. Overall, obesity-targeting weight loss interventions have shown reductions in most inflammatory markers, especially for CRP.

Weight-Loss and Macronutrient Composition

There may be differential amounts of weight loss in response to specific dietary macronutrient (e.g., protein, fat, carbohydrate) composition. Several meta-analyses of weight loss randomized controlled trials (RCTs) examined the efficacy of low-carbohydrate (LC) vs. low-fat (LF) diets on weight change (141–143). One study found non-significant differences for macronutrient composition on the amount of weight loss at 12 months (144); whereas, the other meta-analyses found that LC diets rather than LF diets led to significantly greater weight loss at 12 months, but the weight loss differences between diets were small (141–143). Additionally, two large RCTs did not observe differential effects of macronutrient intakes on the amount of weight loss (145, 146). Specifically, the POUNDS LOST trial did not find differences in 4 diets that varied in macronutrient composition on changes in body composition, abdominal fat, or hepatic fat (145). The DIETFIT study examined the effects of a healthy LF vs. a healthy LC diet on weight change in 609 overweight participants. There were no significant differences between the two diets in terms of weight loss (−5.3 kg HLF and −6.0 kg HLC) nor were there between-group differences for BMI, body fat percent, or waist circumference at 12 months (146). It appears that a reduction in total energy intake may be more important for weight loss rather than manipulating the macronutrient content of the diet. However, the literature is mixed, and further study is required.

Weight-Loss, Macronutrient Composition, and Biomarkers

Fasting glucose and insulin may impact response to weight loss diets with different macronutrient composition. Researchers suggest that a LC diet may provide greater weight loss in overweight and obese women who are insulin resistant (147, 148); in contrast, normoglycemic participants lose more weight on an LF diet (149). A recent study found that overweight/obese participants who were insulin resistant (HOMA-IR >4) lost significantly more weight on a high-fat (HF) high-protein (HP) diet; however, it should be noted the diet was also very low in carbohydrates (40% fat, 25% protein, 35% carbohydrates) compared to a HF-average protein diet (40% fat, 15% protein, 45% carbohydrate) (149). Rock et al. (133) found that women who were insulin sensitive lost greater weight at 12 months in the LF vs. LC diet group. However, a large RCT did not reveal differential effects for the LF vs. LC diets on weight loss by baseline insulin status (146, 150). Our understanding of macronutrient composition on weight loss in obese insulin-sensitive and insulin resistant individuals requires further study.

Furthermore, it is possible that there is a differential weight loss response to diet composition and that biomarkers associated with breast cancer risk may mediate this association. For example, a LC vs. LF weight loss diet was associated with increased adiponectin concentrations in obese women; however, there were no correlations between weight loss and increased adiponectin (151). Other studies did not find significant differences by intervention arm (caloric-restricted LF vs. LC diet) on favorable changes in adipokine and leptin concentrations at study completion, although leptin concentrations decreased with both diets (152). Weight loss induced by overall caloric restriction rather than the macronutrient content of the diet appears to be more effective in reducing chronic systemic inflammation (140, 153–155) and endocrine markers such as insulin and HOMA (156). Research is needed using large RCTs to understand whether differential weight loss response to macronutrient composition is influenced by biomarkers of breast cancer risk.

Pharmacological Approaches to Obesity and Weight Loss

Although our emphasis has been on weight loss as a remedy to obesity, other approaches are being tried. As previously discussed, increased physical activity has potential to decrease breast cancer risk, at least in part by reducing obesity (39). However, targeting physical activity as an isolated behavioral change whose increase might facilitate decreased obesity is complicated by the interplay between this approach, caloric reduction and their effects on energy balance. Alternatively, pharmacological approaches to weight, and hence obesity, reduction have been considered. Metformin, the first-line treatment for type II diabetes, which has been extensively studied regarding its cancer preventive activity, including breast cancer (157), has exhibited efficacy in reducing weight in a number of studies. Weight reduction is expected to disrupt the association between obesity and cancer, suggesting a possible mechanistic basis for the anti-cancer effect of metformin (158). In a study of 154 consecutive non-diabetic, overweight/obese individuals, metformin-treated patients had a mean weight loss of 5.8 ± 7.0 kg in contrast to a loss of 0.8 ± 3.5 kg in an untreated group (159). A meta-analysis of 13 studies addressing the effects of metformin on simple obesity showed that metformin is effective in reducing body weight in this population, without inducing

hypoglycemia (160). The Diabetes Prevention Program is a clinical trial that randomized 3234 participants with elevated glucose and overweight/obesity, to metformin, intensive lifestyle intervention (ILS), or placebo. Whereas, at 1-year follow-up, only 28.5% of participants in the metformin arm had lost at least 5% of their weight, 62.6% in the ILS group and 13.4% in the placebo group had achieved this goal (161). In contrast, between years 6 and 15, after unmasking, maintenance of mean weight loss was 6.2% with metformin, 3.7% with ILS, and 2.8% with placebo, suggesting a benefit to metformin with respect to a long-term weight loss endpoint. Although much remains to be investigated, metformin has exhibited potential to induce weight loss in both diabetic and non-diabetic individuals.

Another agent showing benefits for weight management is liraglutide, a glucagon like peptide-1 (GLP-1) receptor agonist that is approved by the Food and Drug Administration (FDA) as an adjunct to diet and exercise for management of type 2 diabetes. A review of five randomized clinical trials showed that compared to placebo, liraglutide was associated with a higher proportion of patients achieving at least a 5-10% weight loss (162). The main drawbacks to its use are gastrointestinal side effects and the need for injection. In addition, pharmacologic agents that have been investigated for treatment of eating disorders also offer possible interventions to induce weight loss in obese patients (163). One such agent, lisdexamfetamine, a central nervous system amphetamine, has been used in children with severe obesity, although long-term use is discouraged, given its high potential for abuse (164). The state of pharmacologic interventions to induce weight loss thus remains in flux as studies aimed at identifying an improved balance between efficacy and side effects continue.

CONCLUSIONS AND FUTURE DIRECTIONS

Obesity has reached epidemic proportions in the United States and increasingly around the world. Undesirable health-related sequelae are expected to follow as the obese state is increasingly being observed in children and young adults. Obesity is physiologically complex, however, and we have discussed only a few of the endocrine, immunologic and molecular abnormalities that characterize this state. In addressing the need for reducing obesity we have concentrated on evidence derived from weight loss initiatives. However, other approaches are currently being undertaken. For example, physical activity as a major intervention, with or without accompanying diet directives, has potential to improve obesity-related metabolic parameters. Intermittent fasting approaches, including time-restricted feeding, are emerging weight loss strategies, which may also improve metabolic parameters. Bioactive food components such as omega-3-fatty acids are being studied as interventions to facilitate loss of weight. Finally, pharmacologic approaches, including agents such as metformin, need to be investigated in relation to their weight reducing efficacy.

Breast cancer, the most common cancer in postmenopausal women in the U.S., is one of the malignant outcomes associated with chronic obesity. Thus, efforts to improve interventions to prevent breast cancer, along with other serious obesity-associated diseases, require a deeper understanding of the physiological basis of obesity as well as the development of interventions to reduce this high-risk state in the population.

Despite the extensive research that has been ongoing into the multiple facets of obesity on general health and cancer in particular, huge gaps remain in our understanding of mechanisms and associations. Of immediate interest is the disconnect between obesity's positive association with postmenopausal ER-positive breast cancer and its inverse association with premenopausal ER-positive disease; what is the mechanistic basis for this difference? How do the duration and timing in the life cycle influence the chronic nature of obesity that appears to be linked to breast cancer? Additional gaps address the complex molecular mechanisms at the genetic and epigenetic levels which control expression of proteins that contribute to obesity. The integration of various omics data, including transcriptomics, proteomics, epigenomics, microbiomics, and metabolomics, may also assist in elucidating the link between obesity and cancer.

The majority of the epidemiologic studies linking obesity to breast cancer used self-reported anthropometric measures (e.g., BMI, waist circumference) to assess risk. However, more meaningful assessments of body composition compartments (e.g., VAT and SAT), which capture known physiological and metabolic changes associated with breast cancer risk, need to be used in future studies. Also, one must not ignore the enormous effect the obesity epidemic is having on low SES populations, which in the future may potentially lead to associated chronic diseases, including cancer. Lastly, since the majority of the studies were conducted among Caucasian women, research is needed to understand the association between body fat distribution and specific breast cancer subtypes across various racial and ethnic groups.

AUTHOR CONTRIBUTIONS

TA-C, SR, and BD each wrote sections of the manuscript and each reviewed and edited the manuscript for content and cohesion.

REFERENCES

1. Goodwin PJ, Stambolic V. Impact of the obesity epidemic on cancer. *Annu Rev Med*. (2015) 66:281–96. doi: 10.1146/annurev-med-051613-012328
2. Sung H, Siegel RL, Torre LA, Pearson-Stuttard J, Islami F, Fedewa SA, et al. Global patterns in excess body weight and the associated cancer burden. *CA Cancer J Clin*. (2019) 69:88-112. doi: 10.3322/caac.21499
3. WHO. World Health Organization. *Obesity and Overweight*. (2018). Available online at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed February 16, 2018).
4. Andersen CJ, Murphy KE, Fernandez ML. Impact of obesity and metabolic syndrome on immunity. *Adv Nutr*. (2016) 7:66–75. doi: 10.3945/an.115.010207

5. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol*. (2008) 9:367–77. doi: 10.1038/nrm2391
6. Afshin A, Reitsma MB, Murray CJL. Health effects of overweight and obesity in 195 countries. *N Engl J Med*. (2017) 377:1496–7. doi: 10.1056/NEJMc1710026
7. Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K, et al. Body fatness and cancer–viewpoint of the IARC working group. *N Engl J Med*. (2016) 375:794–8. doi: 10.1056/NEJMsr1606602
8. Liu K, Zhang W, Dai Z, Wang M, Tian T, Liu X, et al. Association between body mass index and breast cancer risk: evidence based on a dose-response meta-analysis. *Cancer Manag Res*. (2018) 10:143–51. doi: 10.2147/CMAR.S144619
9. Xia X, Chen W, Li J, Chen X, Rui R, Liu C, et al. Body mass index and risk of breast cancer: a nonlinear dose-response meta-analysis of prospective studies. *Sci Rep*. (2014) 4:7480. doi: 10.1038/srep07480
10. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of obesity among adults and youth: United States, 2015-2016. *NCHS Data Brief*. (2017) 288:1–8.
11. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet*. (2008) 371:569–78. doi: 10.1016/S0140-6736(08)60269-X
12. Horn-Ross PL, Canchola AJ, Bernstein L, Neuhausen SL, Nelson DO, Reynolds P. Lifetime body size and estrogen-receptor-positive breast cancer risk in the California Teachers Study cohort. *Breast Cancer Res*. (2016) 18:132. doi: 10.1186/s13058-016-0790-5
13. Suzuki R, Rylander-Rudqvist T, Ye W, Saji S, Wolk A. Body weight and postmenopausal breast cancer risk defined by estrogen and progesterone receptor status among Swedish women: a prospective cohort study. *Int J Cancer*. (2006) 119:1683–9. doi: 10.1002/ijc.22034
14. Tehard B, Clavel-Chapelon F. Several anthropometric measurements and breast cancer risk: results of the E3N cohort study. *Int J Obes*. (2006) 30:156–63. doi: 10.1038/sj.ijo.0803133
15. Chen Y, Liu L, Zhou Q, Imam MU, Cai J, Wang Y, et al. Body mass index had different effects on premenopausal and postmenopausal breast cancer risks: a dose-response meta-analysis with 3,318,796 subjects from 31 cohort studies. *BMC Public Health*. (2017) 17:936. doi: 10.1186/s12889-017-4953-9
16. Baer HJ, Tworoger SS, Hankinson SE, Willett WC. Body fatness at young ages and risk of breast cancer throughout life. *Am J Epidemiol*. (2010) 171:1183–94. doi: 10.1093/aje/kwq045
17. Premenopausal Breast Cancer Collaborative G, Schoemaker MJ, Nichols HB, Wright LB, Brook MN, Jones ME, et al. Association of body mass index and age with subsequent breast cancer risk in premenopausal women. *JAMA Oncol*. (2018) 4:e181771. doi: 10.1001/jamaoncol.2018.1771
18. Lee KR, Hwang IC, Han KD, Jung J, Seo MH. Waist circumference and risk of breast cancer in Korean women: a nationwide cohort study. *Int J Cancer*. (2018) 142:1554–9. doi: 10.1002/ijc.31180
19. Wang F, Liu L, Cui S, Tian F, Fan Z, Geng C, et al. Distinct effects of body mass index and waist/hip ratio on risk of breast cancer by joint estrogen and progestogen receptor status: results from a case-control study in Northern and Eastern china and implications for chemoprevention. *Oncologist*. (2017) 22:1431–43. doi: 10.1634/theoncologist.2017-0148
20. Chen GC, Chen SJ, Zhang R, Hidayat K, Qin JB, Zhang YS, et al. Central obesity and risks of pre- and postmenopausal breast cancer: a dose-response meta-analysis of prospective studies. *Obes Rev*. (2016) 17:1167–77. doi: 10.1111/obr.12443
21. Connolly BS, Barnett C, Vogt KN, Li T, Stone J, Boyd NF. A meta-analysis of published literature on waist-to-hip ratio and risk of breast cancer. *Nutr Cancer*. (2002) 44:127–38. doi: 10.1207/S15327914NC4402_02
22. Millikan RC, Newman B, Tse CK, Moorman PG, Conway K, Smith LV, et al. Epidemiology of basal-like breast cancer. *Breast Cancer Res Treat*. (2008) 109:123–39. doi: 10.1007/s10549-007-9790-6
23. Harris HR, Willett WC, Terry KL, Michels KB. Body fat distribution and risk of premenopausal breast cancer in the Nurses' Health Study II. *J Natl Cancer Inst*. (2011) 103:273–8. doi: 10.1093/jnci/djq500
24. Pierobon M, Frankenfeld CL. Obesity as a risk factor for triple-negative breast cancers: a systematic review and meta-analysis. *Breast Cancer Res Treat*. (2013) 137:307–14. doi: 10.1007/s10549-012-2339-3
25. Slattery ML, Sweeney C, Edwards S, Herrick J, Baumgartner K, Wolff R, et al. Body size, weight change, fat distribution and breast cancer risk in Hispanic and non-Hispanic white women. *Breast Cancer Res Treat*. (2007) 102:85–101. doi: 10.1007/s10549-006-9292-y
26. Bandera EV, Chandran U, Zirpoli G, Gong Z, McCann SE, Hong CC, et al. Body fatness and breast cancer risk in women of African ancestry. *BMC Cancer*. (2013) 13:475. doi: 10.1186/1471-2407-13-475
27. Amadou A, Ferrari P, Muwonge R, Moskal A, Biessy C, Romieu I, et al. Overweight, obesity and risk of premenopausal breast cancer according to ethnicity: a systematic review and dose-response meta-analysis. *Obes Rev*. (2013) 14:665–78. doi: 10.1111/obr.12028
28. Phipps AI, Chlebowski RT, Prentice R, McTiernan A, Stefanick ML, Wactawski-Wende J, et al. Body size, physical activity, and risk of triple-negative and estrogen receptor-positive breast cancer. *Cancer Epidemiol Biomarkers Prev*. (2011) 20:454–63. doi: 10.1158/1055-9965.EPI-10-0974
29. Dietze EC, Chavez TA, Seewaldt VL. Obesity and triple-negative breast cancer: disparities, controversies, and biology. *Am J Pathol*. (2018) 188:280–90. doi: 10.1016/j.ajpath.2017.09.018
30. Chlebowski RT, Chen Z, Anderson GL, Rohan T, Aragaki A, Lane D, et al. Ethnicity and breast cancer: factors influencing differences in incidence and outcome. *J Natl Cancer Inst*. (2005) 97:439–48. doi: 10.1093/jnci/dji064
31. Zobel EH, Hansen TW, Rossing P, von Scholten BJ. Global changes in food supply and the obesity epidemic. *Curr Obes Rep*. (2016) 5:449–55. doi: 10.1007/s13679-016-0233-8
32. Newton S, Braithwaite D, Akinyemiju TF. Socio-economic status over the life course and obesity: Systematic review and meta-analysis. *PLoS ONE*. (2017) 12:e0177151. doi: 10.1371/journal.pone.0177151
33. Ogden CL, Fakhouri TH, Carroll MD, Hales CM, Fryar CD, Li X, et al. Prevalence of obesity among adults, by household income and education - United States, 2011-2014. *MMWR Morb Mortal Wkly Rep*. (2017) 66:1369–73. doi: 10.15585/mmwr.mm6650a1
34. Vona-Davis L, Rose DP. The influence of socioeconomic disparities on breast cancer tumor biology and prognosis: a review. *J Womens Health*. (2009) 18:883–93. doi: 10.1089/jwh.2008.1127
35. Parise CA, 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
36. Yost K, Perkins C, Cohen R, Morris C, Wright W. Socioeconomic status and breast cancer incidence in California for different race/ethnic groups. *Cancer Causes Control*. (2001) 12:703–11. doi: 10.1023/A:1011240019516
37. Dunn BK, Agurs-Collins T, Browne D, Lubet R, Johnson KA. Health disparities in breast cancer: biology meets socioeconomic status. *Breast Cancer Res Treat*. (2010) 121:281–92. doi: 10.1007/s10549-010-0827-x
38. Lope V, Martin M, Castello A, Ruiz A, Casas AM, Baena-Canada JM, et al. Overeating, caloric restriction and breast cancer risk by pathologic subtype: the EPIGEICAM study. *Sci Rep*. (2019) 9:3904. doi: 10.1038/s41598-019-39346-4
39. de Roon M, May AM, McTiernan A, Scholten R, Peeters PHM, Friedenreich CM, et al. Effect of exercise and/or reduced calorie dietary interventions on breast cancer-related endogenous sex hormones in healthy postmenopausal women. *Breast Cancer Res*. (2018) 20:81. doi: 10.1186/s13058-018-1009-8
40. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*. (2004) 89:2548–56. doi: 10.1210/jc.2004-0395
41. Alkhouri N, Gornicka A, Berk MP, Thapaliya S, Dixon LJ, Kashyap S, et al. Adipocyte apoptosis, a link between obesity, insulin resistance, and hepatic steatosis. *J Biol Chem*. (2010) 285:3428–38. doi: 10.1074/jbc.M109.074252
42. Park J, Morley TS, Kim M, Clegg DJ, Scherer PE. Obesity and cancer–mechanisms underlying tumour progression and recurrence. *Nat Rev Endocrinol*. (2014) 10:455–65. doi: 10.1038/nrendo.2014.94
43. Goodwin PJ. Obesity, insulin resistance and breast cancer outcomes. *Breast*. (2015) 24 (Suppl 2):S56–9. doi: 10.1016/j.breast.2015.07.014
44. Ahima RS, Flier JS. Adipose tissue as an endocrine organ. *Trends Endocrinol Metab*. (2000) 11:327–32. doi: 10.1016/S1043-2760(00)00301-5
45. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol*. (2010) 316:129–39. doi: 10.1016/j.mce.2009.08.018

46. Flier JS, Maratos-Flier E. Leptin's physiologic role: does the emperor of energy balance have no clothes? *Cell Metab*. (2017) 26:24–6. doi: 10.1016/j.cmet.2017.05.013
47. West-Eberhard MJ. Nutrition, the visceral immune system, and the evolutionary origins of pathogenic obesity. *Proc Natl Acad Sci USA*. (2019) 116:723–31. doi: 10.1073/pnas.1809046116
48. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, et al. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nat Med*. (2011) 17:610–7. doi: 10.1038/nm.2353
49. Kanneganti TD, Dixit VD. Immunological complications of obesity. *Nat Immunol*. (2012) 13:707–12. doi: 10.1038/ni.2343
50. Iyengar NM, Hudis CA, Dannenberg AJ. Obesity and cancer: local and systemic mechanisms. *Annu Rev Med*. (2015) 66:297–309. doi: 10.1146/annurev-med-050913-022228
51. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr*. (2004) 92:347–55. doi: 10.1079/BJN20041213
52. Iyengar NM, Zhou XK, Gucalp A, Morris PG, Howe LR, Giri DD, et al. Systemic correlates of white adipose tissue inflammation in early-stage breast cancer. *Clin Cancer Res*. (2016) 22:2283–9. doi: 10.1158/1078-0432.CCR-15-2239
53. Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. *Nat Rev Cancer*. (2004) 4:11-22. doi: 10.1038/nrc1252
54. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest*. (2006) 116:3015–25. doi: 10.1172/JCI28898
55. Howe LR, Subbaramaiah K, Hudis CA, Dannenberg AJ. Molecular pathways: adipose inflammation as a mediator of obesity-associated cancer. *Clin Cancer Res*. (2013) 19:6074–83. doi: 10.1158/1078-0432.CCR-12-2603
56. Gross AL, Newschaffer CJ, Hoffman-Bolton J, Rifai N, Visvanathan K. Adipocytokines, inflammation, and breast cancer risk in postmenopausal women: a prospective study. *Cancer Epidemiol Biomarkers Prev*. (2013) 22:1319–24. doi: 10.1158/1055-9965.EPI-12-1444
57. Subbaramaiah K, Howe LR, Bhardwaj P, Du B, Gravaghi C, Yantiss RK, et al. Obesity is associated with inflammation and elevated aromatase expression in the mouse mammary gland. *Cancer Prev Res*. (2011) 4:329–46. doi: 10.1158/1940-6207.CAPR-10-0381
58. Sun X, Casbas-Hernandez P, Bigelow C, Makowski L, Joseph Jerry D, Smith Schneider S, et al. Normal breast tissue of obese women is enriched for macrophage markers and macrophage-associated gene expression. *Breast Cancer Res Treat*. (2012) 131:1003–12. doi: 10.1007/s10549-011-1789-3
59. Mullooly M, Yang HP, Falk RT, Nyante SJ, Cora R, Pfeiffer RM, et al. Relationship between crown-like structures and sex-steroid hormones in breast adipose tissue and serum among postmenopausal breast cancer patients. *Breast Cancer Res*. (2017) 19:8. doi: 10.1186/s13058-016-0791-4
60. Subbaramaiah K, Morris PG, Zhou XK, Morrow M, Du B, Giri D, et al. Increased levels of COX-2 and prostaglandin E2 contribute to elevated aromatase expression in inflamed breast tissue of obese women. *Cancer Discov*. (2012) 2:356–65. doi: 10.1158/2159-8290.CD-11-0241
61. Bulun SE, Chen D, Moy I, Brooks DC, Zhao H. Aromatase, breast cancer and obesity: a complex interaction. *Trends Endocrinol Metab*. (2012) 23:83–9. doi: 10.1016/j.tem.2011.10.003
62. Subbaramaiah K, Iyengar NM, Morrow M, Elemento O, Zhou XK, Dannenberg AJ. Prostaglandin E2 down-regulates sirtuin 1 (SIRT1), leading to elevated levels of aromatase, providing insights into the obesity-breast cancer connection. *J Biol Chem*. (2019) 294:361–71. doi: 10.1074/jbc.RA118.005866
63. Zahid H, Simpson ER, Brown KA. Inflammation, dysregulated metabolism and aromatase in obesity and breast cancer. *Curr Opin Pharmacol*. (2016) 31:90–6. doi: 10.1016/j.coph.2016.11.003
64. Morris PG, Hudis CA, Giri D, Morrow M, Falcone DJ, Zhou XK, et al. Inflammation and increased aromatase expression occur in the breast tissue of obese women with breast cancer. *Cancer Prev Res*. (2011) 4:1021–9. doi: 10.1158/1940-6207.CAPR-11-0110
65. Iyengar NM, Morris PG, Zhou XK, Gucalp A, Giri D, Harbus MD, et al. Menopause is a determinant of breast adipose inflammation. *Cancer Prev Res*. (2015) 8:349–58. doi: 10.1158/1940-6207.CAPR-14-0243
66. Pigeyre M, Yazdi FT, Kaur Y, Meyre D. Recent progress in genetics, epigenetics and metagenomics unveils the pathophysiology of human obesity. *Clin Sci*. (2016) 130:943–86. doi: 10.1042/CS20160136
67. Speakman JR, Loos RJF, O'Rahilly S, Hirschhorn JN, Allison DB. GWAS for BMI: a treasure trove of fundamental insights into the genetic basis of obesity. *Int J Obes. (Lond)*. (2018) 42:1524–31. doi: 10.1038/s41366-018-0147-5
68. Rohde K, Keller M, la Cour Poulsen L, Bluher M, Kovacs P, Bottcher Y. Genetics and epigenetics in obesity. *Metabolism*. (2019) 92:37–50. doi: 10.1016/j.metabol.2018.10.007
69. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. (2015) 518:197–206. doi: 10.1038/nature14177
70. Ho EV, Klenotich SJ, McMurray MS, Dulawa SC. Activity-based anorexia alters the expression of BDNF transcripts in the mesocorticolimbic reward circuit. *PLoS ONE*. (2016) 11:e0166756. doi: 10.1371/journal.pone.0166756
71. Horstmann A, Kovacs P, Kabisch S, Boettcher Y, Schloegl H, Tonjes A, et al. Common genetic variation near MC4R has a sex-specific impact on human brain structure and eating behavior. *PLoS ONE*. (2013) 8:e74362. doi: 10.1371/journal.pone.0074362
72. Boender AJ, van Rozen AJ, Adan RA. Nutritional state affects the expression of the obesity-associated genes Etv5, Faim2, Fto, and Negr1. *Obesity*. (2012) 20:2420–5. doi: 10.1038/oby.2012.128
73. Millington GW. The role of proopiomelanocortin (POMC) neurones in feeding behaviour. *Nutr Metab*. (2007) 4:18. doi: 10.1186/1743-7075-4-18
74. Kilpelainen TO, Zillikens MC, Stancakova A, Finucane FM, Ried JS, Langenberg C, et al. Genetic variation near IRS1 associates with reduced adiposity and an impaired metabolic profile. *Nature genetics*. (2011) 43:753–60. doi: 10.1038/ng.866
75. Shungin D, Winkler TW, Croteau-Chonka DC, Ferreira T, Locke AE, Magi R, et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nature*. (2015) 518:187–96. doi: 10.1038/nature14132
76. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*. (2007) 316:889–94. doi: 10.1126/science.1141634
77. Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, et al. Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. *Hum Mol Genet*. (2018) 27:3641–9. doi: 10.1093/hmg/ddy271
78. Shah S, Bonder MJ, Marioni RE, Zhu Z, McRae AF, Zhernakova A, et al. Improving phenotypic prediction by combining genetic and epigenetic associations. *Am J Hum Genet*. (2015) 97:75–85. doi: 10.1016/j.ajhg.2015.05.014
79. Akbari ME, Gholamalizadeh M, Doaei S, Mirsafa F. FTO gene affects obesity and breast cancer through similar mechanisms: a new insight into the molecular therapeutic targets. *Nutr Cancer*. (2018) 70:30–6. doi: 10.1080/01635581.2018.1397709
80. Gerken T, Girard CA, Tung YC, Webby CJ, Saudek V, Hewitson KS, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science*. (2007) 318:1469–72. doi: 10.1126/science.1151710
81. Smemo S, Tena JJ, Kim KH, Gamazon ER, Sakabe NJ, Gomez-Marin C, et al. Obesity-associated variants within FTO form long-range functional connections with IRX3. *Nature*. (2014) 507:371–5. doi: 10.1038/nature13138
82. Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origins hypothesis. *Annu Rev Nutr*. (2007) 27:363–88. doi: 10.1146/annurev.nutr.27.061406.093705
83. Thaker VV. Genetic and epigenetic causes of obesity. *Adolesc Med State Art Rev*. (2017) 28:379–405.
84. van Dijk SJ, Molloy PL, Varinli H, Morrison JL, Muhlhausler BS, Members of Epi S. Epigenetics and human obesity. *Int J Obes*. (2015) 39:85–97. doi: 10.1038/ijo.2014.34
85. Lorente-Cebrian S, Gonzalez-Muniesa P, Milagro FI, Martinez JA. MicroRNAs and other non-coding RNAs in adipose tissue and obesity: emerging roles as biomarkers and therapeutic targets. *Clin Sci*. (2019) 133:23–40. doi: 10.1042/CS20180890
86. Kasiappan R, Rajarajan D. Role of MicroRNA regulation in obesity-associated breast cancer: nutritional perspectives. *Adv Nutr*. (2017) 8:868–88. doi: 10.3945/an.117.015800

87. Ling C, Ronn T. Epigenetics in human obesity and Type 2 diabetes. *Cell Metab*. (2019) 29:1028–44. doi: 10.1016/j.cmet.2019.03.009
88. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet*. (2003) 33 (Suppl):245–54. doi: 10.1038/ng1089
89. Kuhnen P, Handke D, Waterland RA, Hennig BJ, Silver M, Fulford AJ, et al. Interindividual variation in DNA methylation at a putative POMC metastable epiallele is associated with obesity. *Cell Metab*. (2016) 24:502–9. doi: 10.1016/j.cmet.2016.08.001
90. Wahl S, Drong A, Lehne B, Loh M, Scott WR, Kunze S, et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. *Nature*. (2017) 541:81–6. doi: 10.1038/nature20784
91. Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. *N Engl J Med*. (1976) 295:349–53. doi: 10.1056/NEJM197608122950701
92. Tobi EW, Slieker RC, Luijk R, Dekkers KF, Stein AD, Xu KM, et al. DNA methylation as a mediator of the association between prenatal adversity and risk factors for metabolic disease in adulthood. *Sci Adv*. (2018) 4:eaao4364. doi: 10.1126/sciadv.aao4364
93. Daraei A, Izadi P, Khorasani G, Nafissi N, Naghizadeh MM, Younosi N, et al. Epigenetic changes of the ESR1 gene in breast tissue of healthy women: a missing link with breast cancer risk factors? *Genet Test Mol Biomark*. (2017) 21:464–70. doi: 10.1089/gtmb.2017.0028
94. Campanella G, Gunter MJ, Polidoro S, Krogh V, Palli D, Panico S, et al. Epigenome-wide association study of adiposity and future risk of obesity-related diseases. *Int J Obes*. (2018) 42:2022–35. doi: 10.1038/s41366-018-0064-7
95. Samblas M, Milagro FI, Martinez A. DNA methylation markers in obesity, metabolic syndrome, and weight loss. *Epigenetics*. (2019) 14:421–44. doi: 10.1080/15592294.2019.1595297
96. Rossi EL, de Angel RE, Bowers LW, Khatib SA, Smith LA, Van Buren E, et al. Obesity-associated alterations in inflammation, epigenetics, and mammary tumor growth persist in formerly obese mice. *Cancer Prev Res*. (2016) 9:339–48. doi: 10.1158/1940-6207.CAPR-15-0348
97. Schlaeppi K, Bulgarelli D. The plant microbiome at work. *Mol Plant-Micro Interact*. (2015) 28:212–7. doi: 10.1094/MPMI-10-14-0334-FI
98. Ley RE. Obesity and the human microbiome. *Curr Opin Gastroenterol*. (2010) 26:5–11. doi: 10.1097/MOG.0b013e328333d751
99. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. (2006) 444:1027–31. doi: 10.1038/nature05414
100. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. *Nature*. (2009) 457:480–4. doi: 10.1038/nature07540
101. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Nat Acad Sci USA*. (2004) 101:15718–23. doi: 10.1073/pnas.0407076101
102. Backhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Nat Acad Sci USA*. (2007) 104:979–84. doi: 10.1073/pnas.0605374104
103. Barko PC, McMichael MA, Swanson KS, Williams DA. The Gastrointestinal microbiome: a review. *J Vet Int Med*. (2018) 32:9–25. doi: 10.1111/jvim.14875
104. Parida S, Sharma D. The power of small changes: comprehensive analyses of microbial dysbiosis in breast cancer. *Biochim et Biophy Acta Rev Cancer*. (2019) 1871:392–405. doi: 10.1016/j.bbcan.2019.04.001
105. Fernandez MF, Reina-Perez I, Astorga JM, Rodriguez-Carrillo A, Plaza-Diaz J, Fontana L. Breast cancer and its relationship with the microbiota. *Int J Environ Res Pub Health*. (2018) 15:E1747. doi: 10.3390/ijerph15081747
106. Eliassen AH, Colditz GA, Rosner B, Willett WC, Hankinson SE. Adult weight change and risk of postmenopausal breast cancer. *JAMA*. (2006) 296:193–201. doi: 10.1001/jama.296.2.193
107. Parker ED, Folsom AR. Intentional weight loss and incidence of obesity-related cancers: the Iowa Women's Health Study. *Int J Obes Relat Metab Disord*. (2003) 27:1447–52. doi: 10.1038/sj.ijo.0802437
108. Rosner B, Eliassen AH, Toriola AT, Chen WY, Hankinson SE, Willett WC, et al. Weight and weight changes in early adulthood and later breast cancer risk. *Int J Cancer*. (2017) 140:2003–14. doi: 10.1002/ijc.30627
109. Chlebowski RT, Luo J, Anderson GL, Barrington W, Reding K, Simon MS, et al. Weight loss and breast cancer incidence in postmenopausal women. *Cancer*. (2019) 125:205–12. doi: 10.1002/cncr.31687
110. Neuhouser ML, Aragaki AK, Prentice RL, Manson JE, Chlebowski R, Carty CL, et al. Overweight, obesity, and postmenopausal invasive breast cancer risk: a secondary analysis of the Women's health initiative randomized clinical trials. *JAMA Oncol*. (2015) 1:611–21. doi: 10.1001/jamaoncol.2015.1546
111. Emaus MJ, van Gils CH, Bakker MF, Bisschop CN, Monninkhof EM, Bueno-de-Mesquita HB, et al. Weight change in middle adulthood and breast cancer risk in the EPIC-PANACEA study. *Int J Cancer*. (2014) 135:2887–99. doi: 10.1002/ijc.28926
112. Hardefeldt PJ, Penninkilampi R, Edirimanne S, Eslick GD. Physical activity and weight loss reduce the risk of breast cancer: a meta-analysis of 139 prospective and retrospective studies. *Clin Breast Cancer*. (2018) 18:e601–e12. doi: 10.1016/j.clbc.2017.10.010
113. McCawley GM, Ferriss JS, Geffel D, Northup CJ, Modesitt SC. Cancer in obese women: potential protective impact of bariatric surgery. *J Am Coll Surg*. (2009) 208:1093–8. doi: 10.1016/j.jamcollsurg.2009.01.045
114. Telgenkamp I, Kusters Y, Schalkwijk CG, Houben A, Kooi ME, Lindeboom L, et al. Contribution of liver fat to weight loss-induced changes in serum hepatokines: a randomized-controlled trial. *J Clin Endocrinol Metab*. (2019) 104:2719–27. doi: 10.1210/jc.2018-02378
115. Chlebowski RT, Reeves MM. Weight loss randomized intervention trials in female cancer survivors. *J Clin Oncol*. (2016) 34:4238–48. doi: 10.1200/JCO.2016.69.4026
116. Playdon M, Thomas G, Sanft T, Harrigan M, Ligibel J, Irwin M. Weight loss intervention for breast cancer survivors: a systematic review. *Curr Breast Cancer Rep*. (2013) 5:222–46. doi: 10.1007/s12609-013-0113-0
117. van Gemert WA, Schuit AJ, van der Palen J, May AM, Iestra JA, Wittink H, et al. Effect of weight loss, with or without exercise, on body composition and sex hormones in postmenopausal women: the SHAPE-2 trial. *Breast Cancer Res*. (2015) 17:120. doi: 10.1186/s13058-015-0633-9
118. Stone SA, Han CJ, Senn T, Korde LA, Allott K, Reding S, et al. Sex hormones in women with elevated breast cancer risk undergoing weight loss. *West J Nurs Res*. (2019). doi: 10.1177/0193945918820672. [Epub ahead of print].
119. Foster-Schubert KE, Alfano CM, Duggan CR, Xiao L, Campbell KL, Kong A, et al. Effect of diet and exercise, alone or combined, on weight and body composition in overweight-to-obese postmenopausal women. *Obesity*. (2012) 20:1628–38. doi: 10.1038/oby.2011.76
120. Campbell KL, Foster-Schubert KE, Alfano CM, Wang CC, Wang CY, Duggan CR, et al. Reduced-calorie dietary weight loss, exercise, and sex hormones in postmenopausal women: randomized controlled trial. *J Clin Oncol*. (2012) 30:2314–26. doi: 10.1200/JCO.2011.37.9792
121. Fabian CJ, Kimler BF, Donnelly JE, Sullivan DK, Klemp JR, Petroff BK, et al. Favorable modulation of benign breast tissue and serum risk biomarkers is associated with > 10 % weight loss in postmenopausal women. *Breast Cancer Res Treat*. (2013) 142:119–32. doi: 10.1007/s10549-013-2730-8
122. Chen Y, Wen YY, Li ZR, Luo DL, Zhang XH. The molecular mechanisms between metabolic syndrome and breast cancer. *Biochem Biophys Res Commun*. (2016) 471:391–5. doi: 10.1016/j.bbrc.2016.02.034
123. Doyle SL, Donohoe CL, Lysaght J, Reynolds JV. Visceral obesity, metabolic syndrome, insulin resistance and cancer. *Proc Nutr Soc*. (2012) 71:181–9. doi: 10.1017/S002966511100320X
124. Lien LF, Haqq AM, Arlotto M, Slentz CA, Muehlbauer MJ, McMahon RL, et al. The STEDMAN project: biophysical, biochemical and metabolic effects of a behavioral weight loss intervention during weight loss, maintenance, and regain. *Omics*. (2009) 13:21–35. doi: 10.1089/omi.2008.0035
125. Beeken RJ, Croker H, Heinrich M, Obichere A, Finer N, Murphy N, et al. The impact of diet-induced weight loss on biomarkers for colorectal cancer: an exploratory study (INTERCEPT). *Obesity*. (2017) 25 (Suppl 2):S95–S101. doi: 10.1002/oby.21984
126. Diabetes Prevention Program Research G. The diabetes prevention program (DPP): description of lifestyle intervention. *Diabet Care*. (2002) 25:2165–71. doi: 10.2337/diacare.25.12.2165
127. Brekke HK, Bertz F, Rasmussen KM, Bosaeus I, Ellegard L, Winkvist A. Diet and exercise interventions among overweight and obese lactating women:

randomized trial of effects on cardiovascular risk factors. *PLoS ONE*. (2014) 9:e88250. doi: 10.1371/journal.pone.0088250
128. Mason C, Xiao L, Duggan C, Imayama I, Foster-Schubert KE, Kong A, et al. Effects of dietary weight loss and exercise on insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in postmenopausal women: a randomized controlled trial. *Cancer Epidemiol Biomarkers Prev*. (2013) 22:1457–63. doi: 10.1158/1055-9965.EPI-13-0337
129. De Pergola G, Zamboni M, Pannacciulli N, Turcato E, Giorgino F, Armellini F, et al. Divergent effects of short-term, very-low-calorie diet on insulin-like growth factor-I and insulin-like growth factor binding protein-3 serum concentrations in premenopausal women with obesity. *Obes Res*. (1998) 6:408–15. doi: 10.1002/j.1550-8528.1998.tb00372.x
130. Fontana L, Villareal DT, Das SK, Smith SR, Meydani SN, Pittas AG, et al. Effects of 2-year calorie restriction on circulating levels of IGF-1, IGF-binding proteins and cortisol in nonobese men and women: a randomized clinical trial. *Aging Cell*. (2016) 15:22–7. doi: 10.1111/acel.12400
131. Proenca AR, Sertie RA, Oliveira AC, Campana AB, Caminhotto RO, Chimin P, et al. New concepts in white adipose tissue physiology. *Braz J Med Biol Res*. (2014) 47:192–205. doi: 10.1590/1414-431X20132911
132. Miller GD, Isom S, Morgan TM, Vitolins MZ, Blackwell C, Brosnihan KB, et al. Effects of a community-based weight loss intervention on adipose tissue circulating factors. *Diab Metab Syndr*. (2014) 8:205–11. doi: 10.1016/j.dsx.2014.09.003
133. Rock CL, Flatt SW, Pakiz B, Quintana EL, Heath DD, Rana BK, et al. Effects of diet composition on weight loss, metabolic factors and biomarkers in a 1-year weight loss intervention in obese women examined by baseline insulin resistance status. *Metabolism*. (2016) 65:1605–13. doi: 10.1016/j.metabol.2016.07.008
134. Merra G, Gratteri S, De Lorenzo A, Barrucco S, Perrone MA, Avolio E, et al. Effects of very-low-calorie diet on body composition, metabolic state, and genes expression: a randomized double-blind placebo-controlled trial. *Eur Rev Med Pharmacol Sci*. (2017) 21:329–45.
135. Abbenhardt C, McTiernan A, Alfano CM, Wener MH, Campbell KL, Duggan C, et al. Effects of individual and combined dietary weight loss and exercise interventions in postmenopausal women on adiponectin and leptin levels. *J Intern Med*. (2013) 274:163–75. doi: 10.1111/joim.12062
136. You T, Berman DM, Ryan AS, Nicklas BJ. Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women. *J Clin Endocrinol Metab*. (2004) 89:1739–46. doi: 10.1210/jc.2003-031310
137. Salehi-Abargouei A, Izadi V, Azadbakht L. The effect of low calorie diet on adiponectin concentration: a systematic review and meta-analysis. *Horm Metab Res*. (2015) 47:549–55. doi: 10.1055/s-0035-1549878
138. Salas-Salvado J, Bullo M, Garcia-Lorda P, Figueredo R, Del Castillo D, Bonada A, et al. Subcutaneous adipose tissue cytokine production is not responsible for the restoration of systemic inflammation markers during weight loss. *Int J Obes*. (2006) 30:1714–20. doi: 10.1038/sj.ijo.0803348
139. Bianchi VE. Weight loss is a critical factor to reduce inflammation. *Clin Nutr ESPEN*. (2018) 28:21–35. doi: 10.1016/j.clnesp.2018.08.007
140. Nicklas JM, Sacks FM, Smith SR, LeBoff MS, Rood JC, Bray GA, et al. Effect of dietary composition of weight loss diets on high-sensitivity c-reactive protein: the Randomized POUNDS LOST trial. *Obesity*. (2013) 21:681–9. doi: 10.1002/oby.20072
141. Sackner-Bernstein J, Kanter D, Kaul S. Dietary intervention for overweight and obese adults: comparison of low-carbohydrate and low-fat diets. a meta-analysis. *PLoS ONE*. (2015) 10:e0139817. doi: 10.1371/journal.pone.0139817
142. Tobias DK, Chen M, Manson JE, Ludwig DS, Willett W, Hu FB. Effect of low-fat diet interventions versus other diet interventions on long-term weight change in adults: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol*. (2015) 3:968–79. doi: 10.1016/S2213-8587(15)00367-8
143. Johnston BC, Kanters S, Bandayrel K, Wu P, Naji F, Siemieniuk RA, et al. Comparison of weight loss among named diet programs in overweight and obese adults: a meta-analysis. *JAMA*. (2014) 312:923–33. doi: 10.1001/jama.2014.10397
144. Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy WS Jr, Brehm BJ, et al. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Arch Intern Med*. (2006) 166:285–93. doi: 10.1001/archinte.166.3.285
145. de Souza RJ, Bray GA, Carey VJ, Hall KD, LeBoff MS, Loria CM, et al. Effects of 4 weight-loss diets differing in fat, protein, and carbohydrate on fat mass, lean mass, visceral adipose tissue, and hepatic fat: results from the POUNDS LOST trial. *Am J Clin Nutr*. (2012) 95:614–25. doi: 10.3945/ajcn.111.026328
146. Gardner CD, Trepanowski JF, Del Gobbo LC, Hauser ME, Rigdon J, Ioannidis JPA, et al. Effect of low-fat vs low-carbohydrate diet on 12-month weight loss in overweight adults and the association with genotype pattern or insulin secretion: The DIETFITS randomized clinical trial. *JAMA*. (2018) 319:667–79. doi: 10.1001/jama.2018.0245
147. Cornier MA, Donahoo WT, Pereira R, Gurevich I, Westergren R, Enerback S, et al. Insulin sensitivity determines the effectiveness of dietary macronutrient composition on weight loss in obese women. *Obes Res*. (2005) 13:703–9. doi: 10.1038/oby.2005.79
148. Pittas AG, Das SK, Hajduk CL, Golden J, Saltzman E, Stark PC, et al. A low-glycemic load diet facilitates greater weight loss in overweight adults with high insulin secretion but not in overweight adults with low insulin secretion in the CALERIE Trial. *Diabetes Care*. (2005) 28:2939–41. doi: 10.2337/diacare.28.12.2939
149. Hjorth MF, Bray GA, Zohar Y, Urban L, Miketinas DC, Williamson DA, et al. Pretreatment fasting glucose and insulin as determinants of weight loss on diets varying in macronutrients and dietary fibers-The POUNDS LOST Study. *Nutrients*. (2019) 11:E586. doi: 10.3390/nu11030586
150. Gardner CD, Offringa LC, Hartle JC, Kapphahn K, Cherin R. Weight loss on low-fat vs. low-carbohydrate diets by insulin resistance status among overweight adults and adults with obesity: a randomized pilot trial. *Obesity*. (2016) 24:79–86. doi: 10.1002/oby.21331
151. Summer SS, Brehm BJ, Benoit SC, D'Alessio DA. Adiponectin changes in relation to the macronutrient composition of a weight-loss diet. *Obesity*. (2011) 19:2198–204. doi: 10.1038/oby.2011.60
152. Llanos AA, Krok JL, Peng J, Pennell ML, Olivo-Marston S, Vitolins MZ, et al. Favorable effects of low-fat and low-carbohydrate dietary patterns on serum leptin, but not adiponectin, among overweight and obese premenopausal women: a randomized trial. *Springerplus*. (2014) 3:175. doi: 10.1186/2193-1801-3-175
153. Rock CL, Flatt SW, Byers TE, Colditz GA, Demark-Wahnefried W, Ganz PA, et al. Results of the exercise and nutrition to enhance recovery and good health for you (ENERGY) trial: a behavioral weight loss intervention in overweight or obese breast cancer survivors. *J Clin Oncol*. (2015) 33:3169–76. doi: 10.1200/JCO.2015.61.1095
154. Song X, Kestin M, Schwarz Y, Yang P, Hu X, Lampe JW, et al. A low-fat high-carbohydrate diet reduces plasma total adiponectin concentrations compared to a moderate-fat diet with no impact on biomarkers of systemic inflammation in a randomized controlled feeding study. *Eur J Nutr*. (2016) 55:237–46. doi: 10.1007/s00394-015-0841-1
155. van Bussel BC, Henry RM, Ferreira I, van Greevenbroek MM, van der Kallen CJ, Twisk JW, et al. A healthy diet is associated with less endothelial dysfunction and less low-grade inflammation over a 7-year period in adults at risk of cardiovascular disease. *J Nutr*. (2015) 145:532–40. doi: 10.3945/jn.114.201236
156. Veum VL, Laupsa-Borge J, Eng O, Rostrup E, Larsen TH, Nordrehaug JE, et al. Visceral adiposity and metabolic syndrome after very high-fat and low-fat isocaloric diets: a randomized controlled trial. *Am J Clin Nutr*. (2017) 105:85–99. doi: 10.3945/ajcn.115.123463
157. Heckman-Stoddard BM, Gandini S, Puntoni M, Dunn BK, DeCensi A, Szabo E. Repurposing old drugs to chemoprevention: the case of metformin. *Semin Oncol*. (2016) 43:123–33. doi: 10.1053/j.seminoncol.2015.09.009
158. Chan AT. Metformin for cancer prevention: a reason for optimism. *Lancet Oncol*. (2016) 17:407–9. doi: 10.1016/S1470-2045(16)00006-1
159. Seifarth C, Schehler B, Schneider HJ. Effectiveness of metformin on weight loss in non-diabetic individuals with obesity. *Exp Clin Endocrinol Diab*. (2013) 121:27-31. doi: 10.1055/s-0032-1327734
160. Ning HH, Le J, Wang Q, Young CA, Deng B, Gao PX, et al. The effects of metformin on simple obesity: a meta-analysis. *Endocrine*. (2018) 62:528–34. doi: 10.1007/s12020-018-1717-y

161. Apolzan JW, Venditti EM, Edelstein SL, Knowler WC, Dabelea D, Boyko EJ, et al. Long-term weight loss with metformin or lifestyle intervention in the diabetes prevention program outcomes study. *Ann Intern Med*. (2019). doi: 10.7326/M18-1605
162. Mehta A, Marso SP, Neeland IJ. Liraglutide for weight management: a critical review of the evidence. *Obes Sci Pract*. (2017) 3:3-14. doi: 10.1002/osp4.84
163. Crow SJ. Pharmacologic treatment of eating disorders. *Psychiatr Clin North Am*. (2019) 42:253–62. doi: 10.1016/j.psc.2019.01.007
164. Srivastava G, O'Hara V, Browne N. Use of lisdexamfetamine to treat obesity in an adolescent with severe obesity and binge eating. *Children*. (2019) 6:E22. doi: 10.3390/children6020022

Conflict of Interest Statement: TA-C, SR, and BD are employees of the National Cancer Institute, National Institutes of Health in Rockville, MD.

frontiers in Genetics

ORIGINAL RESEARCH
published: 27 September 2019
doi: 10.3389/fgene.2019.00885

Glyphosate Primes Mammary Cells for Tumorigenesis by Reprogramming the Epigenome in a TET3-Dependent Manner

Manon Duforestel[1,2,3,4†], Arulraj Nadaradjane[1,2,3,4†], Gwenola Bougras-Cartron[1,2,3,4†], Joséphine Briand[1,2,3,4†], Christophe Olivier[1,2,5], Jean-Sébastien Frenel[1,2,3,4†], François M. Vallette[1,2,3,4†], Sophie A. Lelièvre[6,7] and Pierre-François Cartron[1,2,3,4†]*

[1] CRCINA, INSERM, Université de Nantes, Nantes, France, [2] Equipe Apoptose et Progression tumorale, LaBCT, Institut de Cancérologie de l'Ouest, Saint Herblain, France, [3] Cancéropole Grand-Ouest, réseau Epigénétique (RepiCGO), Nantes, France, [4] LabEX IGO, Université de Nantes, Nantes, France, [5] Service de toxicologie, Faculté de pharmacie de Nantes, Nantes, France, [6] Department of Basic Medical Sciences, Purdue University, West Lafayette, IN, United States, [7] Purdue University Center for Cancer Research, West Lafayette, IN, United States

OPEN ACCESS

Edited by:
Rui Henrique, Portuguese Oncology Institute, Portugal

Reviewed by:
Michael Nicolas Antoniou, King's College London, United Kingdom
Abhijit Shukla, Memorial Sloan Kettering Cancer Center, United States

***Correspondence:**
Pierre-François Cartron
pierre-francois.cartron@inserm.fr

†Members of EpiSAVMEN Consortium

Specialty section:
This article was submitted to Epigenomics and Epigenetics, a section of the journal Frontiers in Genetics

Received: *29 April 2019*
Accepted: *22 August 2019*
Published: *27 September 2019*

Citation:
Duforestel M, Nadaradjane A, Bougras-Cartron G, Briand J, Olivier C, Frenel J-S, Vallette FM, Lelièvre SA and Cartron P-F (2019) Glyphosate Primes Mammary Cells for Tumorigenesis by Reprogramming the Epigenome in a TET3-Dependent Manner. Front. Genet. 10:885. doi: 10.3389/fgene.2019.00885

The acknowledgment that pollutants might influence the epigenome raises serious concerns regarding their long-term impact on the development of chronic diseases. The herbicide glyphosate has been scrutinized for an impact on cancer incidence, but reports demonstrate the difficulty of linking estimates of exposure and response analysis. An approach to better apprehend a potential risk impact for cancer is to follow a synergistic approach, as cancer rarely occurs in response to one risk factor. The known influence of glyphosate on estrogen-regulated pathway makes it a logical target of investigation in breast cancer research. We have used nonneoplastic MCF10A cells in a repeated glyphosate exposure pattern over 21 days. Glyphosate triggered a significant reduction in DNA methylation, as shown by the level of 5-methylcytosine DNA; however, in contrast to strong demethylating agent and cancer promoter UP peptide, glyphosate-treated cells did not lead to tumor development. Whereas UP acts through a DNMT1/PCNA/UHRF1 pathway, glyphosate triggered increased activity of ten-eleven translocation (TET)3. Combining glyphosate with enhanced expression of microRNA (miR) 182-5p associated with breast cancer induced tumor development in 50% of mice. Culture of primary cells from resected tumors revealed a luminal B (ER+/PR-/HER2-) phenotype in response to glyphosate-miR182-5p exposure with sensitivity to tamoxifen and invasive and migratory potentials. Tumor development could be prevented either by specifically inhibiting miR 182-5p or by treating glyphosate-miR 182-5p-cells with dimethyloxallyl glycine, an inhibitor of TET pathway. Looking for potential epigenetic marks of TET-mediated gene regulation under glyphosate exposure, we identified *MTRNR2L2* and *DUX4* genes, the hypomethylation of which was sustained even after stopping glyphosate exposure for 6 weeks. Our findings reveal that low pressure but sustained DNA hypomethylation

occurring *via* the TET pathway primes cells for oncogenic response in the presence of another potential risk factor. These results warrant further investigation of glyphosate-mediated breast cancer risk.

Keywords: DNA methylation, ten-eleven translocation, breast cancer, hypomethylation, epigenetic mark

INTRODUCTION

Cancer results from interactions among genetic, epigenetic, environmental and lifestyle factors. Epigenetic modifications govern heritable changes in phenotypes regulated at the chromatin level without requiring DNA sequence alteration. They are strongly modulated by environmental and lifestyle factors. For instance, epigenetic differences between monozygotic twins have been shown to arise over their life-course (Fraga et al., 2005). In honeybees, fertile queens and sterile workers are alternative forms of the adult female that develop from genetically identical larvae following differential feeding with royal jelly. This specific nutrition is responsible for triggering modifications in the epigenome via a DNA MethylTransferase (DNMT) 3A-dependent mechanism (Kucharski et al., 2008) and histone post-translational modifications (Spannhoff et al., 2011). But, it is worrisome that certain exposures, as in farm environment, in early childhood appear to influence DNA methylation in genes related to asthma and allergy (Michel et al., 2013). Indeed, pollutants are powerful modulators of the epigenome. Over the past five years, 26 records related to the keywords "pollutant; epigenetic; cancer risk" can be found in the web of science (**Supplementary Figure S1**).

Especially, herbicides have been increasingly recognized as epigenetic modifiers. Exposure to Diuron was recently reported to affect the methylome of Pacific oysters (Rondon et al., 2017). In 2015, the International Agency for Research on Cancer (IARC) announced that the hazard of the herbicide glyphosate could be ranked as "probably carcinogenic to humans (Group 2A)". Glyphosate was reported to induce the proliferation of human breast cancer cells via an impact on estrogen receptors (Thongprakaisang et al., 2013). This observation is supported by several other studies demonstrating that glyphosate can affect the activity of estrogen receptor alpha (ERα) and certain phenotypes of ERα positive cells within breast cancer cell populations (Mesnage et al., 2017; De Almeida et al., 2018; Sritana et al., 2018).

The impact of glyphosate on the distribution of methyl groups (or methylome) in the chromatin is extensive. Glyphosate exposure has been reported to induce 9,205 differentially methylated regions (DMRs) across the genome of Arabidopsis thaliana (Kim et al., 2017) and a decrease of DNA methylation in human peripheral blood mononuclear cells (Kwiatkowska et al., 2017).

Here, we present evidence that glyphosate induces global DNA hypomethylation (i.e. overall decrease of 5-methylCytosine (5mC) in the epigenome) in non-neoplastic mammary epithelial MCF10A cells and contributes to tumorigenesis in a "two-hit oncogenic model". Our data also uncover a specific DNA hypomethylation signature of genes (i.e., local DNA hypomethylation) related to the TET3 pathway that might be used as epimark of glyphosate exposure.

RESULTS

Exposure to Glyphosate Promotes TET3-Mediated Global DNA Hypomethylation in MCF10A Cells

DNA hypomethylation has been shown to play a determining role in cancer development (Gaudet et al., 2003; Hervouet et al., 2010; Pacaud et al., 2014). To verify the impact of glyphosate exposure on the global level of DNA methylation, non-neoplastic breast epithelial MCF10A cells were treated with a low dose (10-11 M) of this herbicide every three to four days over 21 days, covering three passage numbers; whereas control cultures were treated with vehicle DMSO (**Figure 1A**). Several articles analyzing the effect of glyphosate on human cells have reported using 10-11 M (Thongprakaisang et al., 2013; Mesnage et al., 2017; Sritana et al., 2018). Indeed, 90% of MCF10A cells were viable as measured by XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay at this concentration (**Supplementary Figure S2**). Importantly, glyphosate 10-11 M is below the concentration detected in biological fluids (milk, serum, urine) (Yoshioka et al., 2011; Acquavella et al., 2004; Steinborn et al., 2016). As a control performed in parallel, MCF10A cells were exposed to carcinogenic UP peptide (0.5 μM) previously described to promote global DNA hypomethylation via the disruption of the DNMT1/PCNA/UHRF1 complex (Pacaud et al., 2014). As expected, there was a decrease in the level of 5mC-DNA in MCF10A cells treated with the UP peptide (**Figure 1B**). There was also a reduction in 5mC content in cells treated with glyphosate (**Figure 1B**), hence suggesting that glyphosate promotes a global DNA hypomethylation as per the definition given in the introduction.

The origin of glyphosate-mediated decrease in DNA methylation was assessed by measuring the levels of activity of maintenance methyltransferase (mMTase) and Ten-eleven translocation (TET), since a decrease of mMTase activity and an increase of TET activity are both causes of DNA hypomethylation. The mMTase activity remained unchanged in MCF10A cells treated with glyphosate (**Figure 1C**) while TET activity significantly increased in these cells (**Figure 1D**). Specifically, an ELISA-based assessment of the amount of the three TET family members, TET1, TET2 and TET3, revealed an overexpression of TET3 in MCF10A cells following exposure to glyphosate (**Figure 1E**).

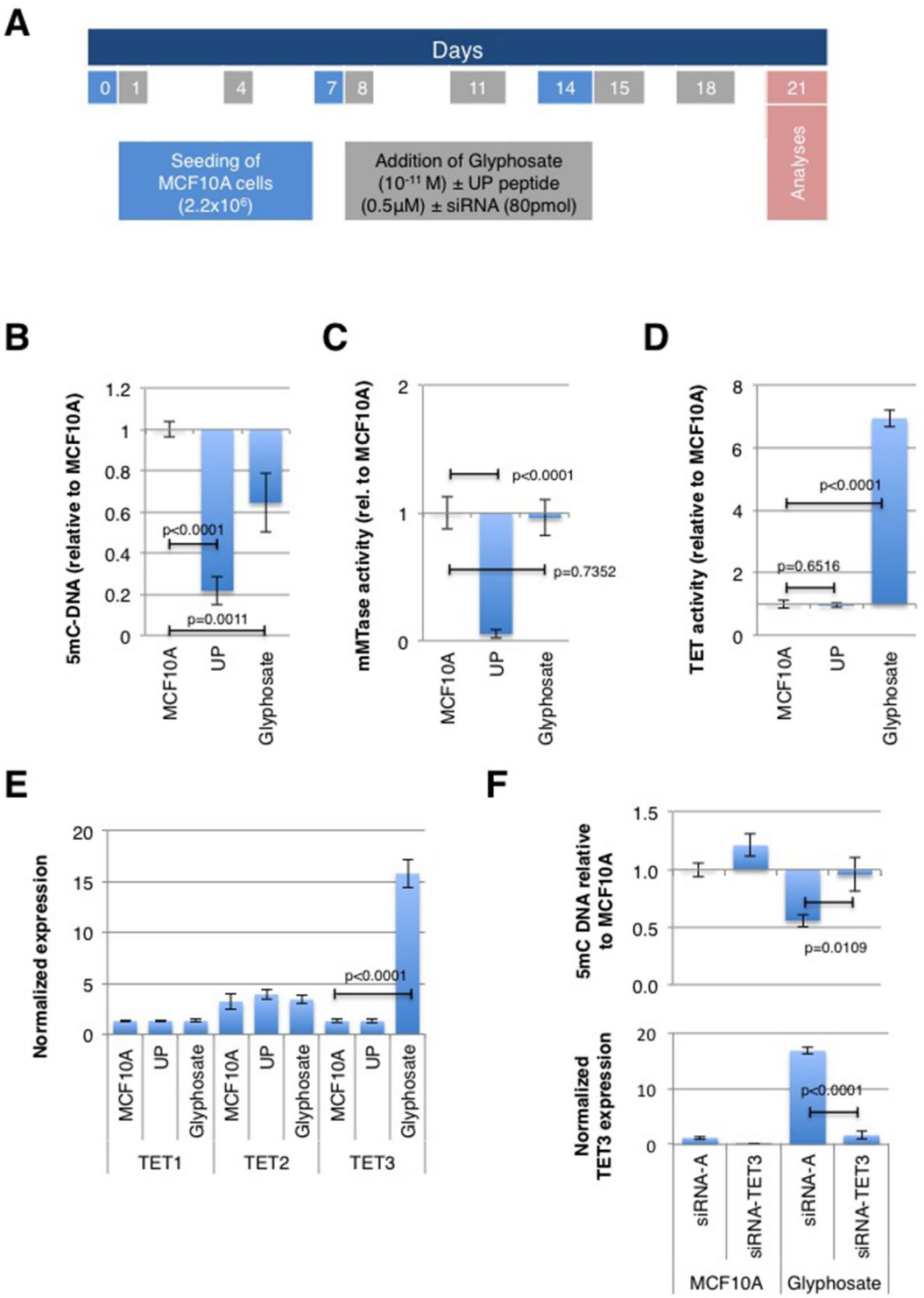


FIGURE 1 | Glyphosate exposure promotes a TET3-mediated global DNA hypomethylation. MCF10A cells were treated according to a timetable shown in **(A)** and analyzed on day 21 of culture. (Explanations for color-coded days are located in corresponding color rectangles underneath the timeline. UP peptide promotes DNMT1/PCNA/UHRF1 disruption). **(B)** ELISA was used to measure the level of 5-methylcytosine (5-mC). **(C)** DMB assay was used to measure maintenance methyltransferase (mMTase). **(D)** TET assay. **(E)** In-Cell ELISA was used to quantify TET proteins. **(F)** MCF10A cells were transfected either with siRNA for TET3 or with control siRNA (siRNA-A) and treated with glyphosate (Glyphosate) or vehicle DMSO (MCF10A) according to a timetable shown in **(A)**. ELISA was used to measure the level of 5mC, and TET3 levels were determined by In-Cell ELISA and normalized to Janus Green staining intensity to account for differences in cell seeding density. For all assays, the bar graph displays the average ± standard deviation values of three independent experiments.

To confirm that glyphosate promotes TET3-mediated global DNA hypomethylation in MCF10A cells, we analyzed the level of DNA methylation in MCF10A cells with siRNA-mediated TET3 down-regulation. ELISA results show that the presence of siRNA-TET3 abrogates TET3 overexpression and prevents DNA hypomethylation in cells exposed to glyphosate (**Figure 1F**).

Glyphosate Exposure Is Tumorigenic for MCF10A Cells in a Two-Factor Hit Model

Global DNA hypomethylation is potentially tumorigenic (Gaudet et al., 2003; Hervouet et al., 2010; Pacaud et al., 2014). Therefore, MCF10A cells exposed to glyphosate were injected subcutaneously in Swiss nude mice. No tumors developed, whereas the control experiment with MCF10A cells exposed to the UP peptide led to visible tumor growth within 21 days in 100% of the mice (**Figure 2A**).

The Knudson's hypothesis for cancer initiation suggests that several oncogenic hits cooperate to promote cancer. This hypothesis initially based on mutations can be transposed to risk factors beyond genetic alterations. Indeed, several microRNAs (miR) have been associated with cancer either as oncomiR (one hit) or suspected to promote cancer phenotype in light of their overexpression in cancers. To investigate the possibility of a two-factor hit oncogenic impact with glyphosate, six miRs associated with poor prognosis of breast cancer [miR-182-5p (Yu et al., 2017), miR-27a (Jiang et al., 2018), miR-500a-5p (Degli Esposti et al., 2017), miR-30a (di Gennaro et al., 2018), miR-495 (Cao et al., 2014), and miR-146a (Wang et al., 2016)] were transfected individually in MCF10A cells. For this purpose, miRs mimics were used, and their increased expression was confirmed by RTqPCR (**Supplementary Figure S3**). Tumor nodules were observed in two out of the four mice with subcutaneous injection of glyphosate-exposed MCF10A overexpressing miR-182-5p, whereas none of the other five miRs were associated with tumor formation (**Figure 2B**). Moreover, no tumor nodules were observed with subcutaneous injection of glyphosate/miR-182-5p/siRNA-TET3-exposed MCF10A, confirming that TET3 is implicated in glyphosate-mediated tumorigenic pathway (**Figure 2C**). The use of the Pan-cancer RNA-seq data available from the KM plotter database revealed that although TET3 overexpression is associated with a favorable overall survival in head and neck squamous cell carcinoma, thymoma, and thyroid carcinoma, it is associated with an unfavorable overall survival in breast cancer, as well as cervical squamous cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, pheochromocytoma, paraganglioma, and uterine corpus endometrial carcinoma (**Supplementary File F1**).

We next compared several molecular signatures and phenotypic traits of primary cultures of tumor cells (PCTC) from glyphosate-induced breast tumors (Glypho-iBPCTC) with the ones of luminal A (MCF-7) and triple negative (MDA-MB-231) breast cancer cells. Only one of the two tumors led to viable Glypho-iBPCTC. In-cell ELISA confirmed that MCF7 and MDA-MB-231 cells were ERα+/PR+/HER2- (luminal A) and ERα-/PR-/HER2- (triple negative), respectively, and revealed that Glypho-iBPCTC were ERα+/PR-/HER2-, hence corresponding to a luminal B type of breast cancer with poorer outcome compared to ER+/PR+/HER2- subtype (Inic et al., 2014) (**Figure 3A**).

Tamoxifen/IC50 in MCF-7 and Glypho-iBPCTC were similar (**Figure 3B**). The QCM™ 24-Well Collagen-based cell invasion assay revealed that all cell strains had similar invasion capacity (**Figure 3C**), although scratch test indicated that Glypho-iBPCTC had the lowest migration ability compared to MCF-7 ($p = 0.0137$) and MDA-MB-231 cells ($p = 0.0002$) (**Figure 3D**). These results confirm that Glypho-iBPCTC display phenotypic traits associated with breast cancer cells *in vitro*.

DMOG, a TET Inhibitor, Prevents Tumor Formation in Glyphosate-Challenged Cells

Some of the nutraceuticals/alicaments currently available target epigenetic pathways involved in normal homeostasis, notably those controlling DNA methylation. Like established epigenetic drugs, these sources of epigenetic modifiers offer great potentials to help determine the epigenetic path targeted by environmental factors and possibly revert the risk of tumorigenesis. MCF10A cells were transfected with miR-182-5p and exposed to 10^{-11} M of glyphosate ($MCF10A^{glyphosate/miR\text{-}182\text{-}5p}$) every 3 to 4 days over a 21-day period. They were also simultaneously treated with 40 µg/ml folate, a promoter of DNA methylation (Hervouet et al., 2009; Cartron et al., 2012), or with 250 µM ascorbic acid, an activator of TET (Minor et al., 2013; Yin et al., 2013), 24 h after every glyphosate +/-miR treatment (**Figure 4A**). $MCF10A^{glyphosate/miR\text{-}182\text{-}5p}$ cells were also treated in a similar manner with two therapeutic agents, an anti-miR-182-5p (50 nM) and dimethyloxallyl glycine (DMOG, 1 mM), a compound that blocks TET enzymatic activity (Zhang et al., 2017) (**Figure 4A**). For all of these conditions, we measured the global level of DNA methylation and tumor incidence compared to untreated $MCF10A^{glyphosate/miR\text{-}182\text{-}5p}$ cells (control) at the end of the 21-day treatment sequence. As expected, folate and DMOG prevented glyphosate-induced DNA demethylation, whereas ascorbic acid further reduced DNA methylation in $MCF10A^{glyphosate/miR\text{-}182\text{-}5p}$ cells, as shown by the level of 5mC (**Figure 4B**). Treatment with anti-miR-182-5p did not modify significantly the level of 5mC compared to control. Both folate and DMOG treatments were confirmed to indeed induce hypermethylation in several cell lines (**Supplementary Figure S4**). Of the two hypermethylating agents, DMOG and folate, only DMOG prevented tumor formation; there was no difference between folate and control treatments (50% of the mice displayed tumors). Ascorbic acid and glyphosate acting synergistically on DNA hypomethylation led to a 50% increase in tumor incidence. In contrast, although without an obvious impact on glyphosate-induced DNA hypomethylation, anti-miR-182-5p was able to prevent tumor formation (**Figure 4C**). These results confirm that both DNA demethylation and miR-182-5p are necessary for tumor onset. Importantly, the extent of DNA demethylation appears to set a threshold for tumor

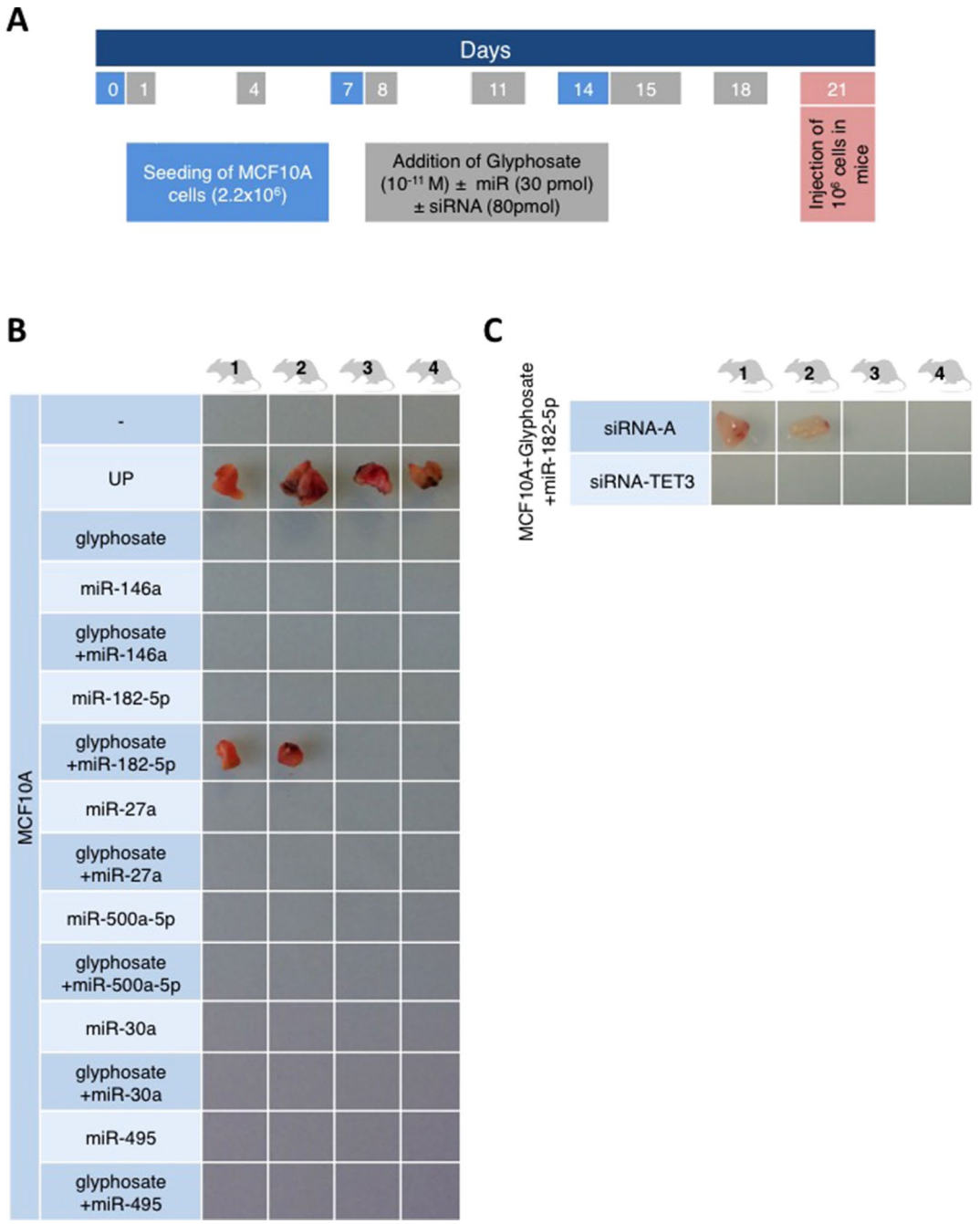


FIGURE 2 | The combination of glyphosate exposure and miR-182 overexpression is tumorigenic for MCF10A cells in a two-factor hit model. **(A)** The timetable illustrates the experiment design. Explanations for color-coded days are located in corresponding color rectangles underneath the timeline. **(B** and **C)** Four mice were injected per condition. miRCury LNA miR mimics and siRNA for TET3 were used to overexpress miRs or siRNA in MCF10A cells. Mice were euthanized 21 days after the injection of cells, and the tumors were resected. The pictures show the resected tumors.

onset (i.e., the more hypomethylated, the higher the risk for tumor development).

Glyphosate Exposure Induces Sustained TET3-Mediated Gene Demethylation

The hypomethylation induced by glyphosate treatment is sufficient for tumor onset when using a two-factor hit model with induced overexpression of miR-182-5p. Therefore, we investigated the possibility that an epimark of hypomethylation might be imprinted in the DNA.

We postulated that the putative epimark induced by glyphosate might be the hypomethylation of TET3-targeted genes because TET3 mediates glyphosate-induced DNA hypomethylation. The chromatin immunoprecipitation (ChIP)

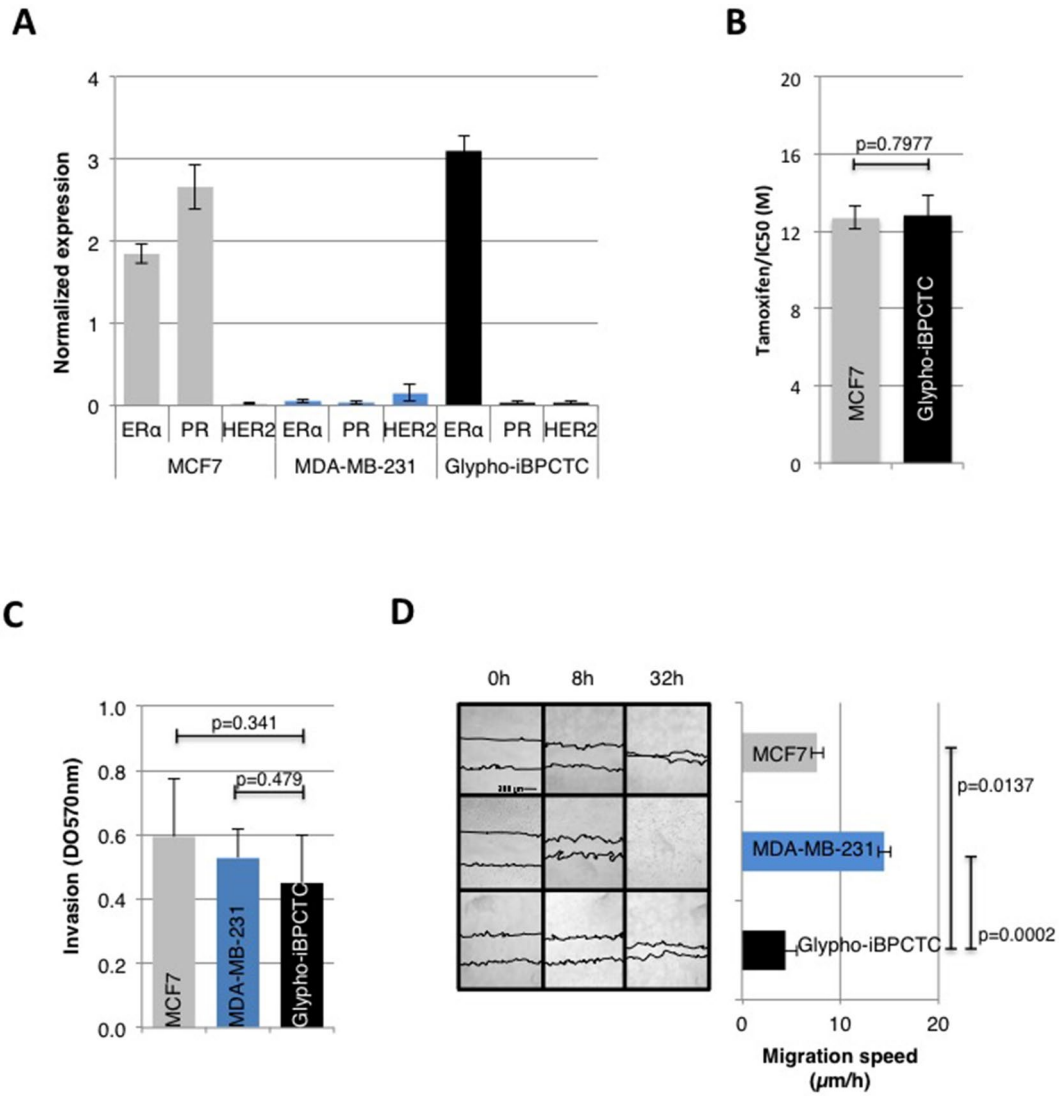


FIGURE 3 | Primary cells from glyphosate-induced breast tumor display characteristics of malignant cells. **(A)** The expression levels of ERα, PR, and HER2 were estimated in MCF7 cells, MDA-MB-231 cells, and Glypho-iBPCTC primary cells using In-Cell ELISA. Normalization to Janus Green staining intensity was performed to account for differences in cell seeding density. The bar graph displays the average ± standard deviation values of three independent experiments. **(B)** Bar graph of the viability of MCF-7 and Glypho-iBPCTC cells treated with increasing doses of tamoxifen (0, 2, 4, 6, 8, 10, 16, 19, 22 µM). Viability was measured by an MTT test, and the results represent the average ± standard deviation values of six independent experiments. The IC50 for each cell type was calculated using the IC50 Calculator (ATT Bioquest). **(C)** Bar graph showing the invasion capacity of MCF-7, MDA-MB-231, and Glypho-iBPCTC cells measured by optical density (absorbance at 570 nm). n = 3. **(D)** Confluent cultures of MCF-7, MDA-MB-231, and Glypho-iBPCTC cells were subjected to the wound healing test. The average migration speed was obtained by calculating the ratio distance/time between each acquisition time. Left: Pictures were acquired immediately after seeding (0 h) and after 8 and 32 h of culture. The bar graph represents the average ± standard deviation values of three independent experiments.

atlas database identifies *MTRNR2L2*, *COL23A1*, *MSH3*, *DHFR,* and *DUX4* as the most frequently present in TET3-ChIP hits. According to this predictive finding, ChIP experiments using anti-TET3 antibody were performed for chromatin obtained from MCF10A cells treated or not with glyphosate for 21 days, such as described in **Figure 1A**. Interestingly, only *MTRNRL2* and *DUX4* genes were immunoprecipitated by TET3 in MCF10A cells treated with glyphosate. *COL23A1*, *MSH3*, and *DHFR* genes were not immunoprecipitated in both untreated and treated MCF10A cells. Thus, the prediction made by the ChIP atlas database was validated for *MTRNRL2* and *DUX4* genes and not for the *COL23A1*, *MSH3*, and *DHFR* genes, suggesting a context-dependent accessibility for this set of TET3-controled genes. Accordingly, quantitative methylation-sensitive restriction enzyme (qMSRE) revealed that *MTRNRL2* and *DUX4* genes were strongly methylated in control cells and became hypomethylated in MCF10A cells exposed to glyphosate (**Figure 5A**). The involvement of TET3 in the glyphosate-induced hypomethylation of *DUX4* and *MTRNR2L2* was confirmed by the abrogation with

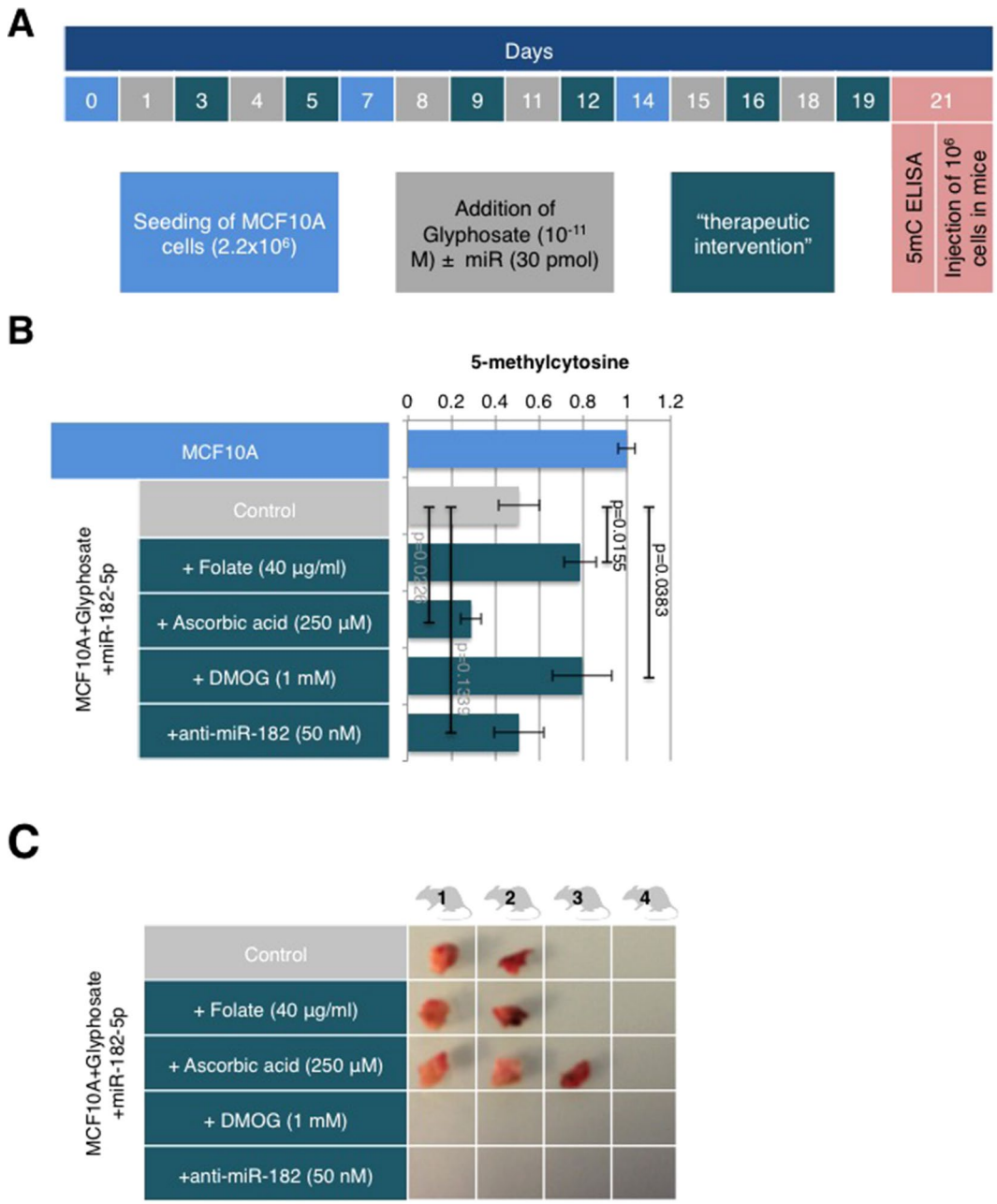


FIGURE 4 | DMOG and anti-miR-182 prevent tumor onset but differentially impact 5-meC level. **(A)** The timetable illustrating the experiment design. Explanations for color-coded days are located in corresponding color rectangles underneath the timeline. Therapeutic interventions on MCF10A cells treated with glyphosate and miR as indicated were performed on days 3, 5, 9, 12, 16, and 19 with folate (40 µg/ml), ascorbic acid (250 µM), DMOG (1 mM), or anti-miR-182 (50 nM). **(B)** MCF10A cells were treated as shown in schedule A. DNA was extracted at day 21 and used in 5mC ELISA. The bar graph illustrates the levels of 5mC for the different conditions. **(C)** Mice were injected with the cells following the treatment schedule A and euthanized 21 days later. Shown are pictures of the resected tumors.

siRNA-TET3 of the glyphosate-induced hypomethylation of these genes (**Figure 5B**). Preliminary investigation of available breast tissue from breast cancer-free women confirmed the demethylation of *DUX4* and *MTRNR2L2* in a woman showing glyphosate exposure based on urinary test. However, the methylation status of the five genes immunoprecipitated by TET3, *MTRNR2L2*, *DUX4COL23A1*, *MSH3,* and *DHFR,* should be kept in consideration in the future because a woman with low glyphosate exposure displayed methylation on the five genes, hence suggesting that an epimark should consider the methylation status of all these genes in future investigations (**Supplementary Figure S5**).

The stability of epigenetic changes is an important factor for long-term risk determination. MCF10A cells were exposed

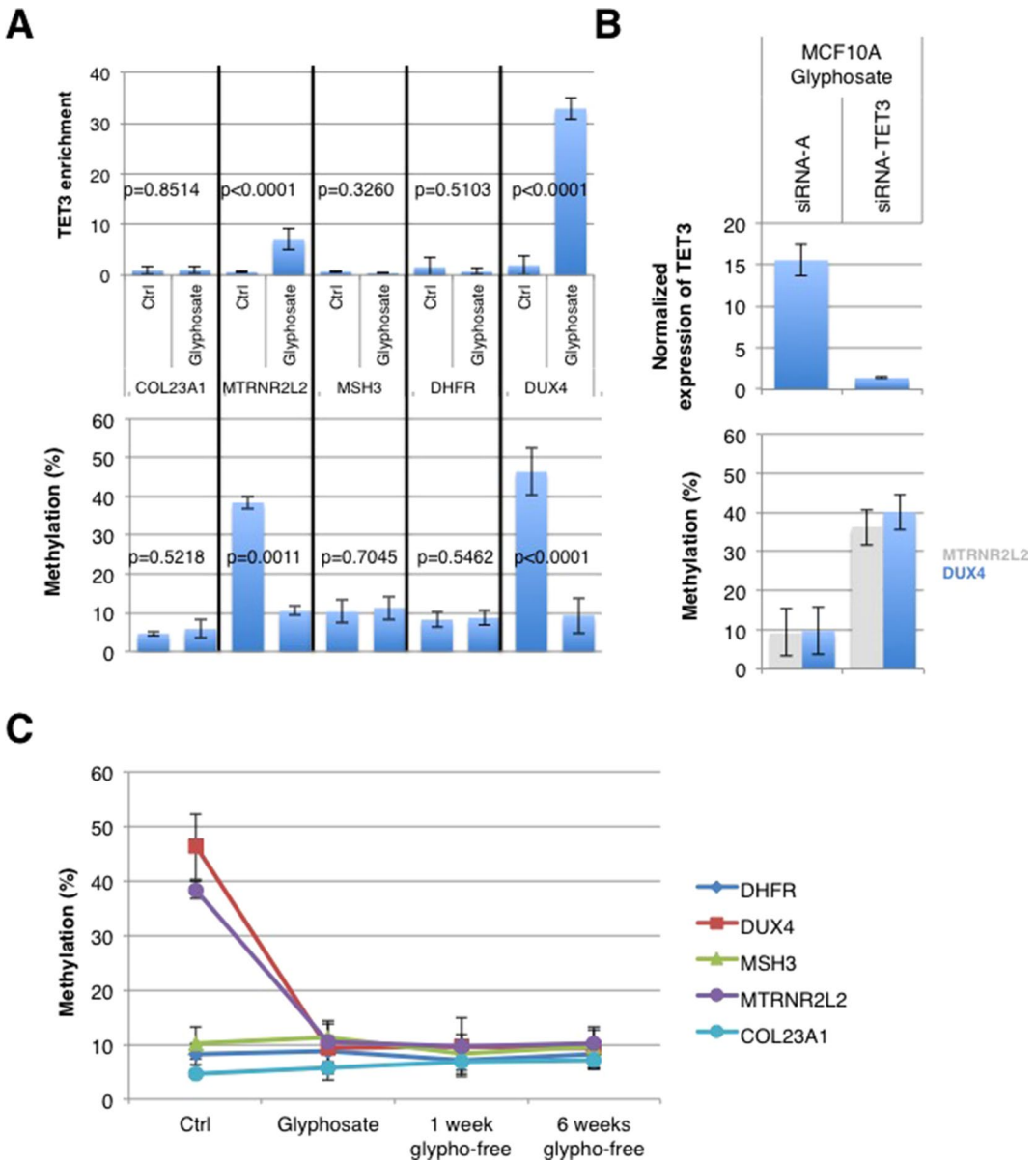


FIGURE 5 | Glyphosate-induced TET3-mediated demethylation affects *MTRNR2L2* and *DUX4* genes. **(A)** MCF10A cells were treated with glyphosate for 21 days as in the schedule shown in **Figure 2**. The graphs illustrate TET3 enrichment (top) following chromatin immunoprecipitation (ChIP) and the methylation level measured by qMSRE (bottom) of five genes defined by the ChIP atlas as being TET3-targeted genes. **(B)** MCF10A cells were treated with glyphosate for 21 days (according to the timetable of **Figure 2**), with siRNA added concomitantly to glyphosate. Bar graph (top) of TET3 expression measured with In-Cell ELISA after treatment with siRNA-TET3 (sc94636) or control siRNA-A (sc94636). Normalization to Janus Green staining intensity was performed to account for differences in cell seeding density. Bar graph (bottom) of methylation levels of *DUX4* and *MTRNR2L2* genes as measured by qMSRE. **(C)** MCF10A cells were treated with glyphosate for 21 days (glyphosate) according to the schedule shown in **Figure 1** and then cultured in glyphosate-free medium for another 1 (1 week glypho-free) or 6 (6 weeks glypho-free) weeks. Shown is the graph of the methylation level of five TET3-dependent genes. "Ctrl" represents MCF10A cells without glyphosate exposure.

to glyphosate for 21 days (as previously described; **Figure 1A**) and then cultured without glyphosate for 1 and 6 weeks. The *DUX4* and *MTRNRL2* hypomethylations remained stable, as shown by qMSRE, even after exposure to glyphosate has seized (**Figure 5C**). bc-GenExMiner and KM plotter indicated that a high expression of *DUX4* is associated with a poor prognosis, suggesting that genes controlled by TET3 might deserve additional scrutiny in breast cancer pathogenesis (**Supplementary File F2**).

DISCUSSION

The impact of glyphosate on human health has been analyzed and discussed for several years now (Gillezeau et al., 2019).

Recently, glyphosate exposure was correlated with shortened gestational lengths (Parvez et al., 2018), and the level of glyphosate excretion was associated with steatohepatitis and advanced liver fibrosis in patients with fatty liver disease (Mills et al., 2019). However, the multiple research studies that investigated the tumorigenic effect of glyphosate as the sole risk factor had not led to convincing evidence of its implication.

It is assumed that only 5–10% of cancers are directly caused by inherited genetic abnormalities. The remaining 90% of cancers are linked to environmental factors that directly or indirectly affect DNA, possibly triggering genetic defects or aberrations in the reading and/or expression of DNA (Perera, 1997; Anand et al., 2008). Environmental and lifestyle factors are pleiotropic and include diet, tobacco, infections, obesity, alcohol, radiation, stress, physical activity, exposure to heavy metals and other pollutants, such as glyphosate. We are reporting that glyphosate exposure is not oncogenic by itself, but it acts as an oncogenic hit factor that, combined with another oncogenic hit, promotes the development of mammary tumors. At the molecular level, our findings demonstrate that glyphosate exposure can predispose breast cells to tumorigenesis *via* epigenetic reprogramming occurring *via* TET3-mediated global and local DNA hypomethylation **(Figure 6)**.

We and others have identified that global DNA hypomethylation promoting tumorigenesis may be caused by a deficiency of the DNMT1/PCNA/UHRF1 complex or of DNMT1 expression as shown in astrocytes, pulmonary fibroblasts, mesothelial cells, and breast cells (Gaudet et al., 2003; Hervouet et al., 2010; Pacaud et al., 2014). We show that glyphosate-mediated DNA hypomethylation is associated with TET3 overexpression instead of the DNMT1 pathway. The lower degree of DNA hypomethylation reached *via* the glyphosate-TET3 path compared to that reached *via* UP peptide-DNMT1 path that is capable of inducing tumor onset suggests that a great intensity of global DNA hypomethylation could act as an oncogenic event, while a moderate intensity of global DNA hypomethylation might be considered a predisposing factor to cancer. The fact that active DNA demethylation orchestrated by TET can occur in resting (nondividing) cells representing the majority of breast cells (in contrast to DNMT activity that requires cell proliferation) confers to TET-mediated mechanism a potentially higher degree of danger for cancer development.

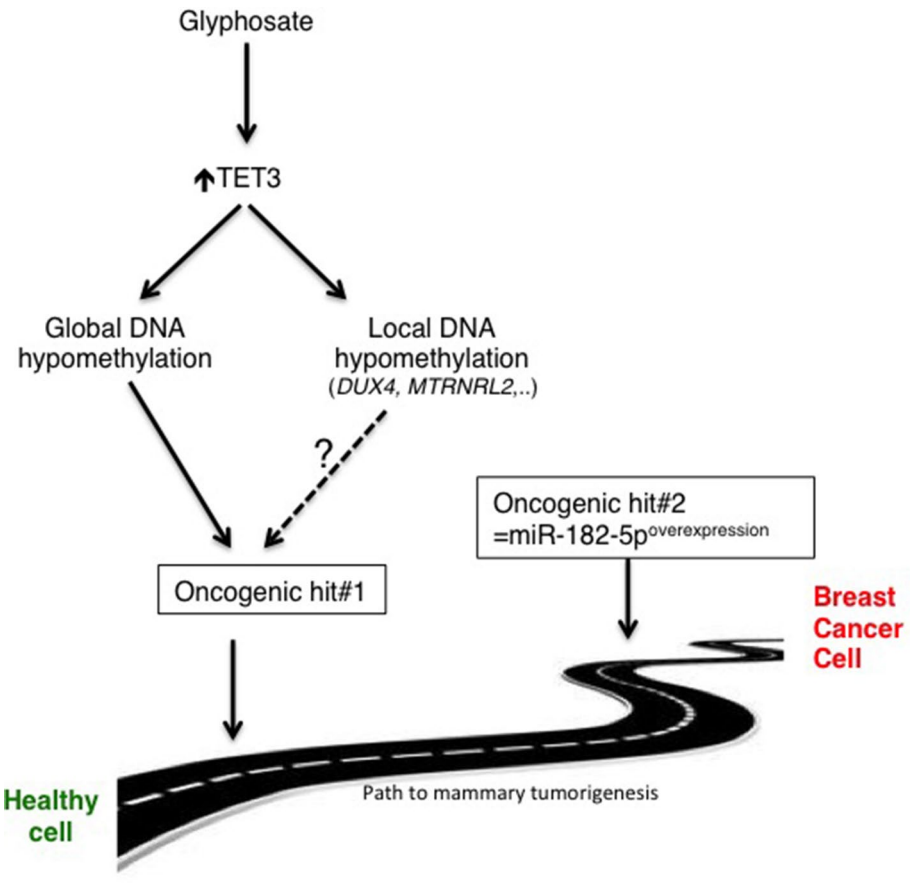


FIGURE 6 | Schematic representation of the proposed glyphosate-induced mammary tumorigenesis path. Whether *DUX4* and *MTRNRL2* are involved in oncogenesis remains to be determined.

The implication of TET proteins in breast cancer growth and metastasis has been strongly documented (Sun et al., 2013; Yang et al., 2015), and the level of hypomethylation of triple-negative breast cancer has been associated with TET1 DNA demethylase activity (Good et al., 2018). In the latter article, it is proposed but not shown that TET1 might act as an oncogene by leading to aberrant hypomethylation. Our findings demonstrate that the hypothesis of an involvement of TET-mediated DNA hypomethylation in cancer onset was correct. Notably, siRNA-TET3 abolished the presence of glyphosate-induced global and local *DUX4* and *MTRNR2L2* hypomethylation, as well as tumorigenesis. Our data feed the ongoing debate regarding whether TET3 exerts an oncogenic role or a tumor suppressor role. For the latter role, TET3 might act by inhibiting epithelial-to-mesenchymal transition in ovarian and melanoma cancers (Ye et al., 2016; Gong et al., 2017). But our analysis with KM plotter database revealed a potentially unfavorable outcome for breast cancers when TET3 is overexpressed (**Supplementary File F1**).

Our work shows that two epigenetic events (global DNA hypomethylation and overexpression of a miR) cooperate to promote breast cancer. Other epigenetic events described to be involved in breast cancer development include the reduction of H3K9 acetylation *via* TIP60 downregulation that promotes ER-negative tumors (Bassi et al., 2017; Judes et al., 2018). Histone acetyltransferase p300 activity and BIM1-mediated histone H2A ubiquitination that remodel chromatin are also two epigenetic events described as promoters for the development of aggressive breast tumors. A body of literature reports that miRs also play a crucial role in mammary tumorigenesis. In addition to oncogenic miRs, there are also miRs acting as tumor suppressors. For example, loss of miR-10b delays oncogene-induced mammary tumorigenesis (Kim et al., 2016), and overexpression of miR-489 inhibits HER2/neu-induced mammary tumorigenesis (Patel et al., 2019). Since the expression of miR depends on epigenetic control, it seems that either an extensive global hypomethylation of DNA (like with UP peptide) or a less extensive global hypomethylation associated with local epigenetic alterations affecting a miR might lead to tumor onset. The mechanisms associated with specific targeting of miR expression remain to be understood.

Breast cancer susceptibility has been statistically linked to epigenetic age acceleration and CpG island methylation (Ambatipudi et al., 2017). An important question is whether exposure to pollutants that are detrimental to epigenetic homeostasis might replace or synergize with age-related epigenetic changes and thus lead to the increase in earlier onset of breast cancer that is now documented. This possibility is further supported by our preliminary observation that the luminal B subtype of tumor (ER+/PR-/HER2-) triggered by glyphosate exposure combined with miR-182-5p overexpression is associated with poorer outcomes than the frequent ER+/PR+/HER2-luminal A type of tumor. Indeed, luminal B type of tumors have been found to be most common in young patients (Goksu et al., 2014). This phenotype obtained from one tumor produced in mice will have to be confirmed with additional means; in any case, epigenetic markers of risk would be a prime resource to help curve the incidence. There exist already DNA methylation markers that add to the prediction of tertiary and secondary outcomes over and beyond standard clinical measures (Terry et al., 2016).

In the MCF10A model, glyphosate-induced DNA hypomethylation can be detected *via* the methylation level of only two of the five genes predicted to be controlled by TET3, *MTRNR2L2* and *DUX4* genes. Even if several other factors than glyphosate-induced TET3-mediated DNA hypomethylation (such as chromatin structure, other epimark, etc.) can govern the methylation status of the five genes, *MTRNR2L2, DUX4, COL23A1, MSH3,* and *DHFR,* our preliminary data with human samples support the idea that the study of the methylation status of these five genes might be important to obtain a marker of risk based on a MethylGlypho score. We are now pursuing this direction of research by detecting and analyzing this 5-gene TET3-dependent epimark in blood samples. Possibly, glyphosate-induced methylome reprogramming might be used for the detection of an increased risk for breast cancer in women living in an environment conducive to this type of pollution.

Due to their concomitant expression during tumorigenesis associated with glyphosate-induced DNA hypomethylation, *DUX4* and *MTRNR2L2* may appear as players in this process instead of only be considered potential biomarkers. Results with KM plotter and bc-GenExMiner indicate that *DUX4* level is negatively associated with breast cancer prognosis. No data seems available on *MTRN2L2* in these databases. Based on the literature, *DUX4* could act as an oncogene in various sarcomas and hematological malignancies (Dib et al., 2019), while we could not find information in the literature revealing a putative oncogenic role for *MTRNR2L2*. These TET3-controlled genes are worth further investigation to establish their causal effect in mammary tumorigenesis in future work.

Knowing the epigenetic pathway involved in glyphosate-mediated risk increase might lead to prevention strategies to follow detection of the epigenetic risk. Our findings suggest that TET-specific inhibitor DMOG might be a plausible therapeutic intervention since it gave a satisfactory response on both DNA methylation and tumor incidence. It would act by limiting TET3-mediated global DNA hypomethylation. In contrast, global remethylation of DNA by folate that has been considered for possible preventive effect is insufficient to prevent tumor incidence in the case of glyphosate exposure (Hervouet et al., 2009; Cartron et al., 2012). Another interesting direction would be to limit the intake of ascorbic acid since it not only further reduced DNA methylation but also increased tumor incidence in mice. The epigenetic pathway leading to DNA hypomethylation is an important aspect to consider for further translational work on breast cancer risk.

MATERIALS AND METHODS

Cell Culture and Transfection

MCF10A cells were cultured in DMEM/F12 supplemented with 5% horse serum (Invitrogen, Cergy Pontoise, France), 500 ng/ml hydrocortisone (Sigma-Aldrich, France), 100 ng/ml cholera

toxin (Sigma-Aldrich, France), 10 µg/ml insulin (ThermoFisher, France) and 20 ng/ml epidermal growth factor (EGF, Sigma-Aldrich, France), penicillin (100 U/ml), and 2 mmol/L L-glutamine. MCF7 and MDA-MB-231 cells were cultured in DMEM medium (Invitrogen) all supplemented with 5% FCS and 2 mM l-glutamine. Glyphosate (CAS 1071-83-6, sc-211568) was purchased from Santa-Cruz (France), and a 10^{-8}-M stock solution was prepared in DMSO every week. Glyphosate was diluted directly in fresh cell culture medium and was fed to the cells at the time points indicated in the results section.

For the transfection of RNAs, we used miRCury LNA miR mimics for the has-miR-146a, has-miR-182-5p, has-miR-27a, has-miR-500a-5p, has-miR-30a, and has-miR-495 (Qiagen, France), siRNA for siRNA-T ET3 (sc94636) and control siRNA-A (sc94636) and HIPerfect Transfection Reagent (Qiagen, France). All miRs showed similar transfection efficiency (10- to 15-fold change, as measured by RTqPCR) (**Supplementary Figure S3**).

DNA Extraction, 5mC ELISA, and qMSRE

A QIAcube automate and QIAmp DNA Mini QiaCube kit (Qiagen, France) were used to isolate DNA.

The quantification of 5mC was performed using the 5mC DNA ELISA Kit (Zymo Research-Euromodex, France) according to the manufacturer's instructions. The 5mC DNA ELISA Kit estimates the number of 5mC on DNA without distinction of localization; therefore, we used the term of global DNA methylation level when referring to results obtained *via* this mode of quantification.

Next, DNA methylation was quantified by qMSRE. Digestions were performed with adequate restriction enzymes, HpaII and AciI (NEB, France). Typically, 1 ng of genomic DNA was digested with 40 U of enzymes at 37°C for 2 h in 50 µl of reaction. Control samples were treated in the same way but without addition of the enzyme. Five microliters of digestion mixture were used for qPCR. The QuantiFast SYBR Green PCR Kit and Rotor-Gene Q (Qiagen, France) were used to perform the qPCR. Primers were MSH3: TTTCTCCAG GGCTGGGACTTTG and CCCGACTGGATTCCCCTTTTCT; DHFR: AAACCTCAGCGCTTCACCCAAT and TGATAGG GCTGGAGGAGGAAG; DUX4: CGACACCCTCGGACAGCA and TCAAAGCAGGCTCGCAG; COL23A1: TCTCCAGG CCAGAAACAGTCTT and ATTTAGAGAGGCAGGGTC GAGA; and MTRNR2L2: ACCCCACCTGTTTACCAA and GCTACCTTTGCACGGTTAGGG.

Tumor Xenografts in Nude Mice

Cells were harvested by trypsinization, washed and resuspended in saline buffer. Cell suspensions were injected subcutaneously into the flank of 7 to 8-week-old mice (Janvier, France) in 100 µl of sterile PBS. Tumor volume based on caliper measurements was calculated using the modified ellipsoidal formula [Tumor volume = 1/2 ($length \times width^2$)] according to previously published work (Cartron et al., 2012). At the end of the observation period, the mice with xenograft tumors were euthanized, and the tumor tissues were removed for analysis.

The experimental procedures with animals were in accordance with the guidelines of Institutional Animal Care and the French National Committee of Ethics. In addition, all experiments were conducted according to the Regulations for Animal Experimentation at the *Plateforme Animalerie* in the *Institut de Recherche en Santé de l'Université de Nantes* (IRS-UN) and approved by the French National Committee of Ethics. The number of mice was restricted to four per condition to limit the number of animals to the necessary minimum as in previous studies (Hervouet et al., 2010; Pacaud et al., 2014) based on the fact that we anticipated to detect a highly frequent tumorigenic event (frequency superior to one to four for tumorigenesis).

Establishment of Tumor Cells From Xenografts (PCTCdX)

PCTCdX (here named Glypho-iBPCTC) were obtained after mechanical dissociation. Briefly, resected tumor tissue from mice was cut into pieces of 1–5 mm^3 and plated in a 60-mm^2 tissue culture dish with DMEM containing 10% FBS and antibiotics. Minced pieces of tumor were incubated with 200 U/ml collagenase I (Sigma) and 500 U/ml DNaseI (Sigma) in PBS for 1 h at 37°C with vigorous constant agitation. The single-cell suspension was filtered through a 70-mm cell strainer (BD Falcon), washed with PBS, and then placed in DMEM-10% FBS. Cell cultures were split 1:5 when confluent.

Migration Assay

Cells (3×10^5) were seeded in six-well plates, cultured until they reached 80–90% confluence, and treated with 10 µg/ml of mitomycin C (Sigma, France) for 2 h (to prevent cell proliferation). The monolayer of cells was scratched using a two-well silicone insert (Ibidi, Germany). Cell migration was monitored by microscopy (Incellis Cell Imager, Bertin, France). The images acquired at different time points (0, 4, 8, 24, 28, 32, and 48 h) for each sample were analyzed quantitatively. For each image, distances between one side of the wound and the other side were measured with ImageJ software; the mean value of 10 measurements all along the wound was recorded. The average migration speed was obtained by calculating the ratio distance/time along the time course.

Invasion Assay

All of the procedures were performed according to the manufacturer's instructions (QCM 24-Well Collagen-Based Cell Invasion Assay, Millipore, France). In brief, 200 µl of serum-free medium containing 2×10^5 cells were added into the invasion chamber, with the bottom well of the 24-well plate containing 500 µl of complete medium. After 72 h of incubation at 37°C, the medium was removed, and the cells were stained by placing the chamber in staining solution for 20 min at room temperature. Cells that did not invade were carefully removed from the top side of the chamber using a cotton swab. The stained chamber was inserted into a clean well containing 200 µl of extraction buffer for 15 min at room temperature. A total of 100 µl of extracted (stained) solution from the chamber was

transferred into a 96-well plate, and the optical density was measured 570 nm using a spectrophotometer.

Viability Assay: MTT and XTT Tests

A cell suspension containing 10^5 cells was prepared, and 100 µl was distributed in sixplicates in a 96-well plate. After 24 h of incubation at 37°C and 5% CO_2, cells were exposed to tamoxifen for 48 h. Tamoxifen was first diluted 10 times in dimethyl sulfoxide (DMSO) and then further diluted in DMEM containing 4.5 g/L glucose, 1% SVF, 1% glutamine, 1% penicillin-streptomycin at the desired concentrations. Following treatment, 10 µl of MTT (10 µg/ml) (VWR Chemicals, France) was added in each well, and the cells were incubated for 3 h. Finally, the medium containing MTT was removed, and 200 µl/well of DMSO was added to measure the optical density at 570 nm using a spectrophotometer.

For the XTT test, we used the XTT Assay Kit (ab232856, Abcam, France) according to the manufacturer's instructions. Briefly, 10^5 cells were seeded in 100 µl of culture medium in each well of a 96-well plate. After 24 h of incubation at 37°C and 5% CO_2, cells were treated with adequate drugs. Then, 10 µl/well of XTT mixture was added for an incubation of 2 h at 37°C and 5% CO_2. Finally, absorbance was measured at 450 nm.

Breast Tissue and Urine Samples

Human samples were collected from the Réseau des tumorothèques du Cancéropole Grand-Ouest and Institut de Cancérologie de l'Ouest (ICO, http://www.ico-cancer.fr).

In accordance with regulations, all subjects signed a specific informed consent form for this biocollection approved by an Ethics Committee (CPP OUEST IV, n°18/16), the French State Department for National Education, Higher Education and Research (Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche, N° DC-2015-2457) and the Commission Nationale de l'Informatique et des Libertés (CNIL) (compliance commitment to MR 001). The glyphosate concentration in urine samples was obtained using Glyphosate kit (Novakits, France).

mMTase and TET Activities

TET activity was determined using the Epigenase 5mC-Hydroxylase TET Activity/Inhibition Assay Kit (Colorimetric; Epigentek/Euromedex, France) according to the manufacturer's instructions. Dnmts-magnetic beads (DMB) assays were performed to estimate mMTase, such as initially described (Yokochi and Robertson, 2002). Briefly, a typical methylation reaction required 50 µg of nuclear extract (Nuclear extract kit, Active Motif, France), 125 nM DNA oligonucleotides (probes), and 900 nM tritium-labeled AdoMet (1 mCi/ml; #NET155V001MC; PerkinElmer, France) in reaction buffer (50 mM Tris, pH 8.0, 5 mM EDTA, 10% glycerol, 0.5 mM phenylmethylsulfonyl fluoride). After incubation at 37°C for 1 h, reactions were quenched with an equal volume of magnetic beads suspension and incubated for 15 min at room temperature. Next, the beads were magnetically isolated from the reaction mix, and tritium incorporation was measured by scintillation counting.

In-Cell ELISA

In-cell ELISA was performed using the In-Cell ELISA Kit (Abcam, France) according to the manufacturer's instructions and after a fixation step performed with 4% of paraformaldehyde solution (10 min at room temperature). Primary antibodies were incubated overnight at 4°C. Adequate HRP-conjugated secondary antibodies were incubated for 1 h at room temperature. Detection was performed at 450 nm.

After the washes, cells in each well were incubated with 1X Janus Green Stain for 5 min at room temperature, according to the manufacturer's instructions. Data were expressed in normalized unit, according to the following calculation: (HRPsignal 'minus' HRPsignal in absence of primary antibody)/(Janus Green signal 'minus' Janus Green signal in absence of cells).

Antibodies used were anti-TET1 (sc163446, Santa Cruz, France), anti-TET2 (sc398535, Santa Cruz), anti-TET3 (sc139186, Santa Cruz), anti-ERα (sc8002, Santa Cruz), anti-PR (sc130071, Santa Cruz), and anti-HER2 (sc-393712, Santa Cruz).

ChIP Analyses

ChIP was performed using the ChIP-IT Express kit (Active Motif, France) according to the manufacturer's instructions. The cross-linking step was performed by treating the cells with 37% formaldehyde solution for 15 min at room temperature. Sonication was performed with the Bioruptor Plus (eight cycles 30 s on/90 s off) (Diagenode, France). The QuantiFast SYBR Green PCR Kit and Rotor-Gene Q (Qiagen, France) were used to perform the qPCR. Antibodies used were Anti-IgG (Abcam, AB2410) and anti-TET3 (sc139186, Santa Cruz).

Statistical Analysis

All experiments were done at least in biological triplicates. Differences in means were assessed using Student t test, and the degree of correlation between two parameters was calculated using Pearson's test. $P < 0.05$ was considered significant.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and the **Supplementary Files**.

ETHICS STATEMENT

The experimental procedures with animals were in accordance with the guidelines of Institutional Animal Care and the French National Committee of Ethics. In addition, all experiments were conducted according to the Regulations for Animal Experimentation at the "Plateforme Animalerie" in the "Institut de Recherche en Santé de l'Université de Nantes (IRS-UN)" and approved by the French National Committee of Ethics.

AUTHOR CONTRIBUTIONS

PFC designed experiments and coordinated the project. MD, JB, AN, and PFC performed all experiments. GBC, FMV, JSF, SL and PFC interpreted and discussed the data. PFC wrote the manuscript. SL edited several versions of the manuscript. All authors have reviewed and approved the manuscript.

FUNDING

This work was supported by funding from the LIGUE NATIONALE CONTRE LE CANCER, Comité InterRégional Grand Ouest, département de Loire Atlantique, d'Ille et Vilaine, Vendée et Côte d'Armorto PFC.

ACKNOWLEDGMENTS

MD and JB were supported by fellowships from "EpiSAVMEN/ REGION PAYS DE LA LOIRE" research program and SAL's work was supported in part by a Congressionally-Directed Medical Research Program/Breast Cancer Research Breakthrough Award # W81XWH-17-1-0250.SL and PFC are members of international breast cancer & nutrition (IBCN).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2019.00885/full#supplementary-material

REFERENCES

Acquavella, J. F., Alexander, B. H., Mandel, J. S., Gustin, C., Baker, B., Chapman, P., et al. (2004). Glyphosate biomonitoring for farmers and their families: results from the farm family exposure study. *Environ. Health Perspect.* 112, 321–326. doi: 10.1289/ehp.6667

Ambatipudi, S., Horvath, S., Perrier, F., Cuenin, C., Hernandez-Vargas, H., Le Calvez-Kelm, F., et al. (2017). DNA methylome analysis identifies accelerated epigenetic ageing associated with postmenopausal breast cancer susceptibility. *Eur. J. Cancer* 75, 299–307. doi: 10.1016/j.ejca.2017.01.014

Anand, P., Kunnumakkara, A. B., Kunnumakara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., et al. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res.* 25, 2097–2116. doi: 10.1007/s11095-008-9661-9

Bassi, S., Tripathi, T., Monziani, A., Di Leva, F., and Biagioli, M. (2017). Epigenetics of Huntington's disease. *Adv. Exp. Med. Biol.* 978, 277–299. doi: 10.1007/978-3-319-53889-1_15

Cao, M., Nie, W., Li, J., Zhang, Y., Yan, X., Guan, X., et al. (2014). MicroRNA-495 induces breast cancer cell migration by targeting JAM-A. *Protein Cell* 5, 862–872. doi: 10.1007/s13238-014-0088-2

Cartron, P.-F., Hervouet, E., Debien, E., Olivier, C., Pouliquen, D., Menanteau, J., et al. (2012). Folate supplementation limits the tumourigenesis in rodent models of gliomagenesis. *Eur. J. Cancer* 48, 2431–2441. doi: 10.1016/j.ejca.2012.01.002

De Almeida, L. K. S., Pletschke, B. I., and Frost, C. L. (2018). Moderate levels of glyphosate and its formulations vary in their cytotoxicity and genotoxicity in a whole blood model and in human cell lines with different estrogen receptor status. *3 Biotech.* 8, 438. doi: 10.1007/s13205-018-1464-z

Degli Esposti, D., Aushev, V. N., Lee, E., Cros, M.-P., Zhu, J., Herceg, Z., et al. (2017). miR-500a-5p regulates oxidative stress response genes in breast cancer and predicts cancer survival. *Sci Rep* 7, 15966. doi: 10.1038/s41598-017-16226-3

di Gennaro, A., Damiano, V., Brisotto, G., Armellin, M., Perin, T., Zucchetto, A., et al. (2018). A p53/miR-30a/ZEB2 axis controls triple negative breast cancer aggressiveness. *Cell Death Differ.* 25, 2165–2180. doi: 10.1038/s41418-018-0103-x

Dib, C., Zakharova, V., Popova, E., Kiseleva, E., Chernyak, B., Lipinski, M., et al. (2019). DUX4 pathological expression: causes and consequences in cancer. *Trends Cancer* 5, 268–271. doi: 10.1016/j.trecan.2019.03.001

Fraga, M. F., Ballestar, E., Paz, M. F., Ropero, S., Setien, F., Ballestar, M. L., et al. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl. Acad. Sci. U. S. A.* 102, 10604–10609. doi: 10.1073/pnas.0500398102

Gaudet, F., Hodgson, J. G., Eden, A., Jackson-Grusby, L., Dausman, J., Gray, J. W., et al. (2003). Induction of tumors in mice by genomic hypomethylation. *Science* 300, 489–492. doi: 10.1126/science.1083558

Gillezeau, C., van Gerwen, M., Shaffer, R. M., Rana, I., Zhang, L., Sheppard, L., et al. (2019). The evidence of human exposure to glyphosate: a review. *Environ. Health* 18, 2. doi: 10.1186/s12940-018-0435-5

Goksu, S. S., Tastekin, D., Arslan, D., Gunduz, S., Tatli, A. M., Unal, D., et al. (2014). Clinicopathologic features and molecular subtypes of breast cancer in young women (age ≤ 35). *Asian Pac. J. Cancer Prev.* 15, 6665–6668. doi: 10.7314/APJCP.2014.15.16.6665

Gong, F., Guo, Y., Niu, Y., Jin, J., Zhang, X., Shi, X., et al. (2017). Epigenetic silencing of TET2 and TET3 induces an EMT-like process in melanoma. *Oncotarget* 8, 315–328. doi: 10.18632/oncotarget.13324

Good, C. R., Panjarian, S., Kelly, A. D., Madzo, J., Patel, B., Jelinek, J., et al. (2018). TET1-Mediated Hypomethylation Activates Oncogenic Signaling in Triple-Negative Breast Cancer. *Cancer Res.* 78, 4126–4137. doi: 10.1158/0008-5472.CAN-17-2082

Hervouet, E., Debien, E., Campion, L., Charbord, J., Menanteau, J., Vallette, F. M., et al. (2009). Folate supplementation limits the aggressiveness of glioma *via the* remethylation of DNA repeats element and genes governing apoptosis and proliferation. *Clin. Cancer Res.* 15, 3519–3529. doi: 10.1158/1078-0432.CCR-08-2062

Hervouet, E., Lalier, L., Debien, E., Cheray, M., Geairon, A., Rogniaux, H., et al. (2010). Disruption of Dnmt1/PCNA/UHRF1 interactions promotes tumorigenesis from human and mice glial cells. *PLoS ONE* 5, e11333. doi: 10.1371/journal.pone.0011333

Inic, Z., Zegarac, M., Inic, M., Markovic, I., Kozomara, Z., Djurisic, I., et al. (2014). 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.* 8, 107–111. doi: 10.4137/CMO.S18006

Jiang, G., Shi, W., Fang, H., and Zhang, X. (2018). miR-27a promotes human breast cancer cell migration by inducing EMT in a FBXW7-dependent manner. *Mol. Med. Rep.* 18, 5417–5426. doi: 10.3892/mmr.2018.9587

Judes, G., Dubois, L., Rifaï, K., Idrissou, M., Mishellany, F., Pajon, A., et al. (2018). TIP60: an actor in acetylation of H3K4 and tumor development in breast cancer. *Epigenomics* 10, 1415–1430. doi: 10.2217/epi-2018-0004

Kim, G., Clarke, C. R., Larose, H., Tran, H. T., Haak, D. C., Zhang, L., et al. (2017). Herbicide injury induces DNA methylome alterations in *Arabidopsis*. *PeerJ* 5, e3560. doi: 10.7717/peerj.3560

Kim, J., Siverly, A. N., Chen, D., Wang, M., Yuan, Y., Wang, Y., et al. (2016). Ablation of miR-10b suppresses oncogene-induced mammary tumorigenesis and metastasis and reactivates tumor-suppressive pathways. *Cancer Res.* 76, 6424–6435. doi: 10.1158/0008-5472.CAN-16-1571

Kucharski, R., Maleszka, J., Foret, S., and Maleszka, R. (2008). Nutritional control of reproductive status in honeybees *via* DNA methylation. *Science* 319, 1827–1830. doi: 10.1126/science.1153069

Kwiatkowska, M., Reszka, E., Woźniak, K., Jabłońska, E., Michałowicz, J., and Bukowska, B. (2017). DNA damage and methylation induced by glyphosate in human peripheral blood mononuclear cells (*in vitro* study). *Food Chem. Toxicol.* 105, 93–98. doi: 10.1016/j.fct.2017.03.051

Mesnage, R., Phedonos, A., Biserni, M., Arno, M., Balu, S., Corton, J. C., et al. (2017). Evaluation of estrogen receptor alpha activation by glyphosate-based herbicide constituents. *Food Chem. Toxicol.* 108, 30–42. doi: 10.1016/j.fct.2017.07.025

Michel, S., Busato, F., Genuneit, J., Pekkanen, J., Dalphin, J.-C., Riedler, J., et al. (2013). Farm exposure and time trends in early childhood may influence

DNA methylation in genes related to asthma and allergy. *Allergy* 68, 355–364. doi: 10.1111/all.12097

Mills, P. J., Caussy, C., and Loomba, R. (2019). Glyphosate excretion is associated with steatohepatitis and advanced liver fibrosis in patients with fatty liver disease. *Clin. Gastroenterol. Hepatol.* S1542-3565, 30361–1. doi: 10.1016/j.cgh.2019.03.045

Minor, E. A., Court, B. L., Young, J. I., and Wang, G. (2013). Ascorbate induces ten-eleven translocation (Tet) methylcytosine dioxygenase-mediated generation of 5-hydroxymethylcytosine. *J. Biol. Chem.* 288, 13669–13674. doi: 10.1074/jbc.C113.464800

Pacaud, R., Brocard, E., Lalier, L., Hervouet, E., Vallette, F. M., and Cartron, P.-F. (2014). The DNMT1/PCNA/UHRF1 disruption induces tumorigenesis characterized by similar genetic and epigenetic signatures. *Sci Rep* 4, 4230. doi: 10.1038/srep04230

Parvez, S., Gerona, R. R., Proctor, C., Friesen, M., Ashby, J. L., Reiter, J. L., et al. (2018). Glyphosate exposure in pregnancy and shortened gestational length: a prospective Indiana birth cohort study. *Environ. Health* 17, 23. doi: 10.1186/s12940-018-0367-0

Patel, Y., Soni, M., Awgulewitsch, A., Kern, M. J., Liu, S., Shah, N., et al. (2019). Overexpression of miR-489 derails mammary hierarchy structure and inhibits HER2/neu-induced tumorigenesis. *Oncogene* 38, 445–453. doi: 10.1038/s41388-018-0439-1

Perera, F. P. (1997). Environment and cancer: who are susceptible? *Science* 278, 1068–1073. doi: 10.1126/science.278.5340.1068

Rondon, R., Grunau, C., Fallet, M., Charlemagne, N., Sussarellu, R., Chaparro, C., et al. (2017). Effects of a parental exposure to diuron on Pacific oyster spat methylome. *Environ. Epigenet.* 3, dvx004. doi: 10.1093/eep/dvx004

Spannhoff, A., Kim, Y. K., Raynal, N. J.-M., Gharibyan, V., Su, M.-B., Zhou, Y.-Y., et al. (2011). Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. *EMBO Rep.* 12, 238–243. doi: 10.1038/embor.2011.9

Sritana, N., Suriyo, T., Kanitwithayanun, J., Songvasin, B. H., Thiantanawat, A., and Satayavivad, J. (2018). Glyphosate induces growth of estrogen receptor alpha positive cholangiocarcinoma cells *via* non-genomic estrogen receptor/ERK1/2 signaling pathway. *Food Chem. Toxicol.* 118, 595–607. doi: 10.1016/j.fct.2018.06.014

Steinborn, A., Alder, L., Michalski, B., Zomer, P., Bendig, P., Martinez, S. A., et al. (2016). Determination of glyphosate levels in breast milk samples from Germany by LC-MS/MS and GC-MS/MS. *J. Agric. Food Chem.* 64, 1414–1421. doi: 10.1021/acs.jafc.5b05852

Sun, M., Song, C.-X., Huang, H., Frankenberger, C. A., Sankarasharma, D., Gomes, S., et al. (2013). HMGA2/TET1/HOXA9 signaling pathway regulates breast cancer growth and metastasis. *Proc. Natl. Acad. Sci. U. S. A.* 110, 9920–9925. doi: 10.1073/pnas.1305172110

Terry, M. B., McDonald, J. A., Wu, H. C., Eng, S., and Santella, R. M. (2016). Epigenetic biomarkers of breast cancer risk: across the breast cancer prevention continuum. *Adv. Exp. Med. Biol.* 882, 33–68. doi: 10.1007/978-3-319-22909-6_2

Thongprakaisang, S., Thiantanawat, A., Rangkadilok, N., Suriyo, T., and Satayavivad, J. (2013). Glyphosate induces human breast cancer cells growth *via* estrogen receptors. *Food Chem. Toxicol.* 59, 129–136. doi: 10.1016/j.fct.2013.05.057

Wang, Q., Wang, C., Guo, J., and Zhnag, Z. (2016). Expression of miR-146a in triple negative breast cancer and its clinical significance. *Int. J. Clin. Exp. Pathol.* 9, 11832–11837. doi: 10.1111/j.0105-2896.2009.00867.x

Yang, L., Yu, S.-J., Hong, Q., Yang, Y., and Shao, Z.-M. (2015). Reduced expression of TET1, TET2, TET3 and TDG mRNAs are associated with poor prognosis of patients with early breast cancer. *PLoS One* 10, e0133896. doi: 10.1371/journal.pone.0133896

Ye, Z., Li, J., Han, X., Hou, H., Chen, H., Zheng, X., et al. (2016). TET3 inhibits TGF-β1-induced epithelial-mesenchymal transition by demethylating miR-30d precursor gene in ovarian cancer cells. *J. Exp. Clin. Cancer Res.* 35, 72. doi: 10.1186/s13046-016-0350-y

Yin, R., Mao, S.-Q., Zhao, B., Chong, Z., Yang, Y., Zhao, C., et al. (2013). Ascorbic acid enhances Tet-mediated 5-methylcytosine oxidation and promotes DNA demethylation in mammals. *J. Am. Chem. Soc.* 135, 10396–10403. doi: 10.1021/ja4028346

Yokochi, T., and Robertson, K. D. (2002). Preferential methylation of unmethylated DNA by Mammalian *de novo* DNA methyltransferase Dnmt3a. *J. Biol. Chem.* 277, 11735–11745. doi: 10.1074/jbc.M106590200

Yoshioka, N., Asano, M., Kuse, A., Mitsuhashi, T., Nagasaki, Y., and Ueno, Y. (2011). Rapid determination of glyphosate, glufosinate, bialaphos, and their major metabolites in serum by liquid chromatography-tandem mass spectrometry using hydrophilic interaction chromatography. *J. Chromatogr. A* 1218, 3675–3680. doi: 10.1016/j.chroma.2011.04.021

Yu, J., Shen, W., Gao, B., Zhao, H., Xu, J., and Gong, B. (2017). MicroRNA-182 targets FOXF2 to promote the development of triple-negative breast cancer. *Neoplasma* 64, 209–215. doi: 10.4149/neo_2017_206

Zhang, J., Zhang, S., Wang, Y., Cheng, H., Hao, L., Zhai, Y., et al. (2017). Effect of TET inhibitor on bovine parthenogenetic embryo development. *PLoS One* 12, e0189542. doi: 10.1371/journal.pone.0189542

Conflict of Interest Statement: 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 in Public Health

SYSTEMATIC REVIEW
published: 06 November 2019
doi: 10.3389/fpubh.2019.00298

Mobilizing Breast Cancer Prevention Research Through Smartphone Apps: A Systematic Review of the Literature

Lauren C. Houghton[1,2]*, *Renata E. Howland*[1] and *Jasmine A. McDonald*[1,2]*

[1] Mailman School of Public Health, Columbia University Irving Medical Center, New York, NY, United States, [2] Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center, New York, NY, United States

OPEN ACCESS

Edited by:
Rabih Shakib Talhouk, American University of Beirut, Lebanon

Reviewed by:
Harm Van Marwijk, Brighton and Sussex Medical School, United Kingdom
Nuno Ribeiro, University of Porto, Portugal

***Correspondence:**
Lauren C. Houghton
lh2746@cumc.columbia.edu
Jasmine A. McDonald
jam2319@cumc.columbia.edu

Specialty section:
This article was submitted to Family Medicine and Primary Care, a section of the journal Frontiers in Public Health

Received: 04 April 2019
Accepted: 02 October 2019
Published: 06 November 2019

Citation:
Houghton LC, Howland RE and McDonald JA (2019) Mobilizing Breast Cancer Prevention Research Through Smartphone Apps: A Systematic Review of the Literature. Front. Public Health 7:298. doi: 10.3389/fpubh.2019.00298

Background: Breast cancer rates have been increasing worldwide, particularly among young women, suggesting important interactions between genes and health behaviors. At the same time, mobile technology, including smartphones applications (apps), has emerged as a new tool for delivering healthcare and health-related services. As of 2018, there were nearly 600 publicly available breast cancer apps designed to provide disease and treatment information, to manage disease, and to raise overall awareness. However, the extent to which apps are incorporated into breast cancer prevention research is unknown. Therefore, the objective of this review was to determine how mobile applications are being used for breast cancer prevention among women across the cancer control continuum.

Methods: Using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we searched PubMed and Web of Science Core Collection databases using the keywords breast cancer, smartphone, mobile application, and phone app. Full-length journal articles available in English that addressed the research question were included. We categorized articles by prevention type (primary, secondary, and tertiary) and phase of research (protocol, development, feasibility, pilot, measurement, and effectiveness), and identified common themes and gaps.

Results: Our search yielded 82 studies (69 unique) that used apps in breast cancer prevention research across 20 countries. Approximately half of the named apps were publicly available. The majority (73%) of studies targeted tertiary prevention; 15% targeted secondary and 13% targeted primary prevention. Apps were used across all phases of research with the predominant phase being feasibility in tertiary prevention (34%), effectiveness in secondary prevention (63%), and development (30%) and effectiveness (30%) in primary prevention. Common uses included assessing outcomes relevant to clinical care coordination, quality of life, increasing self-efficacy and screening behaviors, and tracking and managing health behaviors.

Conclusions: We identified the following gaps: few effectiveness studies in tertiary prevention, minimal use of apps for breast cancer etiology or early detection, and few

interventions in those at average risk of breast cancer. These findings suggest that while mobile apps can inform breast cancer prevention across the continuum, more work is needed to incorporate apps into primary prevention.

Keywords: breast cancer, cancer control continuum, mobile application, smartphone, prevention, systematic review

INTRODUCTION

Breast cancer rates have been increasing worldwide, particularly among young women (1). Such rapid changes in the incidence of early onset breast cancer cannot be attributed solely to genetics, but rather to interactions between health behaviors and genes. Given many behavioral risk factors for breast cancer are modifiable, public health prevention and intervention studies have long sought to change individual health behaviors and more recent work recognizes that a multi-faceted approach is needed to address these behaviors because they are complex in nature (2).

At the same time, mobile technologies, including smartphone applications (hereafter referred to as apps), have emerged as new tools for delivering healthcare and health-related services in the field of cancer and particularly breast cancer. In fact, nearly half of all cancer apps are targeted toward breast cancer (3). A recent review suggests there are nearly 600 publicly available breast cancer apps designed to provide disease and treatment information, to manage disease, and to raise overall awareness (4). With the widespread availability and use of applications, researchers have an opportunity to leverage this ubiquitous technology for breast cancer prevention. However, the extent to which apps are incorporated into breast cancer prevention research across the cancer control continuum is unknown.

Given that the use of apps for breast cancer prevention is still in the early stages of adoption, the authors agreed that a systematic review with a broad research scope was warranted. Therefore, we performed a systematic review to answer the question: how are mobile apps being used for breast cancer prevention research across the cancer control continuum, including tertiary, secondary, and primary prevention, in women? Since the use of apps in research is relatively new, we also sought to identify at what phases of the research process mobile apps were being used for breast cancer research, including protocol, development, feasibility, pilot, effectiveness, and measurement studies. In addition to the systematic review, we sought to find common themes and gaps across the body of literature.

METHODS

Search Strategy

In order to conduct this systematic review, we utilized the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (5). We systematically reviewed PubMed and Web of Science Core Collection databases in December 2018 (updated February 7, 2019 to ensure the most recent articles were captured). Search terms included breast cancer, smartphone, mobile application, and phone app. These terms were applied to all fields in order to capture the greatest number of articles. We also employed the controlled vocabulary of Medical Subject Headings (MeSH), available in PubMed only, including subheadings, for breast neoplasms and mobile apps. **Supplementary Table 1** includes the complete search string as it was conducted in PubMed. We searched for additional articles using the terms mHealth, health app, breast cancer app, iPhone application, and Android application. Our search contained no restrictions regarding language or year of publication. All references were exported to Endnote (X8, Thompson Reuters). We first removed duplicate citations using the automatic feature and then manually reviewed articles for additions that had minor differences in the way information was indexed.

Inclusion/Exclusion Criteria

Records were screened in Endnote and included if they were published as an original research article in English. The primary reviewer [RH] then reviewed the full-text article for relevance to the study question. Articles were excluded if study participants were providers or caregivers; if breast cancer prevention was not an explicit goal or implication of the research; if the article did not include a mobile application or only discussed that the research could be potentially adapted into a mobile application; or if the smartphone was examined as a carcinogen. We also excluded books or book chapters, meeting abstracts, non-empirical records (e.g., reviews, editorials, and letters), non-English records, and records where the full-text were unavailable. When inclusion was unclear, authors LH and JAM independently reviewed the articles and then all authors discussed until a consensus was met. LH and JAM also reviewed 20% of excluded articles for accuracy. In one case where we could not reach consensus, we contacted the corresponding author for clarification. Among all studies that were eligible for qualitative analysis ($n = 82$), we flagged those studies that had multiple publications reporting outcomes across different stages of research (e.g., a protocol and effectiveness study) but were using the same underlying cohort ($n = 23$).

Data Extraction and Analysis

For studies meeting the inclusion criteria, the primary reviewer [RH] extracted the following information from eligible studies: population characteristics, sample size, location of the study (country), mobile application name (where applicable), and study objectives and/or outcomes (e.g., quality of life, efficacy, literacy). We categorized studies by prevention type based on whether they were targeting a secondary cancer event and/or morbidity/mortality (tertiary), early diagnosis and treatment (secondary), or disease prevention (primary). We assigned articles to only one prevention type category. We also categorized studies by research phase based on the study outcome(s).

Studies categorized as Development included those collecting information on participant interest and preferences for a mobile application that was not yet produced. Based on features outlined by Orsmond and Cohn (6), we categorized Feasibility studies as those that reported process outcomes, such as usability of an app (6). We categorized Pilot studies as those studies where the author(s) self-described the study as such and/or the authors(s) mention that a larger study was being planned to evaluate the effectiveness of an intervention. Generally, Pilot studies reported outcomes among a small sample, where the average sample size was ∼35. Effectiveness studies reported outcome measures from a full study; and a Protocol described the protocol for a study, such as for an effectiveness study, usually in the title of the article itself. Measurement studies were those that reported outcomes related to validity or reliability. Some studies were categorized across multiple research phases if papers combined multiple outcomes; therefore, research phase categories were not mutually exclusive.

Our initial analysis tabulated all articles eligible for qualitative analysis by cancer prevention type and by research phase. We then estimated the number of articles published by year. We used the subset of unique studies and tabulated the number of publications by country and continent. Lastly, void of *a priori* hypotheses regarding common themes and gaps in the literature, we comprehensively reviewed unique studies by cancer prevention type to identify common themes and gaps. We then extracted mobile app details and categorized app use by prevention type and the availability of the app in the Apple and/or Android app store.

RESULTS

We identified 199 records through our search, excluding duplicate records (**Figure 1**). Of these, we first screened the record title, abstract, and reference type for eligibility and excluded 83 records as ineligible. We then assessed the remaining 116 articles for eligibility through full-text review and further excluded 34 records. We identified 82 studies eligible for qualitative analysis. Of the 82, we identified 23 studies that were part of multiple publications that used the same underlying cohort to report outcomes across different research phases. Therefore, we identified 69 unique studies, 75% ($n = 52$) were tertiary, 12% ($n = 8$) were secondary, and 13% ($n = 9$) were primary.

The Use of Mobile Apps by Cancer Prevention Type and Research Phase

As displayed in **Figure 2**, apps were used across all phases of research with the predominant phase being feasibility in tertiary prevention studies (34%), effectiveness in secondary prevention studies (63%), and development (30%) and effectiveness (30%) in primary prevention studies. Across the cancer prevention continuum, 14 studies were protocols (17%), 23 were development (28%), 23 were feasibility (28%), 11 were pilots (13%), 18 were effectiveness (22%), and 9 were measurement studies (11%). Given 23 articles reported on multiple study phases, the categories were not mutually exclusive and percentages exceed 100%.

Mobile App Use: Growth and Global Reach

The number of studies using apps for breast cancer prevention research increased rapidly over the last 10 years (**Figure 3**). The earliest studies in this review were published in 2010, while the majority (40%) were published in 2018. There was international use of apps in breast cancer prevention research, with the exception of Africa and South America (**Figure 4**). The studies included in this review were conducted in 20 countries, with most studies conducted in the US (43%) and more than one study each occurring in Canada (7–9), China (10–12), Germany (13–15), Ireland (16–18), Korea (19–24), the Netherlands (25–29), Spain (30, 31), and the United Kingdom (32–35). Tertiary prevention studies took place in North America (US, Canada, Mexico), Western Europe (UK, Sweden, Netherlands, Germany, France, Spain Ireland), and Asia (Korea, China, Japan, Singapore). Secondary prevention studies were based in North America (US), Asia (Korea, China, India, Bangladesh), and Eastern Europe (Romania). Primary prevention studies were based in North America (US), Europe (Netherlands), and the Middle East (Kingdom of Saudi Arabia).

Review of Mobile Apps by Cancer Prevention Types: Common Themes

Tertiary Prevention

The majority of mobile apps used for breast cancer prevention research addressed tertiary prevention. We identified 63 studies (53 unique) (**Table 1**) and the articles ranged across research phases including development (24.5%), feasibility with a focus on process (34%), pilots with a focus on outcomes (18.9%), protocols (15.1%), effectiveness (16%), and measurement (11.3%) (**Figure 2**).

We identified two common themes for the use of mobile health apps in tertiary breast cancer prevention: clinical care coordination and health related quality of life during and after a breast cancer diagnosis. Cancer care coordination studies focused on the support and communication between the breast cancer patient and the physician (32, 41, 47, 48, 66, 68), as well as specific aspects of cancer care coordination, such as symptomology (12, 14, 23, 27, 52), medication adherence (23, 34, 38, 45, 66), and ambulatory surgery (7, 8). Research using apps designed to improve health related quality of life focused on general lifestyle management (30, 42, 56, 60, 64, 69), weight management (61, 66, 67), depression and breast cancer related distress (12, 17, 21, 23, 37, 63), social support (12, 40, 50, 51), sleep (20), and physical activity during and after a breast cancer diagnosis (9, 11, 22, 24, 25, 28, 29, 33, 35, 36, 46, 55, 59, 65). The use of mobile apps for tertiary cancer prevention was preferred in contrast to usual standard of care practices. For example, multiple studies reported that cancer patients and survivors were willing, and had a preference for, receiving clinical care coordination support (13, 15, 16) and health-related quality of life interventions (53, 62) through apps.

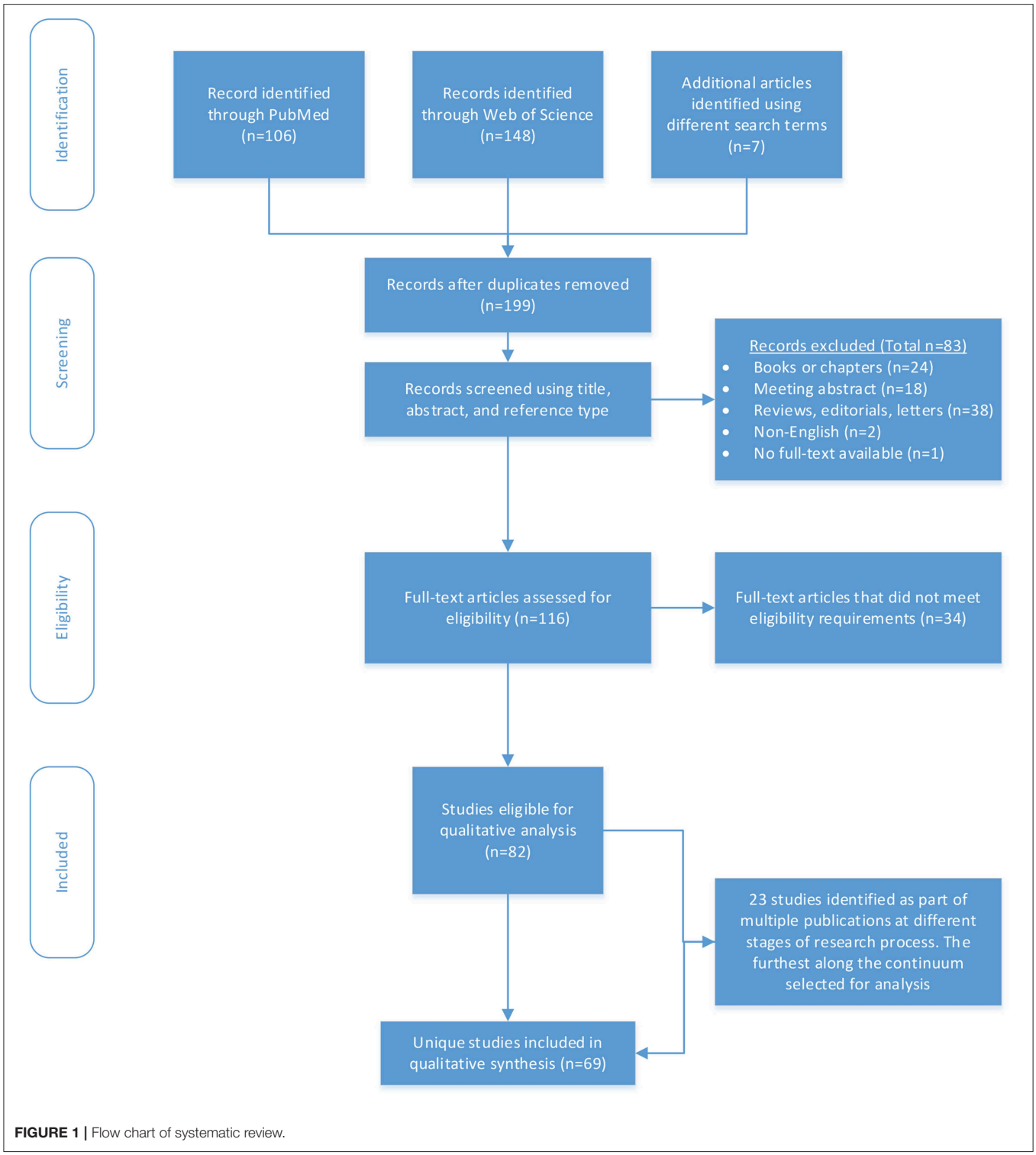


FIGURE 1 | Flow chart of systematic review.

In addition to the two main themes identified, we also found that tertiary prevention apps were used to improve measurement and provide real-time data for assessment and prediction. For example, Timmerman et al. subjectively measured fatigue in 18 cancer survivors by administering the Visual Analog Scale on a smartphone 3 times per day (25). In addition, Langer et al. had cancer patient and spouse dyads systematically record their thoughts via a smart phone twice a day for 14 consecutive days to assess communication (51). Information collected from mobile apps was also validated against other

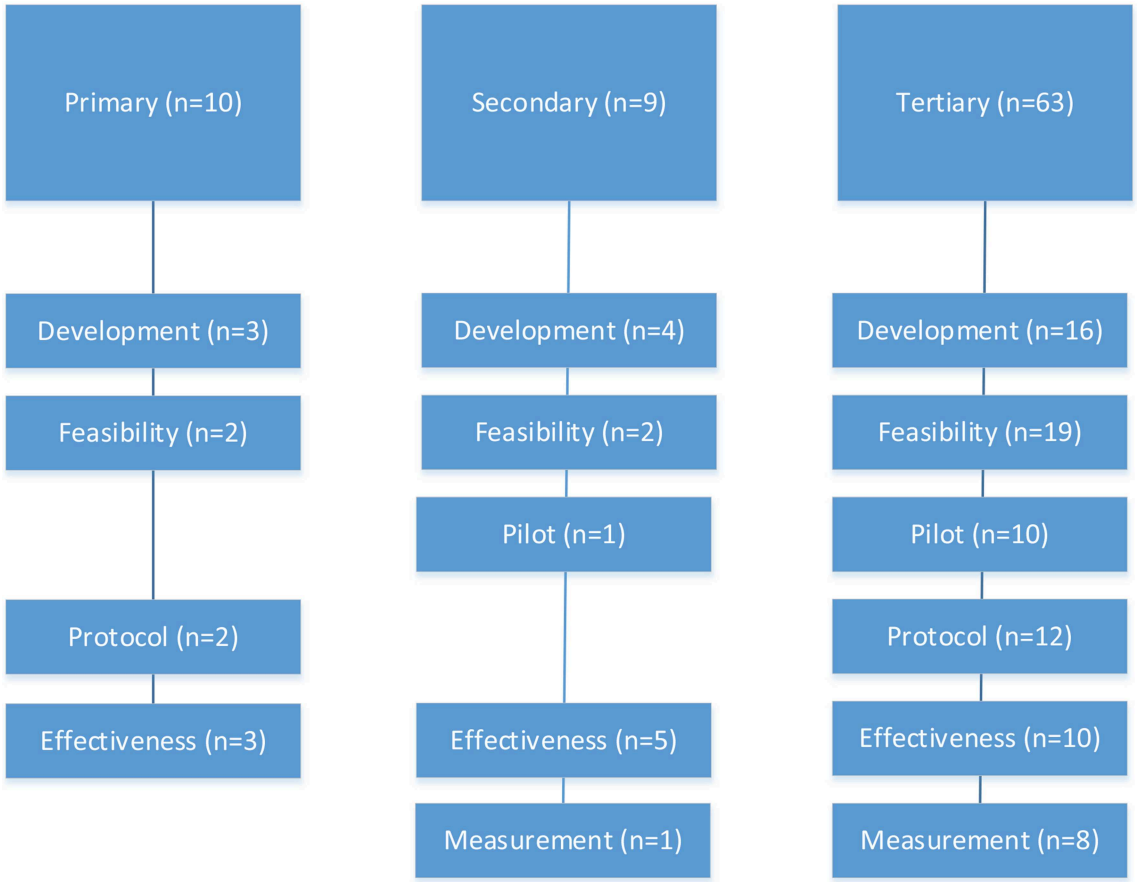


FIGURE 2 | The use of mobile apps across primary, secondary, and tertiary breast cancer prevention by research phase ($n = 82$ eligible studies).

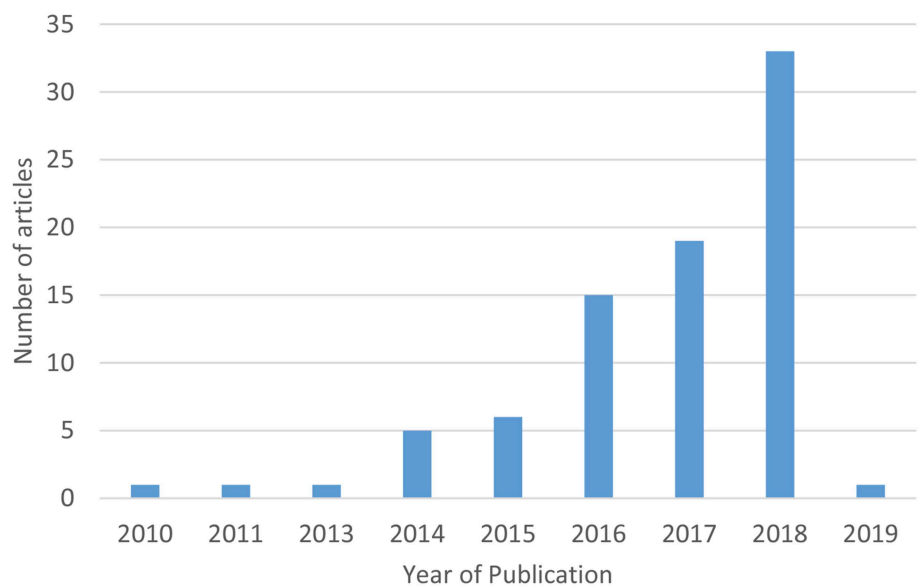


FIGURE 3 | Number of studies using mobile apps for breast cancer prevention research among women by year of publication ($n = 82$ eligible studies). *The initial search was conducted in December 2018 and updated February 7, 2019.

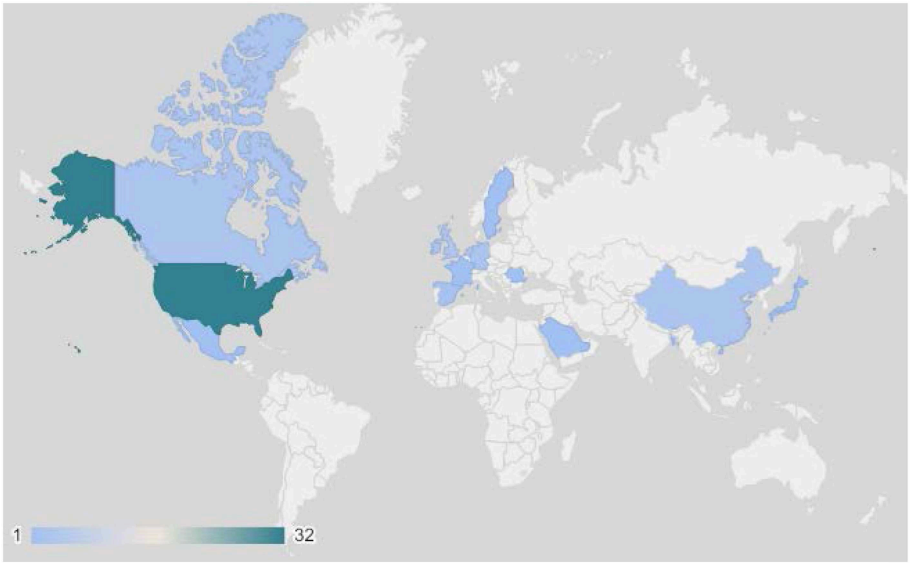


FIGURE 4 | Number of publications by country ($n = 69$ unique studies).

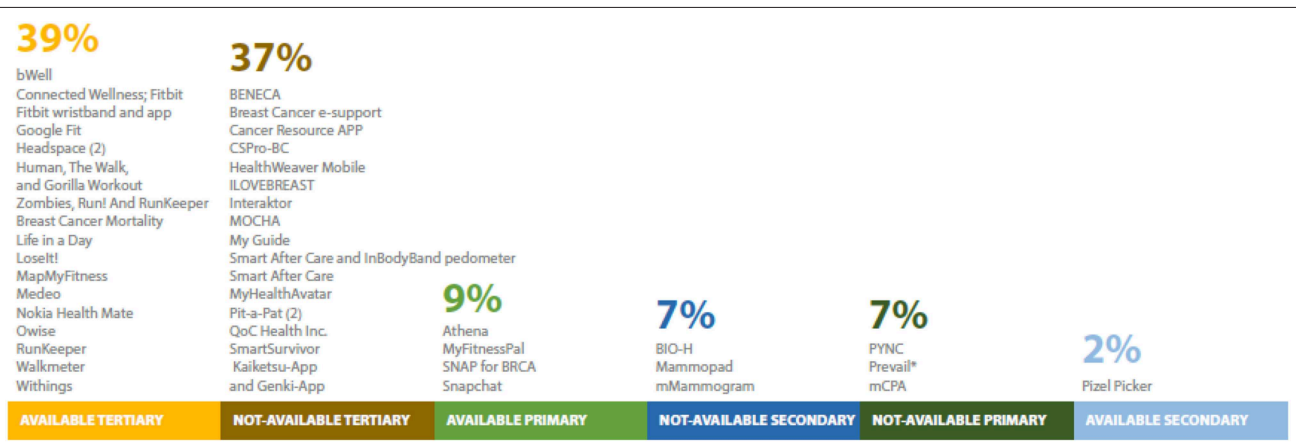


FIGURE 5 | Names and number of publicly-available apps used for breast cancer prevention research ($n = 69$ unique studies). Twenty-one studies excluded because no app name was provided or no app was developed. *Name provided at request of author.

metrics. For instance, Kim et al. found that daily self-reported depression ratings collected by a mobile mental-health application provided comparable results as traditional one-time in-clinic assessment of depression and that higher accuracy of depression was achieved with greater adherence to mobile app use (21). Lastly, information collected via mobile applications was utilized to improve prediction of breast cancer-specific mortality and breast cancer recurrence (31, 57). While risk modeling is a common tool used in clinical practice to inform individuals of their individual cancer risk, Parades-Aracil et al. integrated these risk models into an app making the risk measurement tool more accessible for clinical use.

The vast majority of the apps we identified for clinical care coordination were not named in the study or publicly available, but rather developed for each specific study. In contrast, studies using apps to improve health related quality of life were more readily available for public use in the Apple and/or Android app store (**Figure 5**).

Secondary Prevention

We identified 9 studies (8 unique) that used apps for secondary breast cancer prevention in the following phases: development (37.5%), feasibility (25%), pilot (12.5%), and effectiveness (62.5%); with three articles reporting on multiple study phases (see **Table 2**).

We identified only one theme in the studies of secondary prevention; with one exception (72), all studies that involved human subjects were effectiveness studies that targeted breast cancer screening behaviors, especially among underserved populations and high-risk women (18, 19, 73–75). For example, Eden et al. found that among rural women aged 40–49 years, apps

TABLE 1 | Articles using mobile apps for tertiary breast cancer prevention ($n = 63$ eligible studies).

References	Type of study	Population (sample size)	Location	Outcomes
Ainsworth et al. (36)	Feasibility	Breast cancer survivors (40)	US	App use and experience
Akechi et al. (37)	Protocol	Breast cancer survivors (444)	Japan	Fear of recurrence
Ali et al. (38)	Development	Patients undergoing treatment for cancer (423)	Singapore	App interest and preferences
Armstrong et al. (8)	Effectiveness	Women undergoing breast reconstruction (65)	Canada	Post-surgical follow-up
Armstrong et al. (39)*	Protocol	Women undergoing breast reconstruction (72)	Canada	Post-surgical follow-up
Banas et al. (40)	Development	Breast cancer survivors, Hispanic (31)	US	App interest and preferences
Baseman et al. (41)	Feasibility	Breast cancer survivors and providers (11)	US	App interest and preferences
Brett et al. (34)	Development	Women undergoing treatment for breast cancer (20)	UK	App use and experience
Buscemi et al. (42)	Feasibility + Pilot	Breast cancer survivors, Hispanic (25)	US	App use and experience, Quality of life
Iacobelli et al. (43)*	Development	Breast cancer survivors, Hispanic (9)	US	App interest and preferences
Yanez et al. (44)*	Protocol	Breast cancer survivors, Hispanic (80)	US	Quality of life
Chalela et al. (45)	Protocol	Women undergoing treatment for breast cancer (120)	US	Medication adherence
Delrieu et al. (46)	Protocol	Women undergoing treatment for breast cancer (60)	France	Physical activity, app use
Douma et al. (28)	Feasibility + Measurement	Women undergoing treatment for breast cancer (72)	Netherlands	Physical activity, app use
Drewes et al. (13)	Development	Women undergoing treatment for breast cancer and physicians (168)	Germany	App interest and preferences
Egbring et al. (14)	Effectiveness	Women undergoing treatment for breast cancer (139)	Germany	Daily functional activity
El Shafie et al. (15)	Development	Patients undergoing treatment for cancer (breast or prostate) (200)	Germany	App interest and preferences
Foley et al. (17)	Pilot	Women undergoing treatment for breast cancer (39)	Ireland	Mental health
Gehrke et al. (47)	Development + Feasibility	Breast cancer survivors (11) and their nurses (3)	US	App interest and preferences
Harder et al. (33)	Development + Feasibility	Women undergoing treatment for breast cancer (9)	UK	App interest and preferences
Hwang (7)	Effectiveness	Women undergoing treatment for breast cancer (72)	Canada	Readmission, app use and experience
Kim et al. (23)	Effectiveness	Women undergoing treatment for breast cancer (76)	Korea	Medication adherence
Kim et al. (21)	Measurement	Women undergoing treatment for breast cancer (78)	Korea	Reliability
Klasnja et al. (48)	Effectiveness	Women undergoing treatment for breast cancer (9)	US	Self-management
Klasnja et al. (49)*	Development	Women undergoing treatment for breast cancer (3)	US	App interest and preferences
Kubo et al. (50)	Feasibility + Pilot	Patients undergoing treatment for cancer (28) and their caregivers (14)	US	App use and experience, distress and quality of life
Langer et al. (51)	Measurement	Women undergoing treatment for breast cancer and their partners (107 couples)	US	Relationship satisfaction
Langius-Eklof et al. (52)	Protocol	Patients undergoing treatment for cancer (150)	Sweden	Symptom distress
Lloyd et al. (53)	Development	Breast cancer survivors (279)	US	App interest and preferences
Lozano-Lozano et al. (30)	Protocol	Breast cancer survivors (80)	Spain	Quality of life
Lozano-Lozano et al. (54)*	Measurement	Breast cancer survivors (20)	US	Validity and test-retest reliability
Lyons et al. (55)	Protocol	Breast cancer survivors (120)	US	Physical activity
McCarroll et al. (56)	Pilot	Breast and endometrial cancer survivors (50)	US	Physical activity
Min et al. (20)	Feasibility	Women undergoing treatment for breast cancer (30)	Korea	App use and experience
O'Brien et al. (16)	Development	Breast clinic sample (200)	Ireland	App use and experience

(Continued)

TABLE 1 | Continued

References	Type of study	Population (sample size)	Location	Outcomes
Ormel et al. (29)	Feasibility + Pilot	Patient undergoing treatment for cancer or cancer survivors (32)	Netherlands	Physical activity, use and experience
Paredes-Aracil et al. (57)	Measurement	Breast cancer survivors (272)	Spain	Model validation and calibration
Paredes-Aracil et al. (31)*	Measurement	Breast cancer survivors (287)	Spain	Model validation and calibration
Park et al. (24)	Effectiveness	Women undergoing treatment for breast cancer (356)	Korea	Physical activity
Lee et al. (58)*	Feasibility	Breast cancer survivors (88)	Korea	App use and experience
Phillips et al. (59)	Protocol	Breast cancer survivors (256)	US	Physical activity, use and experience
Phillips et al. (59)	Feasibility	Breast cancer survivors (279)	US	App interest and preferences
Pope et al. (60)	Feasibility + Pilot	Breast cancer survivors (10)	US	Physical activity, use and experience
Quintiliani et al. (61)	Feasibility + Pilot	Breast cancer survivors (10)	US	App use and experience, weight management
Raghunathan et al. (62)	Development	Patients undergoing cancer treatment (631)	US	App interest and preferences
Ritvo et al. (9)	Protocol	Breast cancer survivors (107)	Canada	Physical activity, use and experience
Roberts et al. (35)	Development	Cancer survivors (breast, prostate, colorectal) (32)	UK	App interest and preferences
Rosen et al. (63)	Feasibility + Effectiveness	Breast cancer survivors (112)	US	Quality of life, use and experience
Smith et al. (64)	Development	Breast cancer survivors, African American (96)	US	App interest and preferences
Soto-Perez-De-Celis et al. (65)	Pilot + Feasibility	Patients undergoing cancer treatment (40)	Mexico	Physical activity, use and experience
Stubbins et al. (66)	Effectiveness	Breast cancer survivors (33)	US	Weight management
Timmerman et al. (25)	Measurement	Cancer survivors (18)	Netherlands	Physical activity, reliability
Uhm et al. (22)	Effectiveness	Breast cancer survivors (356)	Korea	Physical activity
Valle et al. (67)	Feasibility + Pilot	Breast cancer survivors, African American (35)	US	Weight management and physical activity
Walker et al. (68)	Development	Breast cancer survivors and nurses (12)	US	App use and experience
Weaver et al. (32)	Pilot	Patients undergoing treatment for cancer (breast or colorectal) (26)	UK	Medication use and perceived support
Xiaosheng et al. (11)	Protocol	Breast cancer survivors (60)	China	Quality of life
Young-Afat et al. (27)	Feasibility	Women undergoing treatment for breast cancer (15)	Netherlands	App use and experience
Zhang et al. (69)	Feasibility	Cancer survivors and workshop attendees (∼150)	Europe	App use and experience
Zhu et al. (70)	Effectiveness	Women undergoing treatment for breast cancer (114)	China	Self-efficacy
Zhu et al. (12)*	Feasibility	Women undergoing treatment for breast cancer (13)	China	App use and experience
Zhu et al. (71)*	Protocol	Women undergoing treatment for breast cancer (108)	China	Self-efficacy
Zhu et al. (71)*	Development	Women undergoing treatment for breast cancer (114)	China	Quality of life

**Duplicate articles are indented.*
US, United States; UK, United Kingdom.

were effective at reducing decisional conflict and increasing self-efficacy around mammography (73). Two studies used mobile apps to increase breast-screening practices in Korean women. Heo et al. successfully introduced an app to increase breast self-examination among young Korean women (average 29.5 ± 5.9 years) (19). In addition, Lee et al. found that in comparison to the usual care control group that received a printed brochure, Korean American women in the intervention group that received access to a mobile mammography app with health navigator services, showed significantly increased knowledge of breast cancer and greater readiness for mammography (75). Similar to Lee et al., other studies also examined if breast cancer screening is improved when pairing mobile apps with community health navigators (18, 74).

Two developmental studies used apps to innovate breast cancer detection strategies. The SmartIHC-Analyzer mobile app automates scoring of Ki-67 protein, a hallmark for assessing cell proliferation rate during cancer progression (76). The Pixel Picker mobile app rapidly detects breast cancer cells (10).

TABLE 2 | Articles using mobile apps for secondary breast cancer prevention ($n = 9$ eligible studies).

References	Type of study	Population (sample size)	Location	Outcomes
Cardos et al. (72)	Feasibility	Community sample of females (16)	Romania	App use and experience
Eden et al. (73)	Pilot + Effectiveness	Clinic sample of females (100)	US	Decisional conflict and intention to screen
Ginsburg et al. (74)	Effectiveness	Women with abnormal clinical breast examination (556)	Bangladesh	Adherence to screening
Heo et al. (19)	Development + Effectiveness	Community sample of females (45)	Korea	Adherence to screening
Jiao et al. (10)	Development	N/A	China	Colorimetric detection of breast cancer cells
Keohane et al. (18)	Effectiveness	Breast clinic sample (84)	Ireland	Knowledge of risk
Lee et al. (75)	Effectiveness + Feasibility	Community sample, Korean American women (120)	US	Knowledge and adherence to screening; app use and experience
Lee et al. (58)*	Development	Community sample, Korean American women (14)	US	App interest and preferences
Tewary et al. (76)	Development + Measurement	Breast cancer tissue samples (30)	India	Automated Ki67 proliferation index scoring

Duplicate articles are indented.
US, United States.

TABLE 3 | Articles using mobile apps for primary breast cancer prevention ($n = 10$ eligible studies).

References	Type of study	Population (sample size)	Location	Outcomes
Alanzi et al. (77)	Effectiveness	Community sample of female students (200)	Kingdom of Saudi Arabia	Breast cancer awareness; Guidelines; High-risk;
Businelle et al. (78)	Effectiveness	Hospital sample (92)	US	Smoking lapse; High-risk
Cohen et al. (79)	Feasibility	Community sample of females with BRCA mutation (102)	US	Awareness; Guidelines
Scherr et al. (80)*	Development	Community sample of females with BRCA mutation (14) and healthcare providers who work with BRCA carriers (3)	US	App preferences; Framework
Coughlin et al. (81)	Development	Community sample (5)	US	App preferences; Framework; Literacy
Hartman et al. (82)	Effectiveness	Breast clinic sample (54)	US	Weight gain and physical activity; High-risk; Framework
Kratzke et al. (83)	Development	Community sample of female students (546)	US	App preferences; Framework; Self-efficacy
Loef et al. (26)	Protocol	Healthcare workers (1960)	Netherlands	Infection susceptibility; High-risk
Smith et al. (64)	Protocol	Breast cancer survivors, African American (12)	US	App preferences; Guidelines; Framework
Bravo et al. (84)	Feasibility	Breast clinic sample (15)	US	Acceptability and usability; Literacy

Duplicate articles are indented.
US, United States.

With one exception (10), none of the mobile apps for secondary prevention were publicly available at the time of this review (**Figure 5**).

Primary Prevention

We identified 10 articles (9 unique) that focused on the use of mobile apps for primary breast cancer prevention (see **Table 3**). The articles ranged across the following research phases: development (30%), feasibility (20%), protocols (20%), and effectiveness (30%).

We identified three common themes for the use of mobile health apps in primary breast cancer prevention: knowledge and adherence to screening guidelines, the targeting of high-risk populations, and the incorporation of theoretical frameworks. Primary prevention studies focused on apps that increased breast cancer prevention knowledge and adherence to breast cancer guidelines and surveillance (77, 79, 80, 83–85). Six of the 9 studies used existing guidelines to inform their apps (77, 80, 81, 83, 85). For example, in designing an app to help women reduce their risk of breast cancer through healthy behaviors, Coughlin et al. (81) included evidence-based information provided by the National Cancer Institute, the Centers for Disease Control and Prevention, and the American Cancer Society. In addition, a protocol study that provided healthy food recipes through the app aimed to assess adherence to diet and physical activity guidelines for cancer survivors set out by the American Institute for Cancer Research (85) and the investigators of an effectiveness study based the content of their app on the Saudi

Cancer Foundation guidelines (77). Four studies focused on encouraging healthy behaviors that reduced the risk of breast cancer (78, 81, 82, 85).

The targeted population for these primary prevention studies was primarily women at high risk for breast cancer (77, 79, 80, 82, 83) including post-menopausal women with high Gail risk scores (82), *BRCA* mutation carriers (79, 80), and African American women, who experience greater breast cancer disparities (85). Some studies also targeted broader populations that engaged in behaviors associated with higher breast cancer risk, such as smoking (78) and night shift work (26). In the latter, Loef et al. described the protocol for an observational cohort of health workers in the Netherlands in which an app will be used to collect daily measures of infection to investigate how night shift work impacts health outcomes that are related to carcinogenesis (26). Therefore, apps are used both to increase knowledge about breast cancer risk and prevention in targeted populations (78, 85), as well as to identify new risk factors in high risk populations (26).

Many of the primary prevention studies incorporated theoretical frameworks for behavior change. The development studies incorporated the Common Sense Model of Behavior Theory (81), Health Information Model (83), and the Messaging Model for Health Communication Campaigns framework (80). One protocol study used both the Health Belief Theory and Theory of Planned Behavior Models (64). One effectiveness study based their study design on a Social Cognitive Theory (82). None of the feasibility studies mentioned a theoretical framework.

In addition to the three themes, we found that several key concepts were vital to implementing primary prevention research with apps, including literacy (specific to health and ehealth), self-efficacy (with a distinction between active and passive information seeking), and user-friendly scheduling tools. For example, literacy and self-efficacy were important in a study among college women that applied a family-based life course approach to breast cancer prevention (83). Given college-age women may adopt healthy lifestyles that are important for cancer risk reduction, Kratze et al. found that the app proved useful in knowledge transfer of breast health awareness while also assisting in daughter-initiated communication with their mothers regarding screenings and health information. The need for user-friendly tools, such as scheduling assistants, emerged in a study of guideline adherence among BRCA carriers. Although their awareness of surveillance guidelines was high, adherence was low and half of respondents indicated they had a difficult time remembering to schedule appointments (79). Thus, the app was designed to remind users when to seek care personalized to their own risk factors. The use of apps was particularly helpful in increasing effectiveness of behavioral interventions because they enabled dynamic tailoring in the case of smoking cessation (78) and easier self-monitoring in the case of tracking diet and physical activity (85).

With regard to app availability, 4 studies used publicly-available apps (**Figure 5**) (77, 79, 82, 84). Other studies used pre-existing apps, including My Fitness Pal (82), Snapchat (77), or incorporated their custom app to be used with FitBit and LoseIt! (81). The studies whose apps were not publicly-available either developed apps for research purposes only (85) or did not mention specific information about their app (26, 83). For one study, the author provided the app name upon contact (78).

DISCUSSION

This systematic review summarizes the emerging literature for breast cancer prevention research using mobile apps. While we found studies across the cancer control continuum, the majority of studies used mobile apps to target tertiary prevention, particularly clinical care coordination and health-related quality of life for breast cancer survivors, as well as to improve the measurement and assessment of symptoms, behaviors, and risk. Fewer mobile apps were used for secondary and primary prevention where outcomes were related to increasing self-efficacy and screening behaviors and tracking and managing health behaviors. The studies reviewed spanned all phases of research in diverse populations in nearly 20 countries. The use of apps in breast cancer research has been increasing since 2010, a trend that will likely continue. Given the ubiquity of smartphones and global burden of breast cancer, there is potential for mobile apps to impact breast cancer trends across the globe.

Progress Since Previous Reviews

Previous reviews have explored the use of cancer apps, but were not systematically conducted (86), specific to breast cancer (87), or focused on research (4). That being said, our findings suggest that some of the gaps identified by past reviews have begun to be addressed. In particular, we identified that many of the primary prevention studies were grounded in theoretical frameworks and were tailored to different cultural and literacy levels, key points that were not being addressed previously as identified by Coughlin et al. (86). Similar to Coughlin et al. (86) and Giunti et al. (4), we also found that the majority of breast cancer apps were designed for tertiary prevention. We further observed that in studies of secondary and primary prevention, many apps provide information about guidelines for early detection of breast cancer for women identified as high risk. However, given that early onset breast cancer is increasing even in women without a family history of breast cancer, larger scale prevention interventions should be considered for additional populations that current risk models and screening strategies do not identify. We also found that apps could be adapted for studies across the cancer control continuum given that healthy behaviors recommended for primary and tertiary prevention overlap. Thus, in this rapidly growing field, while some gaps have been addressed, others gaps and implementation opportunities are emerging.

Research Gaps by Cancer Prevention Types

Tertiary Prevention Gaps

Given that breast cancer is the most commonly diagnosed cancer in women globally (88) and there are an estimated 3.5 million breast cancer survivors in the US alone (89), it makes sense that the majority of the apps were focused on clinical care

coordination and health related quality of life. The majority of the apps we identified for tertiary breast cancer prevention were patient- or survivor-oriented; therefore, they required adherence from the patient/survivor. While this could place a considerable burden on patients/survivors, the repeat and real-time evidence gleaned can be invaluable for patients/survivors in terms of self-management. Furthermore, a small proportion (16%) of studies using apps for tertiary cancer prevention were effectiveness studies. Given the rising rates of breast cancer incidence in low-middle income countries (90), more studies are needed to show the effectiveness of app use, especially in low resource settings.

Secondary Prevention Gaps

While a greater proportion of secondary prevention studies were at the effectiveness stage, we found mixed evidence that apps could modify breast cancer screening behaviors, especially among at-risk populations. Lee et al. showed that a mobile phone-app based intervention, in combination with health navigator services, could effectively improve breast cancer knowledge and readiness for mammography (75). Ginsberg et al. also explored the effectiveness of an app, with or without a health navigator service, to increase Bangladeshi women's adherence to attend a clinic-visit after an abnormal clinical breast examination; however, no significant results were found (74). Similarly, an app in conjunction with genetic clinical counseling did not change women's personal perception of risk (18). Effectiveness studies ought to assess if an app could deliver substantial gains in secondary breast cancer prevention outcomes (e.g., education, screening), alone or in combination with other services. Moreover, given early detection of breast cancer is associated with greater survival rates, effectiveness studies that assess outcomes for the implementation of innovative breast cancer screening/detection apps compared to standard of care, would be of great value. This is especially true for areas where there are barriers to mammography screening and/or timely point-of-care diagnostics.

Primary Prevention Gaps

The majority of primary prevention studies were aimed at improving the transfer of knowledge and adherence to existing cancer prevention guidelines among women at high risk for breast cancer; however, less research has been conducted with populations at average risk, or on modifiable risk factors to prevent breast cancer. Targeted prevention to high-risk populations is logical given that with limited resources and competing disease risk, resources should be allocated to those who will benefit most. However, if maintaining healthy weight, diet and physical activity can reduce cancer incidence by 26% (91), then apps can help promote sustainable, scalable behavioral change that reduces the risk for many additional chronic diseases (e.g., heart disease, diabetes) for women at average risk as well.

Global Implementation Implications

As of early 2019, there were over 5.1 billion mobile phone subscribers and this number is growing given the average annual percent increase of 2.9% (92). One could argue that the adoption of smartphone use is faster than the rate of an epidemic. With smartphones, individuals are readily, in real time, self-monitoring health behaviors. And leveraging this self-tracking for the implementation of breast cancer prevention is at our fingertips. Our review suggests that the use of apps for breast cancer prevention is far-reaching. The global rise in incidence rates of breast cancer coupled with a rapid uptake of mobile platforms creates unique prevention opportunities. That being said, it is unclear if the use of apps for breast cancer prevention will mitigate or create greater gaps in health disparities (93). While low to middle income countries have experienced rapid uptake of mobile platforms (94), in these emerging markets, the young, well-educated and higher-income individuals are more likely to use these mobile platforms (93). Thus, an unintended consequence is the creation of breast cancer health disparities in low resource settings; especially for secondary and tertiary prevention. But, thoughtful app developments and implementation of mHealth tools could lead to more inclusive rather than marginalized research (93).

Opportunities and Recommendations of Mobile App Use Across the Cancer Control Continuum

Given our review, we highlight the following opportunities and/or recommendations with regard to the use of apps across the breast cancer control continuum:

Research is needed to understand the effectiveness of mobile apps for breast cancer primary prevention in women at average risk, but especially in young women. The incidence of invasive breast cancer in young women (age 25–39 years) has risen in the US with an annual percent change of 2.7% for white non-Hispanic women and 3.1% for black non-Hispanic women from 1976 to 2009 (1). Moreover, while global incidence rates for young women under 50 years are similar, independent of country-level income, mortality rates are higher in women in low-middle income and low-income countries (95). Many behavioral risk factors for breast cancer are modifiable, so the potential impact of app technology for breast cancer prevention in young women is particularly powerful given that this age group has come of age with apps and they do not need to be taught or convinced of their usefulness (93).

Breast cancer apps should be readily available. Only about half of the apps in our review were publicly available in the Apple and/or Android app store. The majority of apps readily available for public use were health related apps; whereas, apps catering to secondary prevention (breast cancer screening/detection) and tertiary prevention (continuing cancer care) were not readily available. Even for primary prevention, Cohen et al. found that over 200 potential users from 68 countries outside of the US tried to access the SNAP for BRCA app, but potential users could not download the app as it required a study code (79). Without making developed apps readily available and usable, there is limited possibility of updating, adapting, validating, disseminating, or further testing the app for effectiveness in diverse populations and settings. Researchers should also take advantage of already available apps, especially popular ones (e.g., Fitbit, Headspace), as there is less upfront person time

and financial expenses compared to *de novo* app development. Popular apps carry the benefit of having a strong infrastructure given that software is routinely updated, designs are improved, and new features are added (82). However, an inherent limitation of readily available apps is that the speed of the research does not often advance at the speed of mobile app technology; therefore, researchers have limited control over app developments and the changes that may directly or indirectly impact the study.

Researchers should capitalize on the opportunity apps provide to collect information on exposures and outcomes of interest that have traditionally been difficult to measure. Not only does mobile app technology allow researchers to obtain repeat real-time data, mobile data measurement and collection reduces in-person study staff assistance, while not fully replacing study staff. Study staff will likely remain essential, especially for study implementation in low-middle income and hard to reach populations (84).

Limitations

This review is not without limitations. First, the advent of mobile apps is relatively recent and research in this area is rapidly changing. As a result, articles may have been missed that were not indexed with the search terms selected. To counteract this possibility, we broadened our search to include the full-text rather than just MeSH or keywords. Second, our review may also be missing studies that addressed breast cancer risk factors, such as obesity, but do not make an explicit reference to breast cancer. This likely deflated the number of articles identified as primary prevention; however, a more exhaustive review of all mobile apps being used for breast cancer risk factors was beyond the scope of this study. Finally, we included two databases in our search strategy, so gray literature and clinical trials with unpublished findings were not included.

Conclusions

The use of mobile apps for breast cancer prevention research is rapidly growing. Our systematic review suggests that while some gaps identified in previous reviews have already been addressed, new challenges have emerged. For mobile app interventions to have a global impact across the cancer control continuum, researchers will need to continue to invest in primary and secondary prevention research studies, as well as studies that are farther along in the research phase, in order to demonstrate the potential impact on outcomes relevant to breast cancer.

AUTHOR CONTRIBUTIONS

LH and JM conceptualized the study and all authors (LH, RH, JM) formulated the study design. RH managed the literature search and reviewed all articles and LH and JM independently reviewed a subset of articles. All authors drafted the initial manuscript, reviewed and revised the final manuscript for critical and important intellectual content, approved the final manuscript, and agree to be accountable for all aspects of the work.

FUNDING

The National Cancer Institute supported both JM (5 K01 CA186943) and LH (5 K07 CA218166).

ACKNOWLEDGMENTS

We would like to thank the organizers of the 8th International Breast Cancer and Nutrition Symposium for the excellent feedback that encouraged this review during our session on mobilizing breast cancer research through smartphone apps. We would also like to thank Ms. Eisha Nasar for generating the map included in this review.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2019.00298/full#supplementary-material

REFERENCES

1. Johnson RH, Chien FL, Bleyer A. Incidence of breast cancer with distant involvement among women in the United States, 1976 to 2009. *JAMA.* (2013) 309:800–5. doi: 10.1001/jama.2013.776
2. Sanson-Fisher RW, D'Este CA, Carey ML, Noble N, Paul CL. Evaluation of systems-oriented public health interventions: alternative research designs. *Annu Rev Public Health.* (2014) 35:9–27. doi: 10.1146/annurev-publhealth-032013-182445
3. Bender JL, Yue RYK, To MJ, Deacken L, Jadad AR. A lot of action, but not in the right direction: systematic review and content analysis of smartphone applications for the prevention, detection, and management of cancer. *J Med Internet Res.* (2013) 15:e287. doi: 10.2196/jmir.2661
4. Giunti G, Giunta DH, Guisado-Fernandez E, Bender JL, Fernandez-Luque L. A biopsy of breast cancer mobile applications: state of the practice review. *Int J Med Inform.* (2018) 110:1–9. doi: 10.1016/j.ijmedinf.2017.10.022
5. 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
6. Orsmond GI, Cohn ES. The distinctive features of a feasibility study: objectives and guiding questions. *OTJR (Thorofare NJ).* (2015) 35:169–77. doi: 10.1177/1539449215578649
7. Hwang H. Electronic wound monitoring after ambulatory breast cancer surgery: improving patient care and satisfaction using a smart phone app. *Br Col Med J.* (2016) 58:448–53.
8. Armstrong KA, Coyte PC, Brown M, Beber B, Semple JL. Effect of home monitoring via mobile app on the number of in-person visits following ambulatory surgery a randomized clinical trial. *JAMA Surg.* (2017) 152:622–7. doi: 10.1001/jamasurg.2017.0111
9. Ritvo P, Obadia M, Santa Mina D, Alibhai S, Sabiston C, Oh P, et al. Smartphone-enabled health coaching intervention (iMOVE) to promote long-term maintenance of physical activity in breast cancer survivors: protocol for a feasibility pilot randomized controlled trial. *JMIR Res Protoc.* (2017) 6:e165. doi: 10.2196/resprot.6615
10. Jiao L, Xu Z, Du W, Li H, Yin M. Fast preparation of polydopamine nanoparticles catalyzed by $Fe(2+)/H_2O_2$ for visible sensitive smartphone-enabled cytosensing. *ACS Appl Mater Interfaces.* (2017) 9:28339–45. doi: 10.1021/acsami.7b10564

11. Xiaosheng D, Xiangren Y, Shuyuan H, Dezong G, Mengyao C, Meng D. The effects of combined exercise intervention based on Internet and social media software for postoperative patients with breast cancer: study protocol for a randomized controlled trial. *Trials.* (2018) 19:477. doi: 10.1186/s13063-018-2857-3
12. Zhu JM, Ebert L, Liu XY, Wei D, Chan SWC. Mobile breast cancer e-support program for chinese women with breast cancer undergoing chemotherapy (part 2): multicenter randomized controlled trial. *JMIR Mhealth Uhealth.* (2018) 6:e104. doi: 10.2196/mhealth.9438
13. Drewes C, Kirkovits T, Schiltz D, Schinkoethe T, Haidinger R, Goldmann-Posch U, et al. EHealth acceptance and new media preferences for therapy assistance among breast cancer patients. *JMIR Cancer.* (2016) 2:e13. doi: 10.2196/cancer.5711
14. Egbring M, Far E, Roos M, Dietrich M, Brauchbar M, Kullak-Ublick GA, et al. A mobile app to stabilize daily functional activity of breast cancer patients in collaboration with the physician: a randomized controlled clinical trial. *J Med Internet Res.* (2016) 18:e238. doi: 10.2196/jmir.6414
15. El Shafie RA, Weber D, Bougatf N, Sprave T, Oetzel D, Huber PE, et al. Supportive care in radiotherapy based on a mobile app: prospective multicenter survey. *JMIR Mhealth Uhealth.* (2018) 6:e10916. doi: 10.2196/10916
16. O'Brien C, Kelly J, Lehane EA, Livingstone V, Cotter B, Butt A, et al. Validation and assessment of a technology familiarity score in patients attending a symptomatic breast clinic. *World J Surg.* (2015) 39:2441–9. doi: 10.1007/s00268-015-3134-1
17. Foley NM, O'Connell EP, Lehane EA, Livingstone V, Maher B, Kaimkhani S, et al. PATI: Patient accessed tailored information: a pilot study to evaluate the effect on preoperative breast cancer patients of information delivered via a mobile application. *Breast.* (2016) 30:54–8. doi: 10.1016/j.breast.2016.08.012
18. Keohane D, Lehane E, Rutherford E, Livingstone V, Kelly L, Kaimkhani S, et al. Can an educational application increase risk perception accuracy amongst patients attending a high-risk breast cancer clinic? *Breast.* (2017) 32:192–8. doi: 10.1016/j.breast.2017.02.009
19. Heo J, Chun M, Lee KY, Oh YT, Noh OK, Park RW. Effects of a smartphone application on breast self-examination: a feasibility study. *Healthc Inform Res.* (2013) 19:250–60. doi: 10.4258/hir.2013.19.4.250
20. Min YH, Lee JW, Shin YW, Jo MW, Sohn G, Lee JH, et al. Daily collection of self-reporting sleep disturbance data via a smartphone app in breast cancer patients receiving chemotherapy: a feasibility study. *J Med Internet Res.* (2014) 16:e135. doi: 10.2196/jmir.3421
21. Kim J, Lim S, Min YH, Shin YW, Lee B, Sohn G, et al. Depression screening using daily mental-health ratings from a smartphone application for breast cancer patients. *J Med Internet Res.* (2016) 18:e216. doi: 10.2196/jmir.5598
22. Uhm KE, Yoo JS, Chung SH, Lee JD, Lee I, Kim JI, et al. Effects of exercise intervention in breast cancer patients: is mobile health (mHealth) with pedometer more effective than conventional program using brochure? *Breast Cancer Res Treat.* (2017) 161:443–52. doi: 10.1007/s10549-016-4065-8
23. Kim HJ, Kim SM, Shin H, Jang JS, Kim YI, Han DH. A mobile game for patients with breast cancer for chemotherapy self-management and quality-of-life improvement: randomized controlled trial. *J Med Internet Res.* (2018) 20:e273. doi: 10.2196/jmir.9559
24. Park SW, Lee I, Kim JI, Park H, Lee JD, Uhm KE, et al. Factors associated with physical activity of breast cancer patients participating in exercise intervention. *Support Care Cancer.* (2019) 27:1747–54. doi: 10.1007/s00520-018-4427-3
25. Timmerman JG, Dekker-van Weering MGH, Tonis TM, Hermens HJ, Vollenbroek-Hutten MMR. Relationship between patterns of daily physical activity and fatigue in cancer survivors. *Eur J Oncol Nurs.* (2015) 19:162–8. doi: 10.1016/j.ejon.2014.09.005
26. Loef B, van Baarle D, van der Beek AJ, van Kerkhof LW, van de Langenberg D, Proper KI. Klokwerk plus study protocol: an observational study to the effects of night-shift work on body weight and infection susceptibility and the mechanisms underlying these health effects. *BMC Public Health.* (2016) 16:692. doi: 10.1186/s12889-016-3317-1
27. Young-Afat DA, van Gils CH, Bruinvels DJ, van der Pol CC, Witkamp AJ, Sijtsema S, et al. Patients' and health care providers' opinions on a supportive health app during breast cancer treatment: a qualitative evaluation. *JMIR Cancer.* (2016) 2:e8. doi: 10.2196/cancer.5334
28. Douma JAJ, Verheul HMW, Buffart LM. Feasibility, validity and reliability of objective smartphone measurements of physical activity and fitness in patients with cancer. *BMC Cancer.* (2018) 18:1052. doi: 10.1186/s12885-018-4983-4
29. Ormel HL, van der Schoot GGF, Westerink NDL, Sluiter WJ, Gietema JA, Walenkamp AME. Self-monitoring physical activity with a smartphone application in cancer patients: a randomized feasibility study (SMART-trial). *Support Care Cancer.* (2018) 26:3915–23. doi: 10.1007/s00520-018-4263-5
30. Lozano-Lozano M, Martin-Martin L, Galiano-Castillo N, Alvarez-Salvago F, Cantarero-Villanueva I, Fernandez-Lao C, et al. Integral strategy to supportive care in breast cancer survivors through occupational therapy and a m-health system: design of a randomized clinical trial. *BMC Med Inform Decis Mak.* (2016) 16:150. doi: 10.1186/s12911-016-0394-0
31. Paredes-Aracil E, Palazon-Bru A, Folgado-de la Rosa DM, Ots-Gutierrez JR, Compan-Rosique AF, Gil-Guillen VF. A scoring system to predict breast cancer mortality at 5 and 10 years. *Sci Rep.* (2017) 7:415. doi: 10.1038/s41598-017-00536-7
32. Weaver A, Love SB, Larsen M, Shanyinde M, Waters R, Grainger L, et al. A pilot study: dose adaptation of capecitabine using mobile phone toxicity monitoring–supporting patients in their homes. *Support Care Cancer.* (2014) 22:2677–85. doi: 10.1007/s00520-014-2224-1
33. Harder H, Holroyd P, Burkinshaw L, Watten P, Zammit C, Harris PR, et al. A user-centred approach to developing bWell, a mobile app for arm and shoulder exercises after breast cancer treatment. *J Cancer Surviv.* (2017) 11:732–42. doi: 10.1007/s11764-017-0630-3
34. Brett J, Boulton M, Watson E. Development of an e-health app to support women prescribed adjuvant endocrine therapy after treatment for breast cancer. *Patient Prefer Adher.* (2018) 12:2639–47. doi: 10.2147/ppa.s187692
35. Roberts AL, Potts HWW, Koutoukidis DA, Smith L, Fisher A. Breast, prostate, and colorectal cancer survivors' experiences of using publicly available physical activity mobile apps: qualitative study. *JMIR Mhealth Uhealth.* (2019) 7:e10918. doi: 10.2196/10918
36. Ainsworth MC, Pekmezi D, Bowles H, Ehlers D, McAuley E, Courneya KS, et al. Acceptability of a mobile phone app for measuring time use in breast cancer survivors (life in a day): mixed-methods study. *JMIR Cancer.* (2018) 4:e9. doi: 10.2196/cancer.8951
37. Akechi T, Yamaguchi T, Uchida M, Imai F, Momino K, Katsuki F, et al. Smartphone problem-solving and behavioural activation therapy to reduce fear of recurrence among patients with breast cancer (SMartphone Intervention to LEssen fear of cancer recurrence: SMILE project): protocol for a randomised controlled trial. *BMJ Open.* (2018) 8:e024794. doi: 10.1136/bmjopen-2018-024794
38. Ali EE, Leow JL, Chew L, Yap KY. Patients' perception of app-based educational and behavioural interventions for enhancing oral anticancer medication adherence. *J Cancer Educ.* (2018) 33:1306–13. doi: 10.1007/s13187-017-1248-x
39. Armstrong KA, Coyte PC, Bhatia RS, Semple JL. The effect of mobile app home monitoring on number of in-person visits following ambulatory surgery: protocol for a randomized controlled trial. *JMIR Res Protoc.* (2015) 4:e65. doi: 10.2196/resprot.4352
40. Banas JR, Victorson D, Gutierrez S, Cordero E, Guitleman J, Haas N. Developing a peer-to-peer mHealth application to connect hispanic cancer patients. *J Cancer Educ.* (2017) 32:158–65. doi: 10.1007/s13187-016-1066-6
41. Baseman J, Revere D, Baldwin LM. A mobile breast cancer survivorship care app: pilot study. *JMIR Cancer.* (2017) 3:e14. doi: 10.2196/cancer.8192
42. Buscemi J, Buitrago D, Iacobelli F, Penedo F, Maciel C, Guitleman J, et al. Feasibility of a smartphone-based pilot intervention for Hispanic breast cancer survivors: a brief report. *Transl Behav Med.* (2019) 9:638–45. doi: 10.1093/tbm/iby058
43. Iacobelli F, Adler RF, Buitrago D, Buscemi J, Corden ME, Perez-Tamayo A, et al. Designing an mHealth application to bridge health disparities in Latina breast cancer survivors: a community-supported design approach. *Design Health (Abingdon).* (2018) 2:58–76. doi: 10.1080/24735132.2018.1452871
44. Yanez BR, Buitrago D, Buscemi J, Iacobelli F, Adler RF, Corden ME, et al. Study design and protocol for My Guide: an e-health intervention to improve patient-centered outcomes among Hispanic breast cancer survivors. *Contemp Clin Trials.* (2018) 65:61–8. doi: 10.1016/j.cct.2017.11.018
45. Chalela P, Munoz E, Inupakutika D, Kaghyan S, Akopian D, Kaklamani V, et al. Improving adherence to endocrine hormonal therapy among breast cancer

patients: Study protocol for a randomized controlled trial. *Contemp Clin Trials Commun.* (2018) 12:109–15. doi: 10.1016/j.conctc.2018.10.001
46. Delrieu L, Perol O, Fervers B, Friedenreich C, Vallance J, Febvey-Combes O, et al. A personalized physical activity program with activity trackers and a mobile phone app for patients with metastatic breast cancer: protocol for a single-arm feasibility trial. *JMIR Res Protoc.* (2018) 7:e10487. doi: 10.2196/10487
47. Gehrke A, Lee SS, Hilton K, Ganster B, Trupp R, McCullough C, et al. Development of the cancer survivor profile-breast cancer (CSPro-BC) app: patient and nurse perspectives on a new navigation tool. *J Cancer Surviv.* (2018) 12:291–305. doi: 10.1007/s11764-017-0668-2
48. Klasnja P, Hartzler A, Powell C, Pratt W. Supporting cancer patients' unanchored health information management with mobile technology. *AMIA Annu Symp Proc.* (2011) 2011:732–41.
49. Klasnja P, Hartzler A, Powell C, Phan G, Pratt W. Health weaver mobile: designing a mobile tool for managing personal health information during cancer care. *AMIA Annu Symp Proc.* (2010) 2010:392–6.
50. Kubo A, Altschuler A, Kurtovich E, Hendlish S, Laurent CA, Kolevska T, et al. A pilot mobile-based mindfulness intervention for cancer patients and their informal caregivers. *Mindfulness.* (2018) 9:1885–94. doi: 10.1007/s12671-018-0931-2
51. Langer SL, Romano JM, Todd M, Strauman TJ, Keefe FJ, Syrjala KL, et al. Links between communication and relationship satisfaction among patients with cancer and their spouses: results of a fourteen-day smartphone-based ecological momentary assessment study. *Front Psychol.* (2018) 9:1843. doi: 10.3389/fpsyg.2018.01843
52. Langius-Eklof A, Crafoord MT, Christiansen M, Fjell M, Sundberg K. Effects of an interactive mHealth innovation for early detection of patient-reported symptom distress with focus on participatory care: protocol for a study based on prospective, randomised, controlled trials in patients with prostate and breast cancer. *BMC Cancer.* (2017) 17:466. doi: 10.1186/s12885-017-3450-y
53. Lloyd GR, Oza S, Kozey-Keadle S, Pellegrini CA, Conroy DE, Penedo FJ, et al. Breast cancer survivors' beliefs and preferences regarding technology-supported sedentary behavior reduction interventions. *AIMS Public Health.* (2016) 3:592–614. doi: 10.3934/publichealth.2016.3.592
54. Lozano-Lozano M, Galiano-Castillo N, Martin-Martin L, Pace-Bedetti N, Fernandez-Lao C, Arroyo-Morales M, et al. Monitoring energy balance in breast cancer survivors using a mobile app: reliability study. *JMIR Mhealth Uhealth.* (2018) 6:e67. doi: 10.2196/mhealth.9669
55. Lyons EJ, Baranowski T, Basen-Engquist KM, Lewis ZH, Swartil MC, Jennings K, et al. Testing the effects of narrative and play on physical activity among breast cancer survivors using mobile apps: study protocol for a randomized controlled trial. *BMC Cancer.* (2016) 16:202. doi: 10.1186/s12885-016-2244-y
56. McCarroll ML, Armbruster S, Pohle-Krauza RJ, Lyzen AM, Min S, Nash DW, et al. Feasibility of a lifestyle intervention for overweight/obese endometrial and breast cancer survivors using an interactive mobile application. *Gynecol Oncol.* (2015) 137:508–15. doi: 10.1016/j.ygyno.2014.12.025
57. Paredes-Aracil E, Palazon-Bru A, Folgado-de la Rosa DM, Ots-Gutierrez JR, Llorca-Ferrandiz C, Alonso-Hernandez S, et al. A scoring system to predict recurrence in breast cancer patients. *Surg Oncol.* (2018) 27:681–7. doi: 10.1016/j.suronc.2018.09.005
58. Lee HY, Lee MH, Gao Z, Sadak K. Development and evaluation of culturally and linguistically tailored mobile app to promote breast cancer screening. *J Clin Med.* (2018) 7:E181. doi: 10.3390/jcm7080181
59. Phillips SM, Collins LM, Penedo FJ, Courneya KS, Welch W, Cottrell A, et al. Optimization of a technology-supported physical activity intervention for breast cancer survivors: Fit2Thrive study protocol. *Contemp Clin Trials.* (2018) 66:9–19. doi: 10.1016/j.cct.2018.01.001
60. Pope Z, Lee JE, Zeng N, Lee HY, Gao Z. Feasibility of smartphone application and social media intervention on breast cancer survivors' health outcomes. *Transl Behav Med.* (2019) 9:11–22. doi: 10.1093/tbm/iby002
61. Quintiliani LM, Mann DM, Puputti M, Quinn E, Bowen DJ. Pilot and feasibility test of a mobile health-supported behavioral counseling intervention for weight management among breast cancer survivors. *JMIR Cancer.* (2016) 2:e4. doi: 10.2196/cancer.5305
62. Raghunathan NJ, Korenstein D, Li QS, Tonorezos ES, Mao JJ. Determinants of mobile technology use and smartphone application interest in cancer patients. *Cancer Med.* (2018) 7:5812–9. doi: 10.1002/cam4.1660
63. Rosen KD, Paniagua SM, Kazanis W, Jones S, Potter JS. Quality of life among women diagnosed with breast cancer: a randomized waitlist controlled trial of commercially available mobile app-delivered mindfulness training. *Psychooncology.* (2018) 27:2023–30. doi: 10.1002/pon.4764
64. Smith SA, Whitehead MS, Sheats JQ, Fontenot B, Alema-Mensah E, Ansa B. Formative research to develop a lifestyle application (app) for African American breast cancer survivors. *J Ga Public Health Assoc.* (2016) 6:50–9. doi: 10.21633/jgpha.6.103
65. Soto-Perez-De-Celis E, Kim H, Rojo-Castillo MP, Sun CL, Chavarri-Guerra Y, Navarrete-Reyes AP, et al. A pilot study of an accelerometer-equipped smartphone to monitor older adults with cancer receiving chemotherapy in Mexico. *J Geriatr Oncol.* (2018) 9:145–51. doi: 10.1016/j.jgo.2017.09.008
66. Stubbins R, He T, Yu X, Puppala M, Ezeana CF, Chen S, et al. A behavior-modification, clinical-grade mobile application to improve breast cancer survivors' accountability and health outcomes. *JCO Clin Cancer Inform.* (2018) 2:1–11. doi: 10.1200/cci.18.00054
67. Valle CG, Deal AM, Tate DF. Preventing weight gain in African American breast cancer survivors using smart scales and activity trackers: a randomized controlled pilot study. *J Cancer Surviv.* (2017) 11:133–48. doi: 10.1007/s11764-016-0571-2
68. Walker DK, Hardeman A, Owen L, Frank JS. Information at the point of care an informational application for cancer resources. *Cin Comput Inform Nurs.* (2015) 33:390–5. doi: 10.1097/cin.0000000000000171
69. Zhang X, Deng Z, Parvinzamir F, Dong F. MyHealthAvatar lifestyle management support for cancer patients. *Ecancermedicalscience.* (2018) 12:849. doi: 10.3332/ecancer.2018.849
70. Zhu J, Ebert L, Guo D, Yang S, Han Q, Chan SW. Mobile breast cancer e-support program for Chinese women with breast cancer undergoing chemotherapy (part 1): qualitative study of women's perceptions. *JMIR Mhealth Uhealth.* (2018) 6:e85. doi: 10.2196/mhealth.9311
71. Zhu J, Ebert L, Liu X, Chan SW. A mobile application of breast cancer e-support program versus routine care in the treatment of Chinese women with breast cancer undergoing chemotherapy: study protocol for a randomized controlled trial. *BMC Cancer.* (2017) 17:291. doi: 10.1186/s12885-017-3276-7
72. Cardos RAI, Soflau R, Gherman A, Sucala M, Chiorean A. A mobile intervention for core needle biopsy related pain and anxiety: a usability study. *J Evid Based Psychother.* (2017) 17:21–30. doi: 10.24193/jebp.2017.1.2
73. Eden KB, Scariati P, Klein K, Watson L, Remiker M, Hribar M, et al. Mammography decision aid reduces decisional conflict for women in their forties considering screening. *J Womens Health (Larchmt).* (2015) 24:1013–20. doi: 10.1089/jwh.2015.5256
74. Ginsburg OM, Chowdhury M, Wu W, Chowdhury M, Pal BC, Hasan R, et al. An mHealth model to increase clinic attendance for breast symptoms in rural Bangladesh: can bridging the digital divide help close the cancer divide? *Oncologist.* (2014) 19:177–85. doi: 10.1634/theoncologist.2013-0314
75. Lee H, Ghebre R, Le C, Jang YJ, Sharratt M, Yee D. Mobile phone multilevel and multimedia messaging intervention for breast cancer screening: pilot randomized controlled trial. *JMIR Mhealth Uhealth.* (2017) 5:e154. doi: 10.2196/mhealth.7091
76. Tewary S, Arun I, Ahmed R, Chatterjee S, Chakraborty C. SmartIHC-Analyzer: smartphone assisted microscopic image analytics for automated Ki-67 quantification in breast cancer evaluation. *Anal Methods.* (2017) 9:6161–70. doi: 10.1039/c7ay02302b
77. Alanzi TM, Alobrah A, Alhumaidi R, Aloraifi S. Evaluation of the SnapChat mobile social networking application for breast cancer awareness among Saudi students in the Dammam Region of the Kingdom of Saudi Arabia. *Breast Cancer.* (2018) 10:113–9. doi: 10.2147/bctt.s166135
78. Businelle MS, Ma P, Kendzor DE, Frank SG, Wetter DW, Vidrine DJ. Using intensive longitudinal data collected via mobile phone to detect imminent lapse in smokers undergoing a scheduled quit attempt. *J Med Internet Res.* (2016) 18:e275. doi: 10.2196/jmir.6307
79. Cohen SA, Scherr CL, Nixon DM. An iPhone application intervention to promote surveillance among women with a BRCA mutation: pre-intervention data. *J Genet Couns.* (2018) 27:446–56. doi: 10.1007/s10897-018-0224-x

80. Scherr CL, Feuston JL, Nixon DM, Cohen SA. A two-phase approach to developing SNAP: an iPhone application to support appointment scheduling and management for women with a BRCA mutation. *J Genet Couns.* (2018) 27:439–45. doi: 10.1007/s10897-018-0222-z
81. Coughlin SS, Besenyi GM, Bowen D, De Leo G. Development of the Physical activity and Your Nutrition for Cancer (PYNC) smartphone app for preventing breast cancer in women. *Mhealth.* (2017) 3:5. doi: 10.21037/mhealth.2017.02.02
82. Hartman SJ, Nelson SH, Cadmus-Bertram LA, Patterson RE, Parker BA, Pierce JP. Technology- and phone-based weight loss intervention: pilot RCT in women at elevated breast cancer risk. *Am J Prev Med.* (2016) 51:714–21. doi: 10.1016/j.amepre.2016.06.024
83. Kratzke C, Amatya A, Vilchis H. Differences among college women for breast cancer prevention acquired information-seeking, desired apps and texts, and daughter-initiated information to mothers. *J Community Health.* (2014) 39:291–300. doi: 10.1007/s10900-013-9759-9
84. Bravo C, O'Donoghue C, Kaplan CP, Luce J, Ozanne E. Can mHealth improve risk assessment in underserved populations? Acceptability of a breast health questionnaire app in ethnically diverse, older, low-income women. *J Health Dispar Res Pract.* (2014) 7:6.
85. Smith SA, Whitehead MS, Sheats J, Mastromonico J, Yoo W, Coughlin SS. A community-engaged approach to developing a mobile cancer prevention app: the mCPA study protocol. *JMIR Res Protoc.* (2016) 5:e34. doi: 10.2196/resprot.5290
86. Coughlin SS, Thind H, Liu B, Wilson LC. Towards research-tested smartphone applications for preventing breast cancer. *Mhealth*. (2016) 2:26. doi: 10.21037/mhealth.2016.06.02
87. Davis SW, Oakley-Girvan I. mHealth education applications along the cancer continuum. *J Cancer Educ.* (2015) 30:388–94. doi: 10.1007/s13187-014-0761-4
88. World Health Organization. *Global Status Report on Noncommunicable Diseases 2014*. Geneva: World Health Organization (2014).
89. American Cancer Society. *Cancer Treatment & Survivorship: Facts & Figures 2016–2017*. Atlanta, GA: American Cancer Society (2016).
90. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends–an update. *Cancer Epidemiol Biomarkers Prev.* (2016) 25:16–27. doi: 10.1158/1055-9965.epi-15-0578
91. Colditz GA, Bohlke K. Priorities for the primary prevention of breast cancer. *CA Cancer J Clin.* (2014) 64:186–94. doi: 10.3322/caac.21225
92. The GSMA Corporate Website. *GSMA*. (2019). Available online at: https://www.gsma.com/ (accessed August 27, 2019).
93. Taylor K, Silver L. *Smartphone Ownership Is Growing Rapidly Around the World, but Not Always Equally*. (2019). Available online at: https://www.pewresearch.org/global/2019/02/05/smartphone-ownership-is-growing-rapidly-around-the-world-but-not-always-equally/ (accessed February 28, 2019).
94. 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.* (2015) 24:1495–506. doi: 10.1158/1055-9965.epi-15-0535
95. Bellanger M, Zeinomar N, Tehranifar P, Terry MB. Are global breast cancer incidence and mortality patterns related to country-specific economic development and prevention strategies? *J Glob Oncol.* (2018) 4:1–16. doi: 10.1200/jgo.17.00207

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 in Public Health

MINI REVIEW
published: 08 November 2019
doi: 10.3389/fpubh.2019.00316

Nutrition in the Prevention of Breast Cancer: A Middle Eastern Perspective

*Farah Naja, Lara Nasreddine, Sara Awada, Raeda El Sayed Ahmad and Nahla Hwalla**

Department of Nutrition and Food Science, Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon

This paper reviews the escalating burden of breast cancer (BC) in the Middle East (ME) and the prevalence of modifiable risk factors and underscores opportunities to promote the prevention of the disease. Similar to more developed countries, BC is the most frequent cancer among women in countries of the ME, accounting for one-third of total cancer cases and 24% of total cancer deaths. Average age at BC diagnosis appears to be a decade earlier in Middle Eastern countries compared to the Western countries, and its incidence is predicted to further increase. Although incidence rates of BC are still lower in Middle Eastern countries than Western ones, mortality rates are similar and at times even higher. It is estimated that 30% of BC cases are due to environmental and lifestyle factors, such as obesity and diet and hence can be preventable. The ME suffers from surging rates of obesity, with eight of its countries ranking among the highest worldwide in obesity prevalence among adults aged 18 and above. ME countries with the highest prevalence of obesity that are among the top 20 worldwide include United Arab Emirates (UAE), Lebanon, Egypt, Libya, Qatar, Saudi Arabia, Jordan, and Kuwait with rates ranging from 30% in UAE to 37% in Kuwait. In parallel, studies in the ME have consistently showed a shift in dietary intake whereby traditional diets, rich in fruits and vegetables, are progressively eroding and being replaced by westernized diets high in energy and fat. Accumulating evidence is reporting convincing association between consumption of such westernized diets and higher BC risk. Addressing these risk factors and studying their association with BC in terms of their nature and magnitude in Middle Eastern countries could provide the basis for intervention strategies to lower the risk and alleviate the burden of BC in these countries.

Keywords: breast cancer, diet, risk factors, prevention, obesity, Middle East and North Africa region

OPEN ACCESS

Edited by:
Alessio Molfino,
Sapienza University of Rome, Italy

Reviewed by:
Eron Grant Manusov,
University of Texas Rio Grande Valley Brownsville, United States
Reema Fayez Tayyem,
University of Jordan, Jordan

****Correspondence:***
Nahla Hwalla
nahla@aub.edu.lb

Specialty section:
This article was submitted to Family Medicine and Primary Care, a section of the journal Frontiers in Public Health

Received: *09 April 2019*
Accepted: *14 October 2019*
Published: *08 November 2019*

Citation:
Naja F, Nasreddine L, Awada S, El Sayed Ahmad R and Hwalla N (2019) Nutrition in the Prevention of Breast Cancer: A Middle Eastern Perspective. Front. Public Health 7:316. doi: 10.3389/fpubh.2019.00316

INTRODUCTION

Globally, the most common cancer among women is breast cancer (BC), representing about 25% of all cancers. BC incidence rates vary widely across the world, from 25 per 100,000 in Middle Africa and Eastern Asia to 92 per 100,000 in Western Europe. Incident cases are estimated to increase worldwide by 46.5% by the year 2040 (1).

In the Middle East (ME), the age-standardized incidence rate (ASR) of BC is 45.3 per 100,000 females and is substantially increasing with predictions to reach Western levels (1). According to the World Health Organization (WHO) estimates, BC rates across the ME are expected to double

between 2012 and 2030, which is the highest relative increase of any region globally (2). Although ASR of BC per 100,000 in the ME is lower than that of Europe and the US (45.3 vs. 80.1 and 84.8, respectively), it has a similar mortality rate compared to these countries (13.6 vs. 12.6 and 14.1, respectively) (1). It is noteworthy that, in Middle Eastern countries, the incidence of BC occurs in women at an average age of diagnosis of <50 years, which is around 10 years before it appears in industrialized countries (3, 4). As shown in **Table 1**, limited available data from the Middle East and North Africa (MENA) region shows an increase in ASR of BC per 100,000 females. For example, in Lebanon, over a period of 12 years (1996–2008), ASR of BC has more than quadrupled, from 20 to 95.7. Also, in Jordan between the years 1982 and 2008, ASR increased by more than 6-fold, from 7.6 to 50.4. This illustrates the increasing trajectory of BC in this region (5, 6). It should be cautioned that the reported increases in the incidence of BC in the ME over the last decade may be attributed, in part, to the increase in number of cancer registries, as well as to the wide adoption of mammographic screening programs, an effort supported by several awareness campaigns since 2004 (1, 7, 8).

Prevention strategies have been assessed globally showing that a minimum of 1.3 million cancer deaths and 30% of all cancer cases can be avoided yearly if healthy living and adequate working environments were sustained (9). The WCRF/AICR specified a few environmental and lifestyle factors that showed compelling evidence for their implication in the onset of BC, namely, smoking, diet, obesity, alcohol, sun exposure, physical activity, stress, pollution, and infections (10). Among these factors, obesity and the shift in dietary intake patterns are perceived as important modifiable risk factors of BC. In fact, the increase in BC incidence in Middle Eastern countries was concomitant with the escalating rates of obesity and the shift in dietary patterns (1, 11).

Thus, exploring the underlying factors that are associated with the increased risk of BC in the ME provides a foundation for intervention strategies to mitigate the risk of this cancer in the region. This mini-review examines the escalating burden of BC in the ME and the prevalence of modifiable risk factors and underscores the opportunities to promote prevention of this disease. A total of 71 articles for this minireview were collected from the two search engines PubMed and Google Scholar. Publications in English were selected and search terms included breast cancer, diet/nutrition, and breast cancer, risk factors of breast cancer, prevention of breast cancer, obesity and breast cancer, and breast cancer in the Middle East and North Africa (MENA) region.

OBESITY AND BC

Among the modifiable risk factors strongly associated with BC is weight gain. In a meta-analysis by Cheraghi et al., the effect of obesity and overweight on BC in pre- and post-menopausal periods was examined through 15 cohort studies and 35 case–control studies (12). The results revealed that the BC's incidence increased by 14% among overweight and obese women in

TABLE 1 | Breast cancer age-standardized incidence rates ASR (World Population) per 100,000 females in the MENA countries over time.

Breast cancer	Lebanon	Jordan	Saudi Arabia	Oman	Algeria	Morocco	Egypt*	Israeli Jewish	Israeli Arabs
ASR per 100,000	20 (1996)	7.6 (1982)	–	–	–	–	–	–	–
	69 (2003)	32.8 (1997)	–	–	–	–	–	–	–
	71 (2004)	41.4 (2005)	15.4 (2004)	21.9 (2006)	23.5 (2002)	35 (2004)	49.6 (2001)	–	42.6 (2002)
	95.7 (2008)	50.4 (2008)	22.7 (2008)	21.6 (2008)	65.1 (2007)	36.4 (2005–2007)	Ranges from 35.7 to 63.9 (2009)	94.3 (2008)	56.2 (2008)

Adapted from Lakkis et al. (5).
Lebanese Ministry of Public Health-National Cancer Registry, May 2009.
Abulkhair et al. (6).
**ASRs of Egypt (Aswan), Egypt (Damietta), and Egypt (Minia) are 63.9, 41.4, and 35.7, respectively.*

the post-menopausal stage whereas body mass index (BMI) did not have a significant effect on BC's incidence during the premenopausal stage (12). Similar to these findings, another meta-analysis summarized the results of 9 cohort studies and 22 case–control studies and showed that with every 5 units increase in BMI, BC's risk among postmenopausal women increases by 33% and decreases by 10% among premenopausal women (13). Building on available evidence, the continuous update project (CUP) panel graded the evidence regarding the association between BC and increased body fatness and weight gain as convincing for post-menopausal women whereas the protective effect of body fat against BC in pre-menopausal was graded as probable (14). It is therefore suggested that the risk of BC could be mediated by the menopausal state. More recent data on the distinctive effect of body fat on BC revealed a 12% increase in BC risk among overweight postmenopausal women, which further increased to 25% in obese postmenopausal women (15). High levels of body fat were also associated with an increase in BC risk among postmenopausal women with normal BMI (16, 17).

In several MENA countries, the prevalence of overweight and obesity are at alarmingly high levels where 66–75% of the adult population in the Gulf countries are estimated to be overweight and obese (1, 18). Compared to worldwide figures, eight Middle Eastern countries are among the top 20 countries with the highest prevalence of obesity. These include United Arab Emirates (UAE), Lebanon, Egypt, Libya, Qatar, Saudi Arabia, Jordan, and Kuwait with rates ranging from 30% in UAE to 37% in Kuwait, the latter being among the top 10 countries with highest obesity rates worldwide (19). More specifically, the MENA countries have one of the highest rates of female obesity prevalence on earth and have experienced more rapid increase in incidence of obesity than the developed countries between the years 1990 and 2016 (20). The percent increase in obesity in males and females in 26 years were 170 and 81% in MENA countries as compared to 122 and 75.5%, respectively, in the world. Examining the percent contribution of obesity to BC, data show that the percentages of post-menopausal BC cases attributable to excess BMI among females ranges between 15.2% in Lebanon and 18.5% in Kuwait (**Table 2**) (1).

Several mechanisms were reported, in the literature, attempting to explain the relationship between obesity and BC. It was proposed that the insulin resistance of obesity is linked to metabolic abnormalities that may lead to a decrease in insulin-like growth factor binding protein 1 and insulin-like growth factor binding protein 2, which, in turn, increases the bioavailability of insulin-like growth factor 1 hence, promoting cellular proliferation and inhibiting apoptosis. These events could promote tumorigenesis (21).

Other possible mechanisms supporting the relationship between body fatness and BC are related to increased adiposity. The adipose tissue is an active metabolic organ, with excess adiposity associated with endocrine and metabolic characteristics, altered adipokines (higher leptin and lower adiponectin levels), inflammation, and higher estrogen levels, all of which may inhibit apoptosis and promote tumorigenesis. Many of these factors have been studied and shown to have a link with increased risk of BC, notably in postmenopausal women (22).

TABLE 2 | Percentages of all post-menopausal breast cancer cases among females worldwide in 2012 attributable to excess body mass index, by country from highest to lowest.

Rank	Country	Percentage (%)
1	Samoa	20.2
2	Kuwait	18.5
3	Jordan	18.1
4	Saudi Arabia	17.3
5	United Arab Emirates	17.3
6	Libya	17.1
7	West Bank and Gaza	17.1
8	Puerto Rico	17.1
9	Egypt	16.9
10	Syria	16.4
11	South Africa	16.3
12	Turkey	16.2
13	Bahamas	16.1
14	Qatar	15.6
15	Fiji	15.4
16	Barbados	15.3
17	Lebanon	15.2
18	Belize	15.2

Adapted from Bray et al. (1), IARC World Health Organization, http://gco.iarc.fr/causes/obesity/tools-map.

GENETIC PREDISPOSITION AND BC

BRCA1/2 mutation is a known hereditary risk factor for BC, whereby in Western populations, this mutation confers a lifetime risk of BC of up to 80%, with up to 40% of carriers developing BC by the age of 50 (23). A systematic review of studies examining BRCA1 mutation in the MENA concluded that this mutation is rather frequent in this part of the world and that each region within the MENA appears to have unique mutations (7, 24–31). The authors of this systematic review recommended the development of a mutation database, by each region, for BC screening. National data on BRCA1 mutations may be targeted for this screening to get the best estimation of this cancer-promoting mutation (32). For example, in 2019, and in line with the latter recommendation, the BRCA1 c.131G mutation was considered a founder mutation in the Lebanese population as it was detected among 23% of individuals diagnosed with BRCA mutation, and in Turkey, the positivity prevalence of BRCA1/2 mutation was 19% in high-risk BC patients (31, 33).

DIETARY PATTERNS AND BC

Diet quality has been reported as another modifiable BC risk factor. It is estimated that almost one-third of the BC cases can be prevented through dietary modifications (14, 18, 34, 35).

Meta-analysis studies on dietary patterns and BC revealed that, of three different patterns studied in both developed and developing countries, the prudent diet, which is a diet rich in fruits, vegetables, legumes, poultry, fish, whole grains, and low-fat dairy, had a protective effect on BC with an 11% decreased risk. Alternatively, the Western/unhealthy dietary pattern and the drinker dietary pattern had detrimental effects on BC as they were respectively associated with a 9 and 21% increased risk of BC (34). More specifically, unhealthy dietary patterns, such as those high in sugar, trans fats, refined carbohydrates, and alcohol along with low intake of fibers, antioxidants, and omega 3 fatty acids were shown to increase the risk of BC (15). Similarly, a systematic review of 17 case–control studies identified that dietary patterns that include vegetables, fruits, lean protein, grains, and legumes may reduce the risk of BC, whereas dietary patterns that include high saturated fats, fried foods, sugars, refined grains, and processed meats may increase the risk of BC (36). Also, a 10% increase in ultra-processed foods, such as packaged goods, sugary cereals, and ready meals was found to increase the risk of BC by 12% (37).

Over the last decade, the MENA region was reported as undergoing a shift in the dietary patterns from the traditional healthy Mediterranean type diet to a more westernized diet rich in energy and fat. The diet is becoming energy-dense, sweet, high in fat and processed foods, and low in fiber, cereals, fruits, and vegetables (38). The results of a case–control study from the Kingdom of Saudi Arabia (KSA) suggested a positive association between fats intake, protein, and calories and BC risk (39). This could be associated with the increase in BC incidence among women in these countries. In light of the protective association between the traditional diet and BC risk, increased efforts are needed to promote shifting the dietary patterns to the traditional healthy Mediterranean diet of this region (18).

ALCOHOL AND BC

Alcohol is considered as a promoting factor of human carcinogenesis. It is a well-established modifiable risk factor for BC, being significantly associated with post-menopausal BC and accounting for 5% of worldwide BC deaths (10, 15, 40).

CUP identified four published pooled analyses on the risk of pre- and post-menopausal BC and consumption of alcohol. The results showed that the evidence was consistent, and the increased BC risk remained significant in all studies. In this context, CUP also identified 22 studies that were included in the dose-response meta-analysis, whose results showed a 9% increased risk of BC in the post-menopausal state per 10-g (equivalent to 330 ml of beer and 100 ml of wine) increase in alcohol consumed per day. Hence, CUP graded the evidence for the association between consumption of alcoholic drinks and BC as convincing in postmenopausal women and as probable in pre-menopausal ones CUP, 2018 (14).

Research about the association between alcohol consumption and BC in the ME has been hampered by societal and religious traditions. It was reported that the consumption of alcohol among Middle Eastern women is not viewed as a major problem due to low consumption, and hence it may not be substantially contributing to the rise of BC incidence and deaths in these countries (18).

Many possible mechanisms speculating on the association between alcohol and BC were reported, mainly suggesting that enzymatic degradation of alcohol is linked with a change in the proportions of the two forms of the coenzyme nicotinamide adenine dinucleotide (NAD). The accumulation of its reduced form, nicotinamide-adenine dinucleotide- hydrogen (NADH), means that the breakdown of estradiol to estrone is less favored and estradiol accumulates, hence increasing the rate of aromatization of testosterone to estradiol. The binding of estrogens to its nuclear receptor (ERα) initiates a complex intracellular signal sequence, finally stimulating cell proliferation and cancer (41).

RED AND PROCESSED MEAT AND BC

High intake of red and processed meats was reported to be associated with increased risk of BC. The Women's cohort study in the UK, the NIH-AARP Diet and Health Study, and the Nashville Breast Health study showed that there was an increased risk of BC in both pre- and post-menopausal women who had high consumption of red meat (15). Another prospective cohort study showed that increased consumption of red and processed meat among adolescent females was linked to increased risk of premenopausal BC (18). A meta-analysis of 14 prospective studies on red meats and 12 prospective studies on processed meats indicated that there is a 10% increased risk of BC due to high intake of red meats (120 g/day) and an 8% increased risk due to high intake of processed meats (50 g/day) (42). A case–control study from Iran suggests that consuming red meat is associated with increased risk of BC (43). Previous reports by the WCRF/AICR 2007 stated that the safe intake level of cooked red meat should not exceed 500 g/week (equivalent to 71.4 g/day) and the intake of processed meats should be avoided. Middle Eastern countries have high intakes of red and processed meats, and most of these countries surpass the recommended levels where, for example, the consumption of processed meats in UAE, Algeria, Kuwait, and Lebanon was estimated to be 47, 17.5, 42, and 32 g/week, respectively; as for red meats, it was reported to be 700, 707, 700, and 400 g/week, respectively (44).

CUP graded the evidence of the link between high intake of red and processed meats and increased risk of BC as limited in both pre- and post-menopausal women, which calls for further studies to understand these potential associations. Among the possible reported explanations for the link between meat and BC are the high-fat intake associated with consuming fatty meat, and polycyclic aromatic hydrocarbons and heterocyclic amines formed during meat cooking, which are considered human carcinogens (18, 45, 46).

FRUITS, VEGETABLES, AND BC

Several studies documented the high intake of fruits and vegetables as protective against BC in women (47, 48).

A meta-analysis of 14 cohort studies and 1 case–control study indicated that a high intake of fruits and vegetables combined (>400 g/day for fruits and >300 g/day for vegetables), but not vegetables alone, is associated with an 11% decrease in BC risk (49). Similarly, a meta-analysis of 11 case–control studies and 2 cohort studies showed that a high intake of cruciferous vegetables is significantly linked to a 15% reduction in BC risk. In this meta-analysis, cruciferous vegetables were referred to arugula, broccoli, Brussels sprouts, bok choy, cabbage, canola, cauliflower, collard greens, daikon, horseradish, kale, kohlrabi, mustard, radish, rutabaga, wasabi, and watercress (50). Based on these studies, it was suggested that the intake of cruciferous vegetables and fruits have a protective effect on BC in pre- and post-menopausal women. Studies on food consumption in many countries of the ME and in 22 Arab countries showed a low intake of fruits and vegetables among adults in this region, which is less than the recommended daily intake (above 400 g) among females of all age groups (51). The lowest intakes of fruits and vegetables were seen in Libya (fruits 60.4 g/day and vegetables 134 g/day), Algeria, Yemen, Iran, and Iraq (less than the optimal intake, which is 400 ± 30 g/day) (18, 44).

Several mechanisms may explain the protective effect of fruits and vegetables against BC. Fruits and vegetables are good sources of fiber, which may bind to estrogens, inhibiting the process of enterohepatic reabsorption of estrogen (52). Fruits and vegetables are also very good sources of various antioxidants including glucosinolates, carotenoids, indoles, and isothiocyanates, which can help prevent BC by inducing detoxifying enzymes and decreasing oxidative stress and inflammation (49, 53). More studies in the MENA region are needed to investigate the link between fruits and vegetables' intakes and BC risk in women.

FISH, MARINE N-3 POLYUNSATURATED FATTY ACIDS, AND BC

Fish rich in omega-3 polyunsaturated fatty acids (n-3 PUFA) were reported as being associated with a decreased risk of BC among females. A meta-analysis of five cohort and six prospective case–control studies indicated that there was a 6% reduction in BC risk in the study populations from the United States, Europe, and Asia following a 1/10 increment of n-3/n-6 ratio in the diet (54). Similarly, a meta-analysis of 21 prospective cohort studies showed that a higher intake of dietary marine n-3 PUFA was associated with a lower risk of BC. The risk was decreased by 5% following 0.1 g/day increase in the intake of dietary marine n-3 PUFA (55).

Studies investigating food consumption patterns in countries of the ME have reported low intakes of marine n-3 fats (less than the optimal recommended level by the Academy of Nutrition and Dietetics of 500 mg/day of EPA and DHA of which at least 220 mg should consist of EPA). The lowest intakes were seen in Lebanon Palestine, Syria, Algeria, Iraq, Qatar, Jordan, and Oman (44).

Reports proposing mechanisms by which n-3 PUFA could influence BC risk suggested eicosanoids, n-3 PUFA metabolites, as modulators of cellular processes either by interacting with receptors or by altering signaling pathways. This may result in downregulating the inflammatory cascade, enhancing fatty acid (FA) degradation in association with lowering FA synthesis, and lowering the expression of markers ultimately increasing cell death (56).

FIBER AND BC

Diets rich in fiber were reported to be linked to a reduced BC risk. A meta-analysis of 16 prospective studies indicated that there is an inverse association between the intake of dietary fiber and risk of BC (5% reduction) (57).

Middle Eastern populations, especially Turkey, Egypt, Kuwait, Jordan, Yemen, and UAE, have low intakes of whole grains less than the optimal level of 50 g/day, which constitutes the main source of dietary fibers (44, 58). Other studies targeting specific types of fiber and the BC risk among pre- and post-menopausal women were reported as needed to clarify the mechanisms behind the positive effect of dietary fiber on BC (59). Several mechanisms of action of fiber in protection from BC were proposed in the literature; one mechanism is related to decreasing circulating estrogen levels and increasing fecal excretion of estrogen; hence, the binding of estrogen to its nuclear receptor ERα is hindered, and accordingly, cell multiplication is decreased (57). Another mechanism is the binding of fibers to bile acids, which are suggested to advance cell proliferation, thus allowing decreased chance for mutations and decreasing cancer risk (60). Fermentation of fibers produces butyrate, a short-chain fatty acid, which has been shown to have antineoplastic effects (61).

CARBOHYDRATES AND BC

The association between carbohydrates and BC is unclear. A meta-analysis of 10 prospective cohort studies showed that high dietary glycemic index (GI) is significantly associated with an 8% increased risk of BC, and high dietary glycemic load (GL) is associated with a 3% increased risk of BC (62). Given the limited number of eligible studies to support the association of GI and BC in all countries, including Middle Eastern ones, more studies are needed to examine this association. Nevertheless, reducing the intake of high GI foods, notably refined carbohydrates, in the general population may perhaps offer a benefit in preventing BC (62). Speculating on possible mechanisms regarding the relationship between carbohydrates and BC, the literature suggests that high insulinemia, in response to high glycemic index diets, may inhibit apoptosis and synthesis of IGF1-1 binding proteins 1 and 2, which promote cellular multiplication (63).

VITAMIN D AND BC

A meta-analysis of two randomized clinical trials and one prospective cohort showed that women with 25(OH) D concentration of ≥60 ng/ml had an 80% lower BC incidence rate than women with concentration <20 ng/ml (64). Also, another meta-analysis of 14 case–control studies indicated that

serum 25(OH)D concentration was inversely and significantly associated with 16% decreased BC risk (65). Similarly, a case–control study from KSA in the ME showed an inverse association between serum 25(OH) D, the active form of vitamin D, and the risk of BC in Saudi women (66). However, several other studies have shown no association between dietary and supplemental vitamin D and BC (67–69).

The level of 25(OH) D is considered deficient if it is <25 nmol/L, insufficient if it is between 25 and 49 nmol/L (<20 ng/ml), and inadequate if it is between 50 and 74 nmol/L (70). Studies in the ME showed that the highest prevalence of vitamin D deficiency was found among women (6). For instance, 81% of adolescent girls in Saudi Arabia and 62% in Qatar have vitamin D deficiency (<12 ng/ml). As for adult women, 37% in Jordan and 51% in Iran have vitamin D insufficiency (<20 ng/ml) (71). Altogether, the evidence that 25(OH) D decreases the risk of BC is labeled by CUP as probable (14).

The mechanism by which vitamin D can affect BC has been speculated in the literature, stating that the biologically active form of vitamin D binds to the vitamin D receptor in normal breast epithelium and this complex regulates the cell cycle, promotes differentiation, increases cell-to-cell adhesion, protects cells from DNA damage, regulates cytokines, activates immune cells, and suppresses inflammation, thus reducing malignant transformations (72).

CONCLUSION

In summary, there is sufficient research to suggest an association between obesity and nutrition with BC globally and regionally. In the ME, the rise in the rates of new BC cases, especially among younger women, coupled to the alarming levels of obesity and the shift in dietary patterns toward westernized diet call for action in all countries and at all levels of the society. Policies, strategies, and public health efforts to reduce obesity and promote a healthy lifestyle with emphasis on the prudent diet are needed. It remains important to note that such public health interventions are hampered by the scarcity of research and data that provide a local, context-specific, and culturally adaptable evidence base. The evidence presented in this paper points toward ethnic and context-specific associations between BC and the reported risk factors. This may trigger systematic and well-designed studies in the ME to affirm all these associations, assess the genetic predisposition to BC, and provide data for region-specific evidence-based recommendations for the prevention of BC.

STRENGTHS AND LIMITATIONS

A few limitations ought to be considered when interpreting the findings of this minireview. First, the associations of BC with obesity and nutrition are complex, especially that BC is a disease with a multifactorial and complex etiology of genetics as well as environmental factors. Second, there is a paucity of region-specific studies investigating the association between diet, lifestyle, and BC; hence, most associations of risk factors with BC were conducted in Western countries. Moreover, despite our efforts to include all relevant meta-analyses on BC in MENA, the potential of selection bias could not be ruled out.

AUTHOR CONTRIBUTIONS

NH conceptualized the content of the chapter and acted as lead author of the manuscript. FN and LN provided critical review of the manuscript and participated in the write up. SA conducted the literature search and contributed to the write up of the chapter. RE contributed to the editing and the write up of the chapter.

ACKNOWLEDGMENTS

Publication fees are covered by International Breast Cancer & Nutrition (IBCN) at Purdue University, USA.

REFERENCES

1. 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* (2018) 68:394–424. doi: 10.3322/caac.21492
2. Ginsburg O, Bray F, Coleman MP, Vanderpuye V, Eniu A, Kotha SR, et al. The global burden of women's cancers: a grand challenge in global health. *Lancet*. (2017) 389:847–60. doi: 10.1016/S0140-6736(16)31392-7
3. Najjar H, Easson A. Age at diagnosis of breast cancer in Arab nations. *Int J Surg*. (2010) 8:448–52. doi: 10.1016/j.ijsu.2010.05.012
4. Chouchane L, Boussen H, Sastry KSR. Breast cancer in Arab populations: molecular characteristics and disease management implications. *Lancet Oncol*. (2013) 14:e417–24. doi: 10.1016/S1470-2045(13)70165-7
5. Lakkis NA, Adib SM, Hamadeh G, El Jarrah R, Osman MH. Sociological transition and breast cancer in the Arab world: the experience of Lebanon. *Asian Pac J Cancer Prev*. (2017) 18:1357–64. doi: 10.22034/APJCP.2017.18.5.1357
6. Abulkhair O, Saghir N, Sedky L, Saadedin A, Elzahwary H, Siddiqui N, et al. Modification and implementation of NCCN guidelines^{TM} on breast cancer in the Middle East and North Africa region. *J Natl Compr Cancer Netw*. (2010) 8:S-8–S-15. doi: 10.6004/jnccn.2010.0126
7. Mahfoudh W, Bouaouina N, Ahmed SB, Gabbouj S, Shan J, Mathew R, et al. Hereditary breast cancer in Middle Eastern and North African (MENA) populations: identification of novel, recurrent and founder BRCA1 mutations in the Tunisian population. *Mol Biol Rep*. (2012) 39:1037–46. doi: 10.1007/s11033-011-0829-8
8. Shamseddine A, Saleh A, Charafeddine M, Seoud M, Mukherji D, Temraz S, et al. Cancer trends in Lebanon: a review of incidence rates for the period of 2003–2008 and projections until 2018. *Popul Health Metrics*. (2014) 12:4. doi: 10.1186/1478-7954-12-4
9. IARC. *Press Release N° 223* Lyon: Press Release (2013).
10. WCRF/AICR. *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*.Washington, DC: World Cancer Research Fund; American Institute for Cancer Research (2007).
11. Jomaa L, Hwalla N, Itani L, Chamieh MC, Mehio-Sibai A, Naja F. A Lebanese dietary pattern promotes better diet quality among older adults: findings from a national cross-sectional study. *BMC Geriatr*. (2016) 16:85. doi: 10.1186/s12877-016-0258-6

12. Cheraghi Z, Poorolajal J, Hashem T, Esmailnasab N, Irani AD. Effect of body mass index on breast cancer during premenopausal and postmenopausal periods: a meta-analysis. *PLoS ONE.* (2012) 7:e51446. doi: 10.1371/journal.pone.0051446
13. Suzuki R, Orsini N, Saji S, Key TJ, Wolk A. Body weight and incidence of breast cancer defined by estrogen and progesterone receptor status—a meta-analysis. *Int J Cancer.* (2009) 124:698–712. doi: 10.1002/ijc.23943
14. Continuous Update Project (CUP)/World Cancer Research Fund. *Diet, Nutrition, Physical Activity and Breast Cancer*. Washington, DC: World Cancer Research Fund; American Institute for Cancer Research (2018).
15. Seiler A, Chen MA, Brown RL, Fagundes CP. Obesity, dietary factors, nutrition, and breast cancer risk. *Curr Breast Cancer Rep.* (2018) 10:14–27. doi: 10.1007/s12609-018-0264-0
16. Levitan D. *Do Fat Levels Alter Breast Cancer Risk Even Among Women With Normal BMI?* Cancer Netwrok (2019).
17. Iyengar NM, Arthur R, Manson JE, Chlebowski RT, Kroenke CH, Peterson L, et al. Association of body fat and risk of breast cancer in postmenopausal women with normal body mass index: a secondary analysis of a randomized clinical trial and observational study. *JAMA Oncol.* (2019) 5:155–63. doi: 10.1001/jamaoncol.2018.5327
18. Taha Z, Eltom SE. The role of diet and lifestyle in women with breast cancer: an update review of related research in the Middle East. *BioRes Open Access.* (2018) 7:73–80. doi: 10.1089/biores.2018.0004
19. World Health Organization. *Noncommunicable Diseases (NCD) Country Profiles*. World Health Organization (2018).
20. WHO. *WHO Global Health Observatory*. World Health Organization (2018).
21. Jung U, Choi M-S. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *Int J Mol Sci.* (2014) 15:6184–223. doi: 10.3390/ijms15046184
22. Pimentel I, Lohmann AE, Goodwin PJ. Normal weight adiposity and postmenopausal breast cancer risk. *JAMA Oncol.* (2019) 5:150–1. doi: 10.1001/jamaoncol.2018.5162
23. Easton DF, Hopper JL, Thomas DC, Antoniou A, Pharoah PD, Whittemore AS, et al. Breast cancer risks for BRCA1/2 carriers. *Science.* (2004) 306:2187–91. doi: 10.1126/science.306.5705.2187c
24. Denic S, Al-Gazali L. BRCA1 and BRCA2 mutations in breast cancer patients from Saudi Arabia. *Saudi Med J.* (2003) 24:696.
25. Kadouri L, Bercovich D, Elimelech A, Lerer I, Sagi M, Glusman G, et al. A novel BRCA-1 mutation in Arab kindred from east Jerusalem with breast and ovarian cancer. *BMC Cancer.* (2007) 7:14. doi: 10.1186/1471-2407-7-14
26. El-Harith E-HA, Abdel-Hadi MS, Steinmann D, Dork T. BRCA1 and BRCA2 mutations in breast cancer patients from Saudi Arabia. *Saudi Med J.* (2002) 23:700–4. Available online at: https://pdfs.semanticscholar.org/532e/70bafb95625e84d69532f04d4b5df2f52f94.pdf
27. Riahi A, Kharrat M, Ghourabi M, Khomsi F, Gamoudi A, Lariani I, et al. Mutation spectrum and prevalence of BRCA 1 and BRCA 2 genes in patients with familial and early-onset breast/ovarian cancer from Tunisia. *Clin Genet.* (2015) 87:155–60. doi: 10.1111/cge.12337
28. Uhrhammer N, Abdelouahab A, Lafarge L, Feillel V, Dib AB, Bignon Y-J. BRCA1 mutations in Algerian breast cancer patients: high frequency in young, sporadic cases. *Int J Med Sci.* (2008) 5:197. doi: 10.7150/ijms.5.197
29. Al Hannan F, Keogh MB, Taha S, Al Buainain L. Characterization of BRCA1 and BRCA2 genetic variants in a cohort of Bahraini breast cancer patients using next-generation sequencing. *Mol Genet Genom Med.* 2019:e771. doi: 10.1002/mgg3.771
30. Abdulrashid K, AlHussaini N, Ahmed W, Thalib L. Prevalence of BRCA mutations among hereditary breast and/or ovarian cancer patients in Arab countries: systematic review and meta-analysis. *BMC Cancer.* (2019) 19:256. doi: 10.1186/s12885-019-5463-1
31. Geredeli C, Yasar N, Sakin A. Germline mutations in BRCA1 and BRCA2 in breast cancer patients with high genetic risk in Turkish population. *Int J Breast Cancer.* (2019) 2019:9645147. doi: 10.1155/2019/9645147
32. Laraqui A, Uhrhammer N, EL Rhaffouli H, Sekhsokh Y, Lahlou-Amine I, Bajjou T, et al. BRCA genetic screening in Middle Eastern and North African: mutational spectrum and founder BRCA1 mutation (c.798_799delTT) in North African. *Dis Mark.* (2015) 2015:8. doi: 10.1155/2015/194293
33. Farra C, Dagher C, Badra R, Hammoud MS, Alameddine R, Awwad J, et al. BRCA mutation screening and patterns among high-risk Lebanese subjects. *Hered Cancer Clin Pract.* (2019) 17:4. doi: 10.1186/s13053-019-0105-9
34. Brennan SF, Cantwell MM, Cardwell CR, Velentzis LS, Woodside JV. Dietary patterns and breast cancer risk: a systematic review and meta-analysis. *Am J Clin Nutr.* (2010) 91:1294–302. doi: 10.3945/ajcn.2009.28796
35. Karimi Z, Jessri M, Houshiar-Rad A, Mirzaei H-R, Rashidkhani B. Dietary patterns and breast cancer risk among women. *Public Health Nutr.* (2014) 17:1098–106. doi: 10.1017/S1368980013001018
36. Dandamudi A, Tommie J, Nommsen-Rivers L, Couch S. Dietary patterns and breast cancer risk: a systematic review. *Anticancer Res.* (2018) 38:3209–22. doi: 10.21873/anticanres.12586
37. Doherty M. Study suggests possible link between highly processed foods and cancer. *BMJ* (2018). [Epub ahead of print].
38. Sibai AM, Nasreddine L, Mokdad AH, Adra N, Tabet M, Hwalla N. Nutrition transition and cardiovascular disease risk factors in Middle East and North Africa countries: reviewing the evidence. *Ann Nutr Metab.* (2010) 57:193–203. doi: 10.1159/000321527
39. Al Othaimeen A, Ezzat A, Mohamed G, Muammar T, Al Madouj A. Dietary fat and breast cancer in Saudi Arabia: a case-control study. *East Mediterr Health J.* (2004) 10:879–86. Available online at: https://apps.who.int/iris/bitstream/handle/10665/119492/10_6_2004_879_886.pdf?sequence=1&isAllowed=y
40. Lee Y-CA, Hashibe M. Tobacco, alcohol, and cancer in low and high income countries. *Ann Glob Health.* (2014) 80:378–83. doi: 10.1016/j.aogh.2014.09.010
41. Seitz HK, Pelucchi C, Bagnardi V, Vecchia CL. Epidemiology and pathophysiology of alcohol and breast cancer: update 2012. *Alcohol Alcohol.* (2012) 47:204–12. doi: 10.1093/alcalc/ags011
42. Guo J, Wei W, Zhan L. Red and processed meat intake and risk of breast cancer: a meta-analysis of prospective studies. *Breast Cancer Res Treat.* (2015) 151:191–8. doi: 10.1007/s10549-015-3380-9
43. Fararouei M, Iqbal A, Rezaian S, Gheibi Z, Dianatinasab A, Shakarami S, et al. Dietary habits and physical activity are associated with the risk of breast cancer among young Iranian women: a case-control study on 1010 premenopausal women. *Clin Breast Cancer.* (2019) 19:e127–34. doi: 10.1016/j.clbc.2018.10.011
44. Afshin A, Micha R, Khatibzadeh S, Fahimi S, Shi P, Powles J, et al. The impact of dietary habits and metabolic risk factors on cardiovascular and diabetes mortality in countries of the Middle East and North Africa in 2010: a comparative risk assessment analysis. *BMJ Open.* (2015) 5:e006385. doi: 10.1136/bmjopen-2014-006385
45. Ferguson LR. Meat and cancer. *Meat Sci.* (2010) 84:308–13. doi: 10.1016/j.meatsci.2009.06.032
46. Turati F, Carioli G, Bravi F, Ferraroni M, Serraino D, Montella M, et al. Mediterranean diet and breast cancer risk. *Nutrients.* (2018) 10:326. doi: 10.3390/nu10030326
47. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr Cancer.* (1992) 18:1–29. doi: 10.1080/01635589209514201
48. Murillo G, Mehta RG. Cruciferous vegetables and cancer prevention. *Nutr Cancer.* (2001) 41:17–28. doi: 10.1207/S15327914NC41-1&2_2
49. Aune D, Chan D, Vieira A, Rosenblatt DN, Vieira R, Greenwood D, et al. Fruits, vegetables and breast cancer risk: a systematic review and meta-analysis of prospective studies. *Breast Cancer Res Treat.* (2012) 134:479–93. doi: 10.1007/s10549-012-2118-1
50. Liu X, Lv K. Cruciferous vegetables intake is inversely associated with risk of breast cancer: a meta-analysis. *Breast.* (2013) 22:309–13. doi: 10.1016/j.breast.2012.07.013
51. Ezzati M, Lopez AD, Rodgers AA, Murray CJ. *Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attributable to Selected Major Risk Factors*. World Health Organization (2004).
52. Rose DP. Dietary fiber and breast cancer. *Nutr Cancer.* (1990) 13:1–8. doi: 10.1080/01635589009514040
53. Tamimi RM, Hankinson SE, Campos H, Spiegelman D, Zhang S, Colditz GA, et al. Plasma carotenoids, retinol, and tocopherols and risk of breast cancer. *Am J Epidemiol.* (2005) 161:153–60. doi: 10.1093/aje/kwi030

54. Yang B, Ren X-L, Fu Y-Q, Gao J-L, Li D. Ratio of n-3/n-6 PUFAs and risk of breast cancer: a meta-analysis of 274135 adult females from 11 independent prospective studies. *BMC Cancer.* (2014) 14:105. doi: 10.1186/1471-2407-14-105
55. Zheng J-S, Hu X-J, Zhao Y-M, Yang J, Li D. Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: meta-analysis of data from 21 independent prospective cohort studies. *BMJ.* (2013) 346:f3706. doi: 10.1136/bmj.f3706
56. Joshi AA, Hegde MV, Adekar SP. Omega-3 fatty acids in cancer: insight into the mechanism of actions in preclinical cancer models. In: Hegde MV, Zanwar, AA, Adekar, SP, editors. *Omega-3 Fatty Acids*. Springer (2016). p. 157–71. doi: 10.1007/978-3-319-40458-5_12
57. Aune D, Chan D, Greenwood D, Vieira A, Rosenblatt DN, Vieira R, et al. Dietary fiber and breast cancer risk: a systematic review and meta-analysis of prospective studies. *Ann Oncol.* (2012) 23:1394–402. doi: 10.1093/annonc/mdr589
58. Tayyem RF, Bawadi HA, Shehadah I, Agraib LM, Al-Awwad NJ, Heath DD, et al. Consumption of whole grains, refined cereals, and legumes and its association with colorectal cancer among Jordanians. *Integr Cancer Ther.* (2015) 15:318–25. doi: 10.1177/1534735415620010
59. WCRF/AICR. *Food, Nutrition, Physical Activity, and the Prevention of Breast Cancer: A Global Perspective*. Washington, DC: World Cancer Research Fund; American Institute for Cancer Research (2010).
60. Slavin JL. Mechanisms for the impact of whole grain foods on cancer risk. *J Am Coll Nutr.* (2000) 19:300S−7S. doi: 10.1080/07315724.2000.10718964
61. McIntyre A, Gibson P, Young G. Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. *Gut.* (1993) 34:386–91. doi: 10.1136/gut.34.3.386
62. Dong J-Y, Qin L-Q. Dietary glycemic index, glycemic load, and risk of breast cancer: meta-analysis of prospective cohort studies. *Breast Cancer Res Treat.* (2011) 126:287–94. doi: 10.1007/s10549-011-1343-3
63. Sieri S, Pala V, Brighenti F, Pellegrini N, Muti P, Micheli A, et al. Dietary glycemic index, glycemic load, and the risk of breast cancer in an Italian prospective cohort study. *Am J Clin Nutr.* (2007) 86:1160–6. doi: 10.1093/ajcn/86.4.1160
64. McDonnell SL, Baggerly CA, French CB, Baggerly LL, Garland CF, Gorham ED, et al. Breast cancer risk markedly lower with serum 25-hydroxyvitamin D concentrations ≥60 vs <20 ng/ml (150 vs 50 nmol/L): pooled analysis of two randomized trials and a prospective cohort. *PLoS ONE.* (2018) 13:e0199265. doi: 10.1371/journal.pone.0199265
65. Wang D, de-la-Paz OIV, Zhai J-X, Liu D-W. Serum 25-hydroxyvitamin D and breast cancer risk: a meta-analysis of prospective studies. *Tumor Biol.* (2013) 34:3509–17. doi: 10.1007/s13277-013-0929-2
66. Yousef FM, Jacobs ET, Kang PT, Hakim IA, Going S, Yousef JM, et al. Vitamin D status and breast cancer in Saudi Arabian women: case-control study. *Am J Clin Nutr.* (2013) 98:105–10. doi: 10.3945/ajcn.112.054445
67. Witte JS, Ursin G, Siemiatycki J, Thompson WD, Paganini-Hill A, Haile RW. Diet and premenopausal bilateral breast cancer: a case-control study. *Breast Cancer Res Treat.* (1997) 42:243–51. doi: 10.1023/A:1005710211184
68. John EM, Schwartz GG, Dreon DM, Koo J. Vitamin D and breast cancer risk: the NHANES I epidemiologic follow-up study, 1971–1975 to 1992. *Cancer Epidemiol Prev Biomarkers.* (1999) 8:399–406.
69. Levi F, Pasche C, Lucchini F, La Vecchia C. Dietary intake of selected micronutrients and breast-cancer risk. *Int J Cancer.* (2001) 91:260–3. doi: 10.1002/1097-0215(200002)9999:9999<::aid-ijc1041>3.3.co;2-r
70. International Osteroporosis Foundation. *Vitamin D Status Around the World*. International Osteoporosis Foundation (2012).
71. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? *J Steroid Biochem Mol Biol.* (2014) 144:138–45. doi: 10.1016/j.jsbmb.2013.11.003
72. Narvaez CJ, Matthews D, LaPorta E, Simmons KM, Beaudin S, Welsh J. The impact of vitamin D in breast cancer: genomics, pathways, metabolism. *Front Physiol.* (2014) 5:213. doi: 10.3389/fphys.2014.00213

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 in Medicine

METHODS
published: 10 January 2020
doi: 10.3389/fmed.2019.00314

Radial Profile Analysis of Epithelial Polarity in Breast Acini: A Tool for Primary (Breast) Cancer Prevention

Lawton Manning[1], *Julia Holmes*[2], *Keith Bonin*[1,3]* *and Pierre-Alexandre Vidi*[2,3]*

[1] Department of Physics, Wake Forest University, Winston-Salem, NC, United States, [2] Department of Cancer Biology, Wake Forest School of Medicine, Winston-Salem, NC, United States, [3] Comprehensive Cancer Center of Wake Forest University, Winston-Salem, NC, United States

Preventing cancer is vastly better than treating the disease in terms of a patient's quality of life and healthcare costs. Yet, to screen for chemopreventative drugs or evaluate interventions aimed at lowering cancer risk, quantitative readouts of risk are needed. In the breast and in other organs of epithelial origin, apical-basal polarity is key to homeostasis and is one of the first tissue characteristics lost during cancer initiation. Therefore, apical-basal polarity may be leveraged as an "architectural" determinant of cancer risk. A classic approach to quantify the localization of epithelial polarity markers is visual scoring at the microscope by trained investigators. This approach is time-intensive and limited to low throughput. To increase the speed, accuracy, and scoring volume, we developed an algorithm that essentially replaces the human eye to objectively quantify epithelial polarity in microscopy images of breast glandular units (acini). Acini in culture are identified based on a nuclear stain and the corresponding masks are divided into concentric terraces of equal width. This positional information is used to calculate radial intensity profiles (RP) of polarity markers. Profiles with a steep slope represent polarized structures, whereas more horizontal curves are indicative of non-polarized acini. To compare treatment effects, RP curves are integrated into summary values of polarity. We envision applications of this method for primary cancer prevention research with acini organoids, specifically (1) to screen for chemoprevention drugs, (2) for toxicological assessment of suspected carcinogens and pharmacological hit compounds, and (3) for personalized evaluation of cancer risk and risk-reducing interventions. The RadialProfiler algorithm developed for the MATLAB computing environment and for users without prior informatics knowledge is publicly available on the Open Science Framework (OSF).

Keywords: breast cancer, organoids, apical polarity, chemoprevention screening, toxicology

OPEN ACCESS

Edited by:
Mary Beth Terry,
Columbia University, United States

Reviewed by:
Sergio Britto Garcia,
University of São Paulo, Brazil
Paulo Hilario Nascimento Saldiva,
University of São Paulo, Brazil

***Correspondence:**
Keith Bonin
bonin@wfu.edu
Pierre-Alexandre Vidi
pvidi@wakehealth.edu

Specialty section:
This article was submitted to Pathology, a section of the journal Frontiers in Medicine

Received: *01 May 2019*
Accepted: *11 December 2019*
Published: *10 January 2020*

Citation:
Manning L, Holmes J, Bonin K and Vidi P-A (2020) Radial Profile Analysis of Epithelial Polarity in Breast Acini: A Tool for Primary (Breast) Cancer Prevention. Front. Med. 6:314. doi: 10.3389/fmed.2019.00314

INTRODUCTION

Epithelial Polarity in the Normal Mammary Gland

The mammary gland consists of an arborescence of ducts connecting the glandular elements (called acini, lobules, or alveoli) to the nipple (**Figures 1A,B**). Several (five to ten) of these ductal systems are typically present in each breast (1). The mammary gland is a simple epithelial tissue composed of a single layer of luminal cells lining the ducts and acini (**Figure 1C**). Luminal cells are surrounded

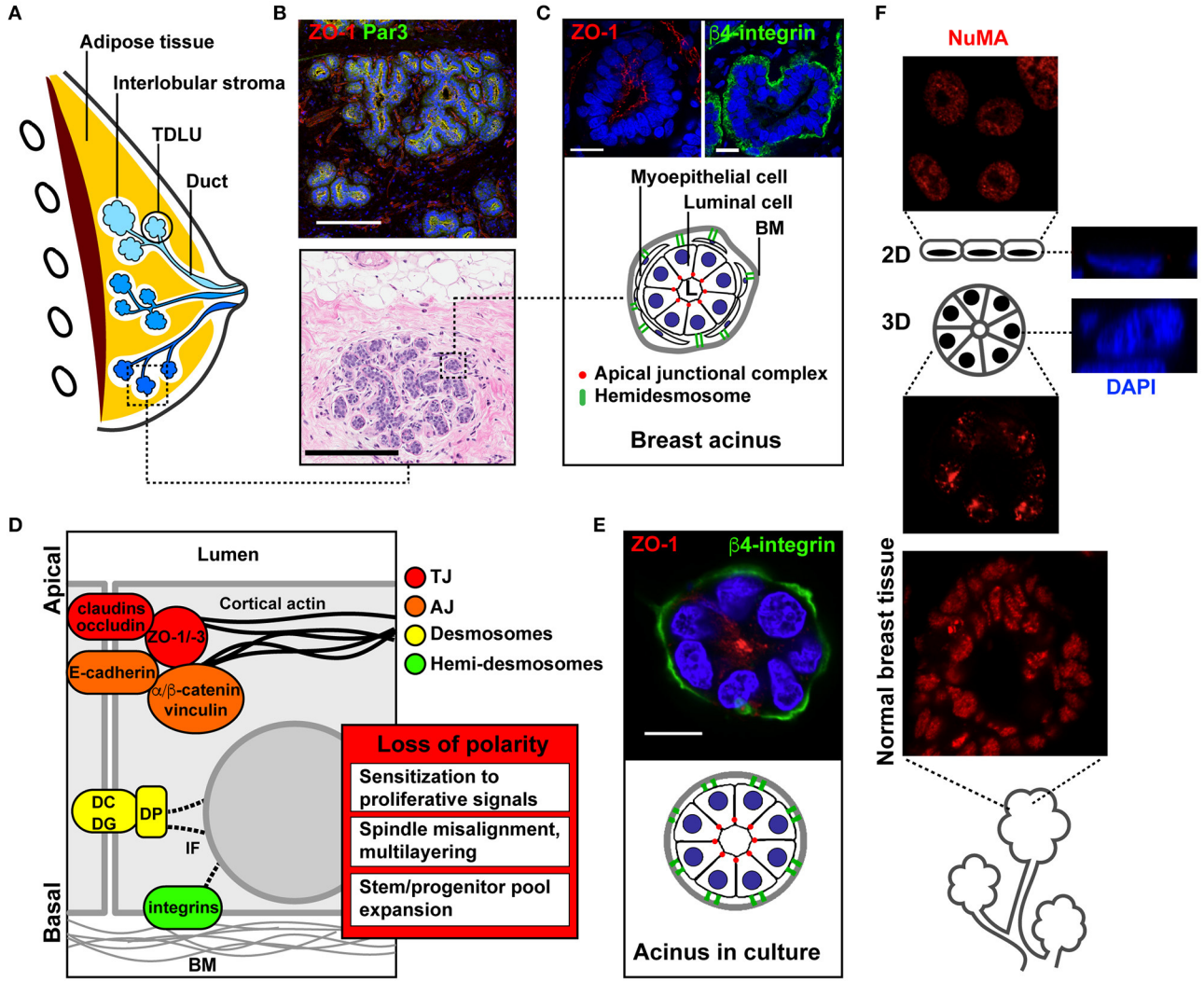


FIGURE 1 | Apical-basal polarity in the normal mammary gland and in culture models of acini. **(A)** Schematic of the breast anatomy. Different ductal systems (or lobes) are shown in distinct shades of blue. TDLU, terminal ductal lobular unit (the initiation site for most breast carcinomas). **(B)** Immunohistochemistry (IHC, bottom) and immunofluorescence (IF, top) images of normal breast tissue sections. **(C)** Schematic and higher magnification images of functional glandular units (acini) stained with the ZO-1 and β4-integrin epithelial polarity markers. **(D)** Schematic of cell junctional complexes along the apical-basal polarity axis of the epithelium, and functional effects of epithelial polarity loss in cancer initiation. BM, basement membrane; DC, desmocollin; DG, desmoglein; DP, desmoplakin; IF, intermediate filaments. **(E)** Schematic and representative confocal images of a breast acinus produced in 3D culture. The IHC image in B is from the Komen Tissue Bank. Scale bars, 200 μm **(B)** and 20 μm **(C,E)**. **(F)** Immunostaining for the structural nuclear protein NuMA in a 2D monolayer culture (top), in a 3D culture of acini (middle), and in normal human breast tissue (bottom). Orthogonal views of stained nuclei (DAPI) in 2D and 3D cultures are shown on the right.

by myoepithelial cells with contractile function to expel the milk toward the nipple. Myoepithelial cells also secrete most of the factors constituting the basement membrane (BM), a specialized form of extracellular matrix (ECM) lining the epithelium and rich in collagen type IV and laminins. In mammary ducts and acini, apical-basal polarity structurally and functionally defines the cellular organization relative to the lumen and BM (2, 3). Apical membranes of luminal cells delineate the luminal space and are segregated from basolateral membranes by cell-cell junctions; these different junctional complexes occupy distinct radial positions along the apical-basal polarity axis of the epithelial layer (**Figure 1D**).

Tight junctions (TJs) are localized closest to the lumen. They consist of integral membrane proteins [claudins, occludin, JAM (4)], as well as cytosolic adaptor and scaffolding factors [zona occludens proteins ZO-1, ZO-2, ZO-3 (5)] bridging the membrane-integral TJ factors with the cytoskeleton. TJs form a seal ensuring the segregation of apical and basolateral membrane lipids and proteins. In addition to this fence function, TJs serve as gates for selective diffusion between basal and luminal interstitial

spaces. Both gate and fence functions are essential for the normal function of the gland, in particular for milk secretion and to control paracellular exchanges between blood and milk (6).

Adherens junctions (AJs) are located next to TJs and are composed of transmembrane cadherins and nectins bound to cytosolic catenins and to afadin. AJs provide attachment of neighboring cells and are physically bound to TJs via ZO-1. During cell differentiation, AJ formation precedes and promotes TJ assembly by nucleating TJ proteins (7, 8). Both TJs and AJs are connected to the actin cytoskeleton, with ZO proteins and catenins directly binding to and organizing F-actin, which leads to the establishment and maintenance of perijunctional actomyosin rings stabilizing junctional complexes (9, 10).

Desmosomes have a similar organization as AJs but, in contrast to AJs that are linked to actin filaments, desmosomes are connected to keratin intermediate filaments. Desmosomes also play an important role in cell-cell adhesion along the basolateral membrane. Together with AJs, desmosomes mechanically couple neighboring epithelial cells, and thereby provide mechanical strength to the tissue, define cell-intrinsic mechanical properties, and constitute mechanotransduction hubs for the integration of physical cues from surrounding cells (11, 12).

Cell-cell contacts in the breast epithelium and other epithelia also comprise gap junctions (GJs) that form channels connecting the cytoplasm of adjacent cells and that enable cell-cell communication via small molecules (13). GJs consist of connexons (connexin hexamers) and are classically represented toward the basal side of epithelial cells. Yet connexin 43 was recently found to be apically localized in the breast epithelium, and to be required for apical polarity establishment and maintenance (14).

Three major polarity complexes regulate the maturation and maintenance of cell-cell adhesion complexes along the apical-basal axis [reviewed in (2, 7)]: the crumbs complex, which defines apical membrane identity, the PAR (partitioning defective) system, which defines the apical-basal boundary, and the scribble complex, which defines basolateral membrane identity. The establishment of the apical-basal polarity axis—and particularly, the orientation of this axis orthogonal to the BM—also depends on cell-ECM interactions, which are critical for differentiation and homeostasis (15, 16). Such cell-ECM contacts involve both luminal and myoepithelial cells and are largely mediated by integrins located at the basal pole of the acini and ducts. Integrins cross-talk with and modulate growth factor receptors signaling, and play important roles in mechanosensing (17–19). Importantly, these ECM receptors initiate a structural continuum between the ECM and the cell nucleus, which defines nuclear shape and genomic functions (20).

As alluded to in the previous paragraphs, the function and relevance of cell-cell junctional complexes and cell-ECM contacts go far beyond their structural role. Polarity factors include tumor suppressors and oncoproteins that localize both at cell-cell junctions and in the cytosol or cell nucleus where they modulate biochemical signals, gene expression, and genome maintenance (21–23). Altered cell polarity causes misregulation of proliferative and survival pathways by shifting the proportion of soluble and membrane bound polarity factors. We also found evidence that cell-ECM interactions are required for an efficient DNA damage response in breast epithelial cells (24). Apical-basal polarity, specifically the PAR system, also defines the orientation of mitotic spindle poles, and hence the relative position of the daughter cells after cytokinesis; spindle orientation parallel to the BM is necessary for the maintenance of a single cell layer and, accordingly, epithelial polarity loss may promote cell multilayering and hyperplasia (25, 26). Epithelial polarity may therefore be considered an architectural biomarker of breast cancer risk and, indeed, disruption of epithelial polarity is one of the first identifiable events and a necessary step for the initiation of carcinoma (7, 27–29).

Epithelial Polarity for Breast Cancer Risk Assessment

Current breast cancer risk assessment methods, such as the Gail model (30) provide population-based estimates of risk. Several genetic breast cancer risk factors have been identified (BRCA1, BRCA2, p53, etc.), yet the majority of breast cancers still have no clear germline mutation origin and cannot be predicted by genetic testing. Molecular assays of breast cancer risk are therefore needed for primary breast cancer prevention research and, ultimately, for personalized cancer prevention.

We propose that breast epithelial polarity, which is a hallmark of homeostasis in the mammary gland, is one of the molecular links between metabolic risk factors (including obesity and pre-diabetes) and cancer initiation. As such, epithelial polarity readouts may provide valid estimates of cancer risk. Loss of epithelial polarity, and in particular TJ and AJ remodeling, is associated with cancer initiation in multiple contexts, often involving tissue inflammation. For example, ulcerative colitis and Crohn's disease are both associated with elevated colorectal cancer risk (31) and are characterized by TJ dysfunctions (32). Similarly, patients with Celiac disease have TJ defects and increased epithelial cell permeability in the small intestine. These patients are at increased risk for adenocarcinoma of the small intestine. For breast cancer, obesity is one of the few modifiable risk factors and is characterized by a chronic state of inflammation and deregulation of cytokine and growth factors in circulation (33, 34). Our group found that cell microenvironments characteristic of obesity lead to the mislocalization of apical polarity proteins and premalignant changes in the mammary gland (14, 35). Apical polarity was also found to be disrupted by omega-6 fatty acids, which may be associated with increased breast cancer risk (36). These observations validate the concept of using epithelial polarity as a readout for primary prevention.

Cell Culture Models of Breast Acini

When cultured with a reconstituted basement membrane (rBM) hydrogel having physical and chemical characteristics similar to that of the basement membrane *in vivo*, non-neoplastic mammary epithelial cells develop into 3D structures resembling mammary gland acini (**Figure 1E**). Acini cultures recapitulate important characteristics of the normal mammary gland, namely single cell-layered structures, proliferation arrest (90–95% Ki67-negative cells) and apical-basal polarity (37, 38). Signaling

pathways are dramatically rewired in 3D acini cultures (39). Moreover, nuclear organization features, such as gene positioning and nucleoskeletal arrangement are strikingly different in acini cultures compared to 2D monolayer cultures (40, 41). **Figure 1F** illustrates a remarkable parallel between distribution patterns of a structural nuclear protein (NuMA) in normal breast tissue and acini cultures.

Mammary epithelial cells can be cultured either embedded in or on top of rBM (38). Micropatterned surfaces have also been developed as an alternative for acinar cultures (42). Acini cultures have the advantage of high reproducibility and manipulability. Compared to mouse models, experiments with acini cultures are cheaper, faster, raise fewer ethical concerns, and typically do not require regulatory approval. A limitation of classic breast acini cultures is the lack of other cell types (myoepithelial cells, fibroblasts, immune cells, adipocytes). Hence, experimentation with acini cultures does not replace, but complements, *in vivo* studies.

In principle, acini models can be used for high-content analyses (HCA) at medium to high throughput—"high-content" referring to complex phenotypic readouts. While many screening platforms have been developed around cancer models to identify new cancer treatments, HCA protocols with normal cells for cancer prevention are scarce. Obviously, readouts based on cell killing cannot be used in the context of prevention. HCA methods to assess epithelial polarity will contribute to fill this gap.

THE RADIALPROFILER ALGORITHM

RadialProfiler identifies and segments single or grouped acini based on a nuclear stain and separates contiguous acini with a watershed algorithm. A filtering step excludes structures smaller or larger than set values, as well as blurred, out-of-focus, acini. Regions of interest (ROIs) corresponding to individual acini are divided into concentric terraces. The number of terraces depends on the size of the acini and the magnification used to capture images. It is set by the user. The concentric terraces are then used to calculate a radial profile of polarity for each acinus. The intensity profiles are normalized to avoid influences from the staining procedure. In addition, the center of the acinus is defined with a radial value of zero and the periphery as a radial value of one, thereby avoiding effects linked to acini sizes. A flowchart of the analysis is shown in **Figure 2**. Steep radial profiles represent polarized structures, whereas more horizontal curves represent non-polarized acini. Radial polarity indexes (*RP*) are calculated from the RP curves for direct comparisons between treatment conditions according to the equation:

$$RP = \sum_{i=1}^{n} |1 - RP_i| \quad (1)$$

Here, RP_i is the radial polarity of the *i*th terrace. The higher the value of the *RP* index, the more centrally concentrated is the polarity marker. Lower *RP* values indicate the polarity markers are more evenly distributed radially. To distinguish between apical and basal marker distributions, positive or negative signs are assigned to *RP* indexes. By definition, *RP* indexes from descending curves (apical) are set to positive values, whereas upward RP curves (basal) yield *RP* indexes with negative values. RadialProfiler was initially implemented in ImageJ (http://rsbweb.nih.gov/ij/) [see (35)], using an approach inspired by the Radial Profile Plot plugin from Paul Baggethun (https://imagej.nih.gov/ij/plugins/radial-profile.html). The algorithm was then translated for MATLAB and the following key improvements were made: (1) addition of watershed to improve threshold-based segmentation, (2) dilation of the identified acini to account for the discrepancy between borders of nuclear-stained images as opposed to true membrane edges, (3) substitution of approximated circles with contour terracing to calculate radial profiles, and (4) addition of an exclusion criteria based on image blur to exclude out-of-focus acini. The RadialProfiler workflow is summarized below.

Image Segmentation

Nuclear stain images are smoothened (by replacing each original pixel intensity value with the average intensity value corresponding to a 3 × 3 kernel size). This step reduces noise before initial segmentation, which is based on the global Otsu thresholding method. Initial segmentation usually leaves errors, such as under-segmentation, where two or more adjacent acini are joined into one, larger ROI in the binary mask. To separate merged structures, the algorithm applies a watershed on the binary mask obtained from Otsu thresholding. Before watershedding is applied, the borders of the identified ROIs are smoothened. To create an image for a watershed, a distance function is performed on the binary mask that reports the distance of each interior pixel to the nearest border pixel, and regional minima are found. The MATLAB watershed function is applied on this distance image, and pixels labeled as 0 in the resulting matrix are then labeled as 0 in the binary image. Finally, acini ROIs are dilated by a certain number of pixels depending on the image magnification. This is done as the true membrane edge of the acinus lies outside of the ROI identified based on the nuclear stain.

Filtering

Binary masks are filtered to exclude (1) structures partially on the border of an image, (2) structures with sizes outside a specified range, and (3) structures for which the level of blur is above a user-defined cutoff. Multiple algorithms have been developed to quantify blur in an image. We compared the different approaches summarized by Pertuz et al. (43) to determine which algorithm performed best at distinguishing blurred, out-of-focus acini based on nuclear stain images. Different levels of Gaussian blur were applied to a subset of images, creating series of images with defined levels of blurriness (**Figure 3A**). We also visually assigned acini from wide field microscopy images to clear and blurry categories (**Figure 3B**). For both approaches, we found that a wavelet-based operator (WAVR in the Focus Measure MATLAB function) was highly sensitive to Gaussian blurring and performed best to parse in-focus from out-of-focus acini. A plot summarizing

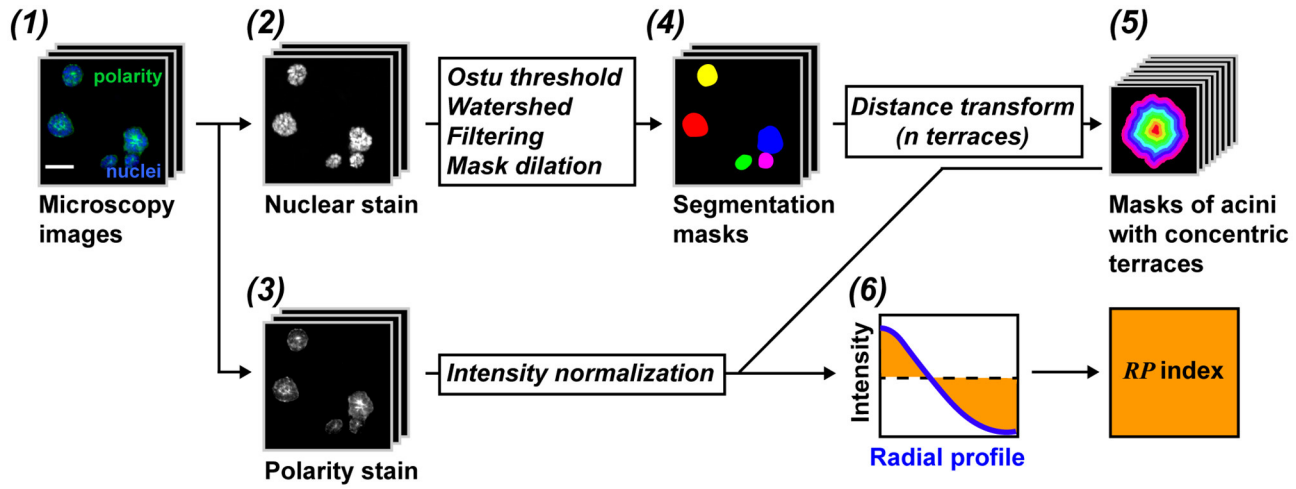


FIGURE 2 | RadialProfiler flowchart. (1) Images are taken from acini cultures stained with a nuclear dye (2) and for a cell polarity marker (3). (4) Acini are segmented based on the DNA dye. Filtering steps exclude structures with inappropriate sizes or structures that are out of focus. (5) Acini are divided into concentric terraces used to calculate radial profiles of polarity. (6) The profiles are normalized and integrated to obtain a summary value of polarity (*RP* index). Scale bar, 50 μm. See text for details.

the results is given **Figure 3C**. The graph shows the WAVR probability density function for acini visually characterized as either in focus or out of focus, revealing low WAVR values for blurry structures. The WAVR values determined from a Gaussian fit were 0.61 ± 0.08 and 0.94 ± 0.2 (mean/SD; $P < 0.00001$, Student's *t*-test) for out-of-focus and in-focus images, respectively. In this example, using a WAVR cutoff of 0.8 lead to the correct identification of 95% of acini deemed out of focus by visual evaluation, while retaining 78% of the structures visually assigned as in focus. This demonstrates that the WAVR blur value effectively distinguishes in-focus from blurry images.

Contour Terracing

Our previous algorithm (35) discarded all acini that were not highly circular in shape because concentric circles were used to assign image pixels to the different radial zones. To lessen the amount of excluded acini and to improve precision, the current RadialProfiler algorithm defines concentric "terraces" within each acinus. This step is performed using a distance transformation similar to the one used for the watershed technique. The distance transformation uses the binary mask (ROI) of an acinus. For each true pixel, the transformation returns the Euclidean distance between that pixel and the closest edge of the structure (i.e., the ROI boundary). By analogy, each acinus is treated as a "mountain," where the edges have lowest height, and the center marks the highest elevation. Acini ROIs are converted into topographical maps with contour lines (or terraces) of equal height ranges going from the base to the peak. Having a set number of terraces (radial bin values in the software interface) is important to normalize results for comparisons between different acini of unequal sizes and between treatment conditions.

RP Index Calculation

To calculate RP curves, the terraces defined in the previous step are imposed on the polarity images. The average pixel intensity in each terrace is calculated and divided by the average pixel intensity for the entire acinus. This normalization step yields RP curves that are not dependent on the staining efficacy (which can be uneven). The number of points for these curves is equal to the number of terraces selected. To obtain an *RP* index value for each acinus, each of the normalized radial intensities (RP_i) are subtracted from one (the average) and the corresponding absolute values are summed—see Equation (1). A negative sign is added to *RP* indexes from RP profiles with a positive slope, to distinguish between apical and basal signal localization.

ANALYSES OF EPITHELIAL POLARITY USING RADIALPROFILER

The RadialProfiler algorithm was developed to analyze acini produced with non-neoplastic HMT-3522 S1 breast epithelial cells (44). We expect that the radial profile method is applicable to acini produced with other normal or pre-malignant epithelial cell lines. Detailed protocols for 3D cell culture of breast acini can be found in ref. (38). Briefly, a thin coat of rBM (e.g., Corning Matrigel™) is applied at the bottom of the culture vessel. Then, a single cell suspension (42,000 cells/cm^2) is added on top of the rBM coat and is overlaid with rBM diluted in culture medium (5% final concentration) to engage the cell surface integrins that are not in contact with the rBM-coated substratum, and to promote the development of 3D structures. Different culture vessels (35 mm dishes, chambered slides, multiwell plates) are used depending on the analysis method (fixed vs. live imaging), and the throughput level (low vs. medium). For live imaging in glass-bottom dishes and plates, a thinner coat of

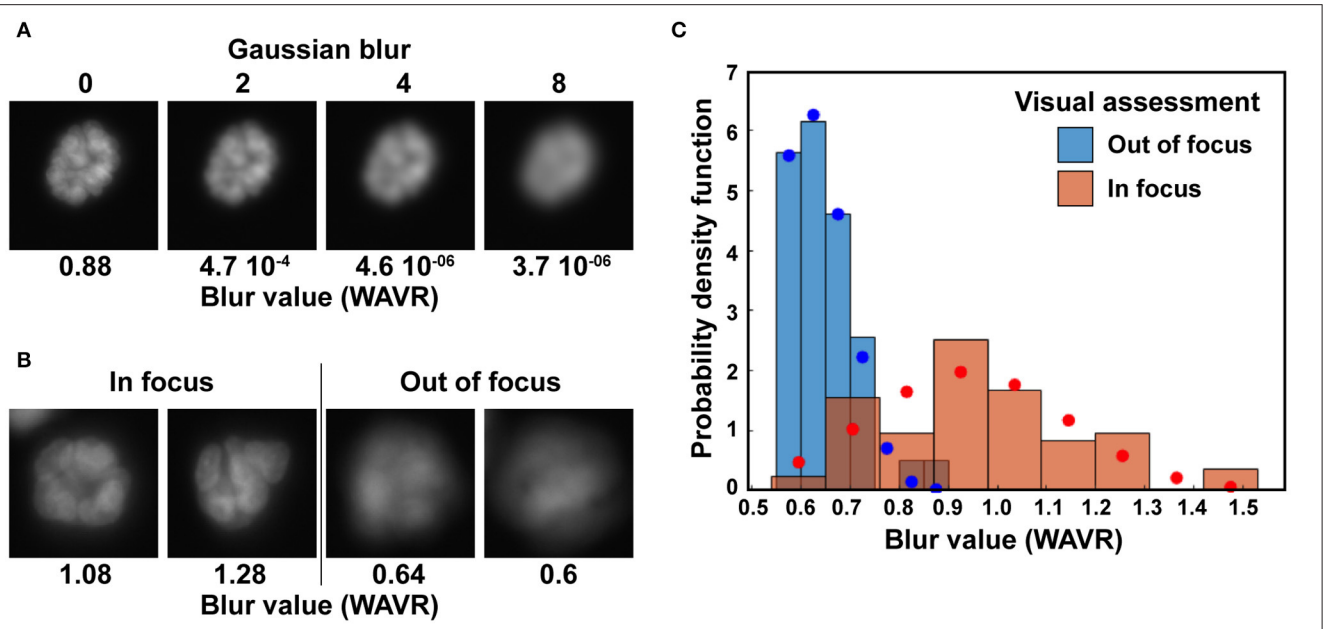


FIGURE 3 | Elimination of out-of-focus acini. **(A)** Illustration of the wavelets (WAVR) blur metric calculated for Hoechst images with different levels of Gaussian blur. **(B)** Representative acini images (Hoechst stain) deemed either in focus or out of focus and their corresponding WAVR values. Images in A and B were taken with an epifluorescence microscope at 20× magnification, using a sCMOS camera. **(C)** Histograms showing the probability density function of WAVR values belonging to acini images visually rated as clear (in focus; $n = 76$) or blurry (out of focus; $n = 39$). Corresponding Levenberg-Marquardt fits of normal distributions are shown in blue circles and red circles for the two distributions.

rBM is applied to enable imaging with high numerical aperture (NA) objectives, which typically have relatively short working distances (<0.2 mm).

RadialProfiler can be applied to quantify epithelial markers detected by immunofluorescence [as described in (35)], or to quantify cortical actin labeled in live acini with the SiR-actin dye (Cytoskeleton Inc.). DAPI and Hoechst are used to counterstain cell nuclei in fixed and live experiments, respectively.

For imaging, our laboratory uses an automated IX83 microscope (Olympus) equipped with a motorized ultrasonic stage and a TruFocus Z drift compensation module. For RadialProfiler analyses, images are taken with either 10× (NA = 0.3) or 20× (NA = 0.45) air lenses, using a sCMOS camera (ORCA-Flash4.0, Hamamatsu). The RadialProfiler software was also tested with images acquired using different imaging systems, including a high content imager (Perkin Elmer Operetta CLS). RadialProfiler and the underlying approach to analyze polarity are agnostic to the imaging system. Fields of view are chosen either in an automated fashion or based on nuclear signals (DAPI or Hoechst) to avoid bias. For live cell analyses, acini are maintained at 37°C and 5% CO_2 using a stage-top incubator (Tokai Hit). The minimal resolution needed depends on the number of radial terraces used by RadialProfiler. To improve statistical power, the number of acini in a single image needs to be maximized, which can be achieved with a low magnification objective. However, the ability to analyze the distribution of polarity markers in an acinus improves with the number of sampled image points. Lenses with higher magnification generally provide higher resolution images, with more pixels per acini, albeit with fewer acini in each field of view. In the end, the choice of magnification is directed by the need to have an individual acinus sampled at enough camera pixels to allow an accurate polarity radial profile analysis with a suitable number of terraces. We determined that using 5–10 bins that are two pixels wide yields accurate measurements. This corresponds to a diameter of 20–40 pixels, which, for a circular acinus, corresponds to 316–1,264 pixels. Acini are not perfect spheres; this value is therefore an estimate. This has been reinforced empirically through our analysis of large data sets.

RadialProfiler operates in two modes, either supervised or unsupervised. The user chooses between these two modes with the first dialog box (**Figure 4A**). The unsupervised mode runs the analysis automatically once the program parameters are set. It retrieves a table listing the normalized radial intensity values and an *RP* index value for each acinus. It also produces images annotated with segmentation results and *RP* index values (**Figure 5**). Results in the table are grouped by experimental conditions. The supervised version performs the same calculations as the unsupervised version but also includes a graphical user interface (**Figure 4B**), allowing the investigator to visually score polarity and assess the quality of the acini identification steps (segmentation efficacy, blurriness, etc.). Individual acini are presented to the user in a randomized order and without providing any treatment information, which enables blind scoring. After completion of visual scoring, a table with *RP* index values and user scores is produced. Additional details on RadialProfiler installation and usage are provided as **Supplementary Information** to this article. Representative results are shown in **Figure 6**.

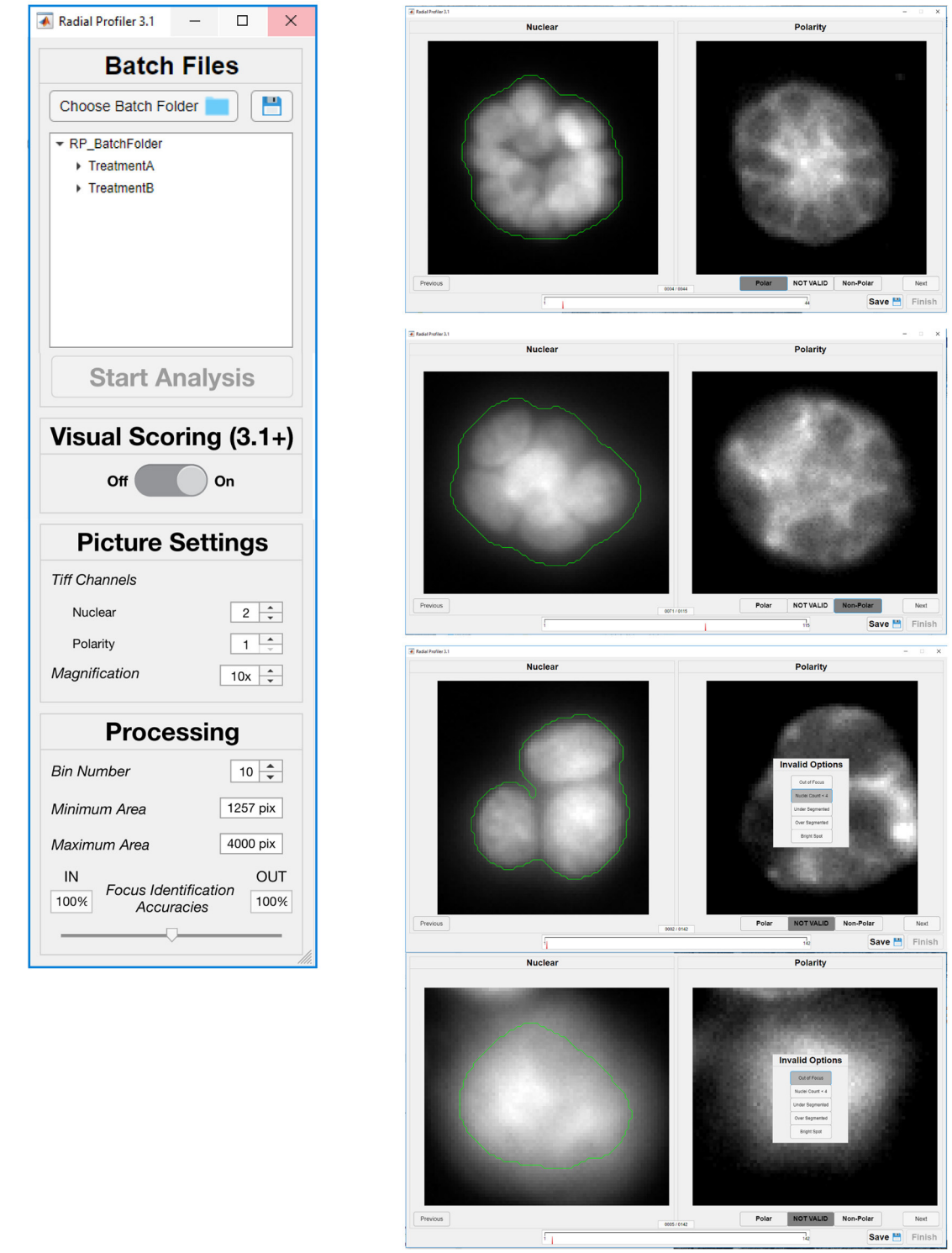


FIGURE 4 | Graphical user interfaces of RadialProfiler. **(A)** Window to select image folders corresponding to the dataset for analysis, and to define analysis parameters. The user chooses between supervised and unsupervised analyses with this first dialog box by turning visual scoring on or off. **(B)** Interface assisting visual scoring of polarity marker distribution. This window appears when the user selects supervised analysis. For each acinus identified by RadialProfiler (in the entire dataset selected in **A**), nuclear stain and polarity images are displayed side-by-side. The user input is a binary choice between ("Polar" or "Non-Polar") or exclusion from analysis. The progress bar (bottom) indicates the number of structures that remain to be scored. Acini appear in a randomized order. See text for details.

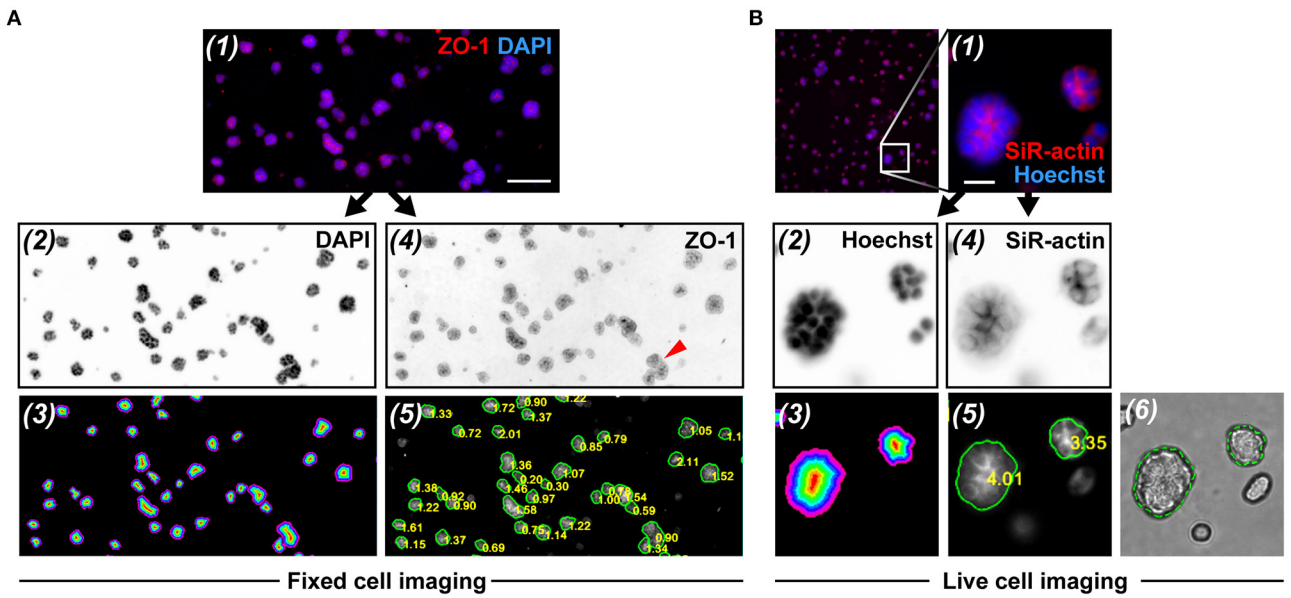


FIGURE 5 | RadialProfiler analysis of wide field fluorescence images from fixed and immunostained acini **(A)**, or of cortical actin staining in live acini **(B)**. The figure shows (1) portions of overlay images, (2) nuclear stain images (inverted to improve visualization), (3) corresponding masks with the concentric terraces, (4) inverted polarity images, and (5) polarity images annotated with acini contours and *RP* indexes. In rare instances (red arrowhead in A-4), acini were under-segmented. In B, an overlay of the bright field image and the corresponding contour ROI validates the segmentation (6). Scale bars, 100 μm **(A)** and 20 μm **(B)**.

DISCUSSION

We developed a method to quantify epithelial polarity in breast acini organoid cultures. The method is based on radial marker profiling and results in a single polarity index to assess establishment or breakdown of apical-basal polarity in populations of acini. This method should be applicable to a wide variety of cell types and treatment conditions. The software interface is user-friendly and circumvents the need to use command lines in MATLAB. RadialProfiler is therefore accessible to biologists and health scientists with minimal knowledge of the computing platform. Importantly, the *RP* index produced by the software successfully distinguishes between non-polar and polar acini, as demonstrated in the analyses presented in **Figure 6**. Similar results were obtained using different imaging platforms.

S1 cell acini are characterized by a small lumen; hence, radial profile curves of apically polarized structures have a maximal value close to the center of the structure. For epithelial cells forming cysts with a larger lumen (e.g., MDCK cells), radial profile maxima will be shifted toward the periphery. In this case, disruption of polarity protein distribution will still alter radial profiles, although we do not expect the method to perform well for structures with a very large lumen. Whereas the *RP* index distinguishes well between apical and basal signals (high positive vs. high negative values), as well as between polarized and uniform signals (high vs. low values), the index is not very sensitive to smaller radial shifts and may not be appropriate to quantify markers with multimodal distributions. The shape of the radial profile curves is however more indicative, and additional curve characteristics can be considered, such as the number of intersections with the average line ($x = 1$), the radial position of the maxima, etc.

RadialProfiler was developed and validated for homotypic cultures of breast acini. Co-cultures including multiple cell types (fibroblasts, adipocytes, immune cells) are better models—albeit more complex—to capture the effects of epithelial-stromal cell interactions on drug pharmacokinetics and phenotypical outcomes (35, 45–47). Acini in co-culture systems can in principle be analyzed using RadialProfiler, as long as epithelial cells can be distinguished from other cell types. For example, a breast epithelial cell line stably expressing a GFP-tagged histone can be co-cultured with other cell types (untagged or tagged with a different chromophore). In this case, GFP signals would be used instead of Hoechst staining to identify and segment acini with the current version of the RadialProfiler. The presence of other cell types should not interfere with immunostaining or cortical actin staining in the acini. Staining of the breast epithelial cells prior to co-culture with a cell tracking dyes would be an alternative approach.

We welcome feedback on RadialProfiler performance in different contexts and plan on further developments for this approach. In particular, operation of RadialProfiler in supervised mode yields rich datasets annotated for polarity by expert investigators. Datasets from supervised analyses also contain information on segmentation and image blur. This "ground truth" information will enable us to integrate machine learning into the next version of the algorithm. RadialProfiler is currently limited to the analysis of acini cultures *in vitro*. However, the general principle

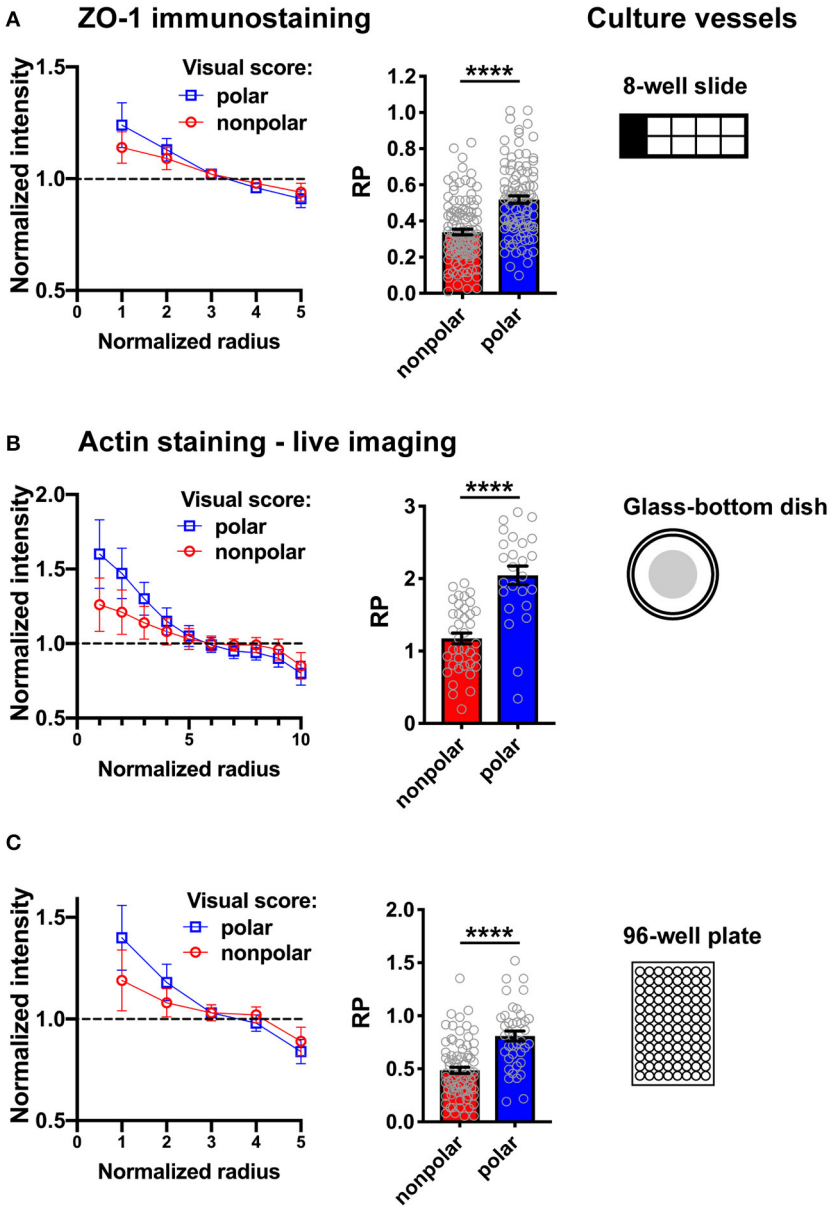


FIGURE 6 | Illustration of RadialProfiler results for HMT-3522 S1 acini in different culture vessels. The supervised version of the software was used to classify acini in polarized and non-polarized categories. Radial profiles (left) and bar graphs of the *RP* indexes (right) are shown for both categories. **(A)** Fixed acini immunostained for ZO-1. **(B,C)** Live imaging of acini stained with SiR-actin. Fluorescence images were captured using a wide field microscope (Olympus; **A,B**) or with an automated spinning disc high content imaging system (Perkin Elmer Operetta; **C**). In C, maximal intensity projections of 10 confocal frames were analyzed. The number of radial bins used for analysis was adapted to the different magnifications and image resolutions. ****$P < 0.0001$ (Student's *t*-test).

to quantify radial profiles is applicable to tissues, and we plan on further developing the computational approach for tissue analyses.

We hope and anticipate that this assay will fill unmet needs in primary prevention of breast cancer and other carcinomas, with applications including (1) chemoprevention drug screening, (2) toxicology assessment of suspected carcinogens and pharmacological lead compounds, and (3) personalized cancer risk diagnosis. High content screening methods for cancer prevention are scarce. Since loss of apical-basal polarity is an early step enabling the initiation of carcinoma, an assay of epithelial polarity may be used to screen for chemoprevention drugs or natural compounds preventing polarity loss or restoring polarity. The RP assay may also be implemented to weed out drug candidates with toxic effects on the epithelial architecture before testing in mice models. Indeed, the vast majority of hit compounds in drug discovery pipelines fail the transition from the initial screen to animal models. Relevant *in vitro*

assays, such as the RP assessment, may be used to rapidly and cheaply screen for toxic effects on normal cells, thereby reducing the need for animal research, which is expensive and raises ethical concerns. More broadly, assays with non-neoplastic cell organoids can be used to assess suspected carcinogens (48–51).

Current breast cancer risk assessment methods provide population-based estimates of risk rather than personalized risk assessment. Genetic testing can identify mutations associated with cancer risk (e.g., BRCA1/2 for breast cancer), yet only a small fraction of malignancies (about 5% for breast cancer) have a known genetic origin. Cell-phenotypical assays, including epithelial polarity readouts, may be used to rapidly assess personalized breast cancer risk, for example for women participating in lifestyle interventions. In these cases, acini cultures and RP analyses may serve as biomarkers for integrative assessment of improvements in metabolic risk factors.

DATA AVAILABILITY STATEMENT

The RadialProfiler MATLAB code, as well as a detailed tutorial describing installation and use of RadialProfiler and test images of breast acini is available on OSF (https://osf.io/g48ac/). The latest version of the code can also be downloaded from the MathWorks website.

AUTHOR CONTRIBUTIONS

LM wrote the computer code. JH performed the experiments. KB and P-AV directed the research and wrote the manuscript. LM and JH edited the manuscript.

FUNDING

This work was funded by the Department of Defense Breast Cancer Research Program (BC170905 to P-AV), the Wake Forest Center for Molecular Signaling (CMS; to KB and P-AV), and the National Cancer Institute's Cancer Center Support Grant (P30CA012197) issued to the Wake Forest University CCC.

ACKNOWLEDGMENTS

P-AV was a member of the International Breast Cancer & Nutrition (IBCN) program. The authors thank Catherine Ulich (Perkin Elmer) for assistance with high content imaging and Kevin Wang for his initial contribution to MATLAB programing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2019.00314/full#supplementary-material

REFERENCES

1. Love SM, Barsky SH. Anatomy of the nipple and breast ducts revisited. *Cancer*. (2004) 101:1947–57. doi: 10.1002/cncr.20559
2. Roignot J, Peng X, Mostov K. Polarity in mammalian epithelial morphogenesis. *Cold Spring Harb Perspect Biol*. (2013) 5:a013789. doi: 10.1101/cshperspect.a013789
3. Rodriguez-Boulan E, Macara IG. Organization and execution of the epithelial polarity programme. *Nat Rev Mol Cell Biol*. (2014) 15:225–42. doi: 10.1038/nrm3775
4. Krug SM, Schulzke JD, Fromm M. Tight junction, selective permeability, and related diseases. *Semin Cell Dev Biol*. (2014) 36:166–76. doi: 10.1016/j.semcdb.2014.09.002
5. Gonzalez-Mariscal L, Betanzos A, Avila-Flores A. MAGUK proteins: structure and role in the tight junction. *Semin Cell Dev Biol*. (2000) 11:315–24. doi: 10.1006/scdb.2000.0178
6. Stelwagen K, Singh K. The role of tight junctions in mammary gland function. *J Mammary Gland Biol Neoplasia*. (2014) 19:131–8. doi: 10.1007/s10911-013-9309-1
7. Martin-Belmonte F, Perez-Moreno M. Epithelial cell polarity, stem cells and cancer. *Nat Rev Cancer*. (2012) 12:23–38. doi: 10.1038/nrc3169
8. Campbell HK, Maiers JL, DeMali KA. Interplay between tight junctions & adherens junctions. *Exp Cell Res*. (2017) 358:39–44. doi: 10.1016/j.yexcr.2017.03.061
9. Van Itallie CM, Fanning AS, Bridges A, Anderson JM. ZO-1 stabilizes the tight junction solute barrier through coupling to the perijunctional cytoskeleton. *Mol Biol Cell*. (2009) 20:3930–40. doi: 10.1091/mbc.e09-04-0320
10. Arnold TR, Stephenson RE, Miller AL. Rho GTPases and actomyosin: partners in regulating epithelial cell-cell junction structure and function. *Exp Cell Res*. (2017) 358:20–30. doi: 10.1016/j.yexcr.2017.03.053
11. Broussard JA, Getsios S, Green KJ. Desmosome regulation and signaling in disease. *Cell Tissue Res*. (2015) 360:501–12. doi: 10.1007/s00441-015-2136-5
12. Rubsam M, Broussard JA, Wickstrom SA, Nekrasova O, Green KJ, Niessen CM. Adherens junctions and desmosomes coordinate mechanics and signaling to orchestrate tissue morphogenesis and function: an evolutionary perspective. *Cold Spring Harb Perspect Biol*. (2018) 10:a029207. doi: 10.1101/cshperspect.a029207
13. Bazzoun D, Lelièvre S, Talhouk R. Polarity proteins as regulators of cell junction complexes: implications for breast cancer. *Pharmacol Ther*. (2013) 138:418–27. doi: 10.1016/j.pharmthera.2013.02.004
14. Adissu HA, Bazzoun D, Wang L, Urazaev A, Tenvooren I, Fostok S, et al. Connexin 43 maintains tissue polarity and regulates mitotic spindle orientation in the breast epithelium. *J Cell Sci*. (2019) 132:jcs223313. doi: 10.1242/jcs.223313
15. Barcellos-Hoff MH, Aggeler J, Ram TG, Bissell MJ. Functional differentiation and alveolar morphogenesis of primary mammary cultures on reconstituted basement membrane. *Development*. (1989) 105:223–35.
16. Bissell MJ, Weaver VM, Lelievre SA, Wang F, Petersen OW, Schmeichel KL. Tissue structure, nuclear organization, and gene expression in normal and malignant breast. *Cancer Res*. (1999) 59:1757–63s.
17. Wang F, Weaver VM, Petersen OW, Larabell CA, Dedhar S, Briand P, et al. Reciprocal interactions between beta1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different perspective in epithelial biology. *Proc Natl Acad Sci USA*. (1998) 95:14821–6. doi: 10.1073/pnas.95.25.14821
18. Glukhova MA, Streuli CH. How integrins control breast biology. *Curr Opin Cell Biol*. (2013) 25:633–41. doi: 10.1016/j.ceb.2013.06.010
19. Bosch-Fortea M, Martin-Belmonte F. Mechanosensitive adhesion complexes in epithelial architecture and cancer onset. *Curr Opin Cell Biol*. (2018) 50:42–9. doi: 10.1016/j.ceb.2018.01.013
20. Simon DN, Wilson KL. The nucleoskeleton as a genome-associated dynamic 'network of networks'. *Nat Rev Mol Cell Biol*. (2011) 12:695–708. doi: 10.1038/nrm3207

21. Balda MS, Matter K. The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression. *EMBO J*. (2000) 19:2024–33. doi: 10.1093/emboj/19.9.2024
22. Feigin ME, Akshinthala SD, Araki K, Rosenberg AZ, Muthuswamy LB, Martin B, et al. Mislocalization of the cell polarity protein scribble promotes mammary tumorigenesis and is associated with basal breast cancer. *Cancer Res*. (2014) 74:3180–94. doi: 10.1158/0008-5472.CAN-13-3415
23. Fang L, Wang Y, Du D, Yang G, Tak Kwok T, Kai Kong S, et al. Cell polarity protein Par3 complexes with DNA-PK via Ku70 and regulates DNA double-strand break repair. *Cell Res*. (2007) 17:100–16. doi: 10.1038/sj.cr.7310145
24. Vidi PA, Chandramouly G, Gray M, Wang L, Liu E, Kim JJ, et al. Interconnected contribution of tissue morphogenesis and the nuclear protein NuMA to the DNA damage response. *J Cell Sci*. (2012) 125:350–61. doi: 10.1242/jcs.089177
25. McCaffrey LM, Macara IG. Epithelial organization, cell polarity and tumorigenesis. *Trends Cell Biol*. (2011) 21:727–35. doi: 10.1016/j.tcb.2011.06.005
26. Macara IG, Guyer R, Richardson G, Huo Y, Ahmed SM. Epithelial homeostasis. *Curr Biol*. (2014) 24:R815–25. doi: 10.1016/j.cub.2014.06.068
27. Lelievre SA. Tissue polarity-dependent control of mammary epithelial homeostasis and cancer development: an epigenetic perspective. *J Mammary Gland Biol Neoplasia*. (2010) 15:49–63. doi: 10.1007/s10911-010-9168-y
28. Royer C, Lu X. Epithelial cell polarity: a major gatekeeper against cancer? *Cell Death Differ*. (2011) 18:1470–7. doi: 10.1038/cdd.2011.60
29. Chatterjee SJ, McCaffrey L. Emerging role of cell polarity proteins in breast cancer progression and metastasis. *Breast Cancer*. (2014) 6:15–27. doi: 10.2147/BCTT.S43764
30. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst*. (1989) 81:1879–86. doi: 10.1093/jnci/81.24.1879
31. Dulai PS, Sandborn WJ, Gupta S. Colorectal cancer and dysplasia in inflammatory bowel disease: a review of disease epidemiology, pathophysiology, and management. *Cancer Prev Res*. (2016) 9:887–94. doi: 10.1158/1940-6207.CAPR-16-0124
32. Vancamelbeke M, Vermeire S. The intestinal barrier: a fundamental role in health and disease. *Expert Rev Gastroenterol Hepatol*. (2017) 11:821–34. doi: 10.1080/17474124.2017.1343143
33. Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. *Nat Rev Cancer*. (2011) 11:886–95. doi: 10.1038/nrc3174
34. Dietze EC, Chavez TA, Seewaldt VL. Obesity and triple-negative breast cancer: disparities, controversies, and biology. *Am J Pathol*. (2018) 188:280–90. doi: 10.1016/j.ajpath.2017.09.018
35. Tenvooren I, Jenks MZ, Rashid H, Cook KL, Muhlemann JK, Sistrunk C, et al. Elevated leptin disrupts epithelial polarity and promotes premalignant alterations in the mammary gland. *Oncogene*. (2019) 38:3855–70. doi: 10.1038/s41388-019-0687-8
36. Yue S, Cardenas-Mora JM, Chaboub LS, Lelievre SA, Cheng JX. Label-free analysis of breast tissue polarity by Raman imaging of lipid phase. *Biophys J*. (2012) 102:1215–23. doi: 10.1016/j.bpj.2012.01.023
37. Petersen OW, Ronnov-Jessen L, Howlett AR, Bissell MJ. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. *Proc Natl Acad Sci USA*. (1992) 89:9064–8. doi: 10.1073/pnas.89.19.9064
38. Vidi PA, Bissell MJ, Lelievre SA. Three-dimensional culture of human breast epithelial cells: the how and the why. *Methods Mol Biol*. (2013) 945:193–219. doi: 10.1007/978-1-62703-125-7_13
39. Furuta S, Bissell MJ. Pathways involved in formation of mammary organoid architecture have keys to understanding drug resistance and to discoveryof druggable targets. *Cold Spring Harb Symp Quant Biol*. (2016) 81:207–17. doi: 10.1101/sqb.2016.81.030825
40. Knowles DW, Sudar D, Bator-Kelly C, Bissell MJ, Lelievre SA. Automated local bright feature image analysis of nuclear protein distribution identifies changes in tissue phenotype. *Proc Natl Acad Sci USA*. (2006) 103:4445–50. doi: 10.1073/pnas.0509944102
41. Meaburn KJ, Misteli T. Locus-specific and activity-independent gene repositioning during early tumorigenesis. *J Cell Biol*. (2008) 180:39–50. doi: 10.1083/jcb.200708204
42. Rodriguez-Fraticelli AE, Auzan M, Alonso MA, Bornens M, Martin-Belmonte F. Cell confinement controls centrosome positioning and lumen initiation during epithelial morphogenesis. *J Cell Biol*. (2012) 198:1011–23. doi: 10.1083/jcb.201203075
43. Pertuz S, Puig D, Garcia MA. Analysis of focus measure operators for shape-from-focus. *Pattern Recognit*. (2013) 46:1415–32. doi: 10.1016/j.patcog.2012.11.011
44. Briand P, Petersen OW, Van Deurs B. A new diploid nontumorigenic human breast epithelial cell line isolated and propagated in chemically defined medium. *In Vitro Cell Dev Biol*. (1987) 23:181–8. doi: 10.1007/BF02623578
45. Gudjonsson T, Ronnov-Jessen L, Villadsen R, Bissell MJ, Petersen OW. To create the correct microenvironment: three-dimensional heterotypic collagen assays for human breast epithelial morphogenesis and neoplasia. *Methods*. (2003) 30:247–55. doi: 10.1016/S1046-2023(03)00031-8
46. Sung KE, Yang N, Pehlke C, Keely PJ, Eliceiri KW, Friedl A, et al. Transition to invasion in breast cancer: a microfluidic *in vitro* model enables examination of spatial and temporal effects. *Integr Biol*. (2011) 3:439–50. doi: 10.1039/C0IB00063A
47. Dumont N, Liu B, Defilippis RA, Chang H, Rabban JT, Karnezis AN, et al. Breast fibroblasts modulate early dissemination, tumorigenesis, and metastasis through alteration of extracellular matrix characteristics. *Neoplasia*. (2013) 15:249–62. doi: 10.1593/neo.121950
48. Meng Q. Three-dimensional culture of hepatocytes for prediction of drug-induced hepatotoxicity. *Expert Opin Drug Metab Toxicol*. (2010) 6:733–46. doi: 10.1517/17425251003674356
49. Kuratnik A, Giardina C. Intestinal organoids as tissue surrogates for toxicological and pharmacological studies. *Biochem Pharmacol*. (2013) 85:1721–6. doi: 10.1016/j.bcp.2013.04.016
50. Grabinger T, Luks L, Kostadinova F, Zimberlin C, Medema JP, Leist M, et al. *Ex vivo* culture of intestinal crypt organoids as a model system for assessing cell death induction in intestinal epithelial cells and enteropathy. *Cell Death Dis*. (2014) 5:e1228. doi: 10.1038/cddis.2014.183
51. Rocco SA, Koneva L, Middleton LYM, Thong T, Solanki S, Karram S, et al. Cadmium exposure inhibits branching morphogenesis and causes alterations consistent with HIF-1α inhibition in human primary breast organoids. *Toxicol Sci*. (2018) 164:592–602. doi: 10.1093/toxsci/kfy112

Conflict of Interest: KB and P-AV have filed a patent application on the use of the radial profile method to measure epithelial polarity.

The remaining 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 in Oncology

MINI REVIEW
published: 24 January 2020
doi: 10.3389/fonc.2020.00021

The Menstrual Cycle and Risk of Breast Cancer: A Review

Håkan Lars Olsson[1]* *and Mona Landin Olsson*[2]

[1] Departments of Oncology and Cancer Epidemiology, Clinical Sciences, Lund University, Lund, Sweden, [2] Department of Endocrinology, Clinical Sciences, Lund University, Lund, Sweden

Cyclic hormonal stimulation of the breast tissue plays a significant role in breast carcinogenesis. Current risk factor models do not include direct measures of cycle characteristics although the effects of possible surrogates of cycle activity such as age at menarche and menopause, parity, and nursing time have been investigated. Future risk models should also include menstrual cycle length, regularity, number of cycles before first full-term pregnancy, and life-time number of cycles. New risk factor models for pre- and postmenopausal breast cancer are proposed here. Furthermore, there is a need for more long-term, prospective studies investigating menstrual cycle characteristics as data currently available are primarily retrospective and collected at one time-point only.

Keywords: breast cancer, menstrual cycle, risk, retrospective, prospective

OPEN ACCESS

Edited by:
Mary Beth Terry,
Columbia University, United States

Reviewed by:
Azin Nahvijou,
Tehran University of Medical Science, Iran
Ahmadreza Niavarani,
Tehran University of Medical Sciences, Iran
Jasmine A. McDonald,
Columbia University, United States

***Correspondence:**
Håkan Lars Olsson
hakan.olsson@med.lu.se

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

Received: *19 August 2019*
Accepted: *08 January 2020*
Published: *24 January 2020*

Citation:
Olsson HL and Olsson ML (2020) The Menstrual Cycle and Risk of Breast Cancer: A Review. Front. Oncol. 10:21. doi: 10.3389/fonc.2020.00021

BACKGROUND

In the 1990s, our research group pioneered studies on menstrual cycle length, menstrual regularity, and the number of menstrual cycles as risk factors for breast cancer (1, 2). Women who developed breast cancer were more likely to have short, regular cycles, and had more cycles before the first full-term pregnancy than healthy women and those with benign breast disease. As the luteal phase is fixed in time, only the follicular phase may vary, thus exposing women with shorter, and more numerous cycles to higher amounts of progesterone during the luteal phase (3). We and others have also shown a greater number of dividing epithelial cells in the luteal phase than in the follicular phase (4–6). Cell division is generally considered a prerequisite for carcinogenesis and women with short and numerous cycles may therefore have a higher risk of developing cancer as a result of increased cell proliferation. Although progesterone protects against endometrial cancer, it appears to have a different effect in increasing breast cancer risk (7). This was confirmed by recent findings investigating breast cancer type 1 susceptibility protein (BRCA1) carcinogenesis, the roles of progesterone and receptor activator of nuclear factor kappa-B ligand (RANKL), and the therapeutic potential of anti-progestins (8, 9).

Furthermore, several studies regarding the risk of exogenous hormones and breast cancer revealed that the combination of progestins and estrogen increased the risk of breast cancer compared with the effects of estrogen alone (10–13). We also showed that shorter menstrual cycles were associated with the cytochrome P450 17 (CYP17) genotype (14).

A list of studies concerning the menstrual cycle is presented in **Table 1** (15–25). These studies indicate that a high number of cycles before the first full-term pregnancy and high life-time menstrual activity (LMA) increased breast cancer risk. Furthermore, a short time interval between menarche and the establishment of regular cycles is another risk factor. In contrast, no relationship was observed between the length of menstrual bleeding and breast cancer (26). Of the studies listed in **Table 1** two (16, 20) included only Asian women and one (24) only American African women.

TABLE 1 | Studies of different menstrual cycle characteristics and breast cancer risk.

Study / year	Type of study	Main effect					
		Short cycles	Long cycles	NC<AFFP	LMA	Regularity	Comment
Olsson et al. (1)	Case-control	+	–	na	na	+	
Olsson et al. (2)	Case-control	+	–	+	na	+	
Bernstein et al. (15)	Case-control	na	na	na	(+)	na	
Yuan et al. (16)	Case-control	+	0	na	na	0	
Rautalahti et al. (17)	Case-control	na	na	na	+	na	
Whelan et al. (18)	Cohort	+	+	na	+	na	Also effect of long cycles
den Tonkelaar et al. (19)	Case-control	na	na	na	+	+	
Chie et al. (20)	Case-control	na	na	+	na	na	
Titus-Ernstoff et al. (21)	Case-control	0	0	Increased risk if short time between puberty to menstrual regularity Reduced risk if early surgical menopause			
Garland et al. (22)	Cohort	–	–	na	+	+	
Clavel-Chapelon and E3N Group (23)	Case-control	na	na	+	+	na	
Beiler et al. (24)	Case-control	+	–	na	na	na	
Chaves-MacGregor et al. (25)	Case-control	na	na	+	+	+	

Short cycles, average cycle in general <26 days; Long cycles, average cycle in general longer than 33 daysl NC<AFFP, number of menstrual cycles before first full term pregnancy; LMA, life time menstrual activity or number of life time cycles; Regularity, regular menstrual cycles; na, not assessed; +, increased risk; –, decreased risk; 0, neutral findings.

LMA is calculated for natural cycles using the following variables: age at menopause and menarche, average cycle length, number of pregnancies, and duration of nursing excluding periods of exogenous hormone use. There are however a number of relevant caveats: first, cycle length may vary during reproductive life and studies thus consider the average cycle length. In retrospective studies, there may be a recall bias for cycle length. Furthermore, there are discrepancies regarding the number of cycles counted during exogenous hormonal treatment (27, 28). In addition, there are few high-quality, long-term (life-time) prospective studies investigating cycle length. In this context and in support of the importance of LMA, it is notable that early menopause or castration protect against breast cancer. Other factors such as extreme physical activity and starvation reduce cyclic activity and thus breast cancer risk (29). Finally, the consistency in results regarding cycle length, the number of cycles before the first full-term pregnancy, and LMA indicate that the crude retrospective assessment of menstrual cycles has an important bearing on investigating breast cancer risk.

Benign breast disease is characterized by irregular menstrual cycles and is more common at the end of reproductive life (1). Irregular cycles cause cystic disease in the breasts and ovaries and women with cystic ovarian disease therefore have a lower incidence of breast cancer (30).

We have postulated that women whose breast size is maintained or increased after hormonal exposure may have a higher risk of cancer than those whose breast size decreases upon such exposure (31). However, this hypothesis requires further investigation of the menstrual cycle. Possible assessment of breast density or magnetic resonance imaging (MRI) images without contrast assessing fibroglandular density may be helpful (32).

Finally, the effects of oral contraceptive (OC) use should be investigated. For example, it is unclear whether lengthening menstrual cycles artificially via administration of OCs in women with naturally short cycles decreases cancer risk. Conversely, it is also unclear whether cancer risk increases in women whose naturally long cycles are artificially shortened by the use of OCs.

A number of risk factors have been identified for breast cancer such as age at menarche, age at first full term pregnancy, parity, age at menopause, obesity (postmenopausal risk), number of menstrual cycles, weight gain, hormone replacement therapy, early oral contraceptive use, breast size, preecclampsia, birth weight, nursing, height, breast density, physical activity, night shift work, radiation exposure, tobacco use, alcohol use, family history, mutation carrier of a predisposing gene. Some of the above factors are still under investigation with partly diverging findings such as for tobacco use, breast size and night shift work and others like preecclampsia and high physical activity are protective. Some factors like radiation exposure, reproductive and genetic factors are more important premenopausally, while obesity is more important for older women.

Development of better methods to describe the menstrual cycle more exact is needed. One method is of course to use a calendar recording the start of each menstruation, another way is to record basal body temperature daily, women in the luteal phase have a higher body temperature, or study the cervical mucus. However, it can be difficult to pinpoint ovulation using these methods, especially if your menstrual cycles are irregular. Research in fertility medicine especially in women with irregular menstruations is mainly driven to better time ovulation through ovulation prediction kits either using urine (measuring LH) or saliva (studying ferning patterns in relation to estrogen increase). Again these latter methods

TABLE 2 | Revised risk factor models for breast cancer taking the menstrual cycle into account.

Classic	Revised premenopausal	Revised postmenopausal
Family history	Family history	Family history
Germline mutations	Germline mutations	Germline mutations
Polygenic risk score	Polygenic risk score	Polygenic risk score
Breast density	Breast density	(Breast density)
Age at menarche	NC<AFFP	LMA
AFFP	(parity, AFFP)	(parity, AFFP)
Age at menopause	OC use	HRT use
HRT use	Regular cycles	Regular cycles
	Physical activity	Weight/weight gain

NC<AFFP, number of menstrual cycles before first full term pregnancy; LMA, life time menstrual activity or number of life time cycles.
AM, age at menarche; AAFP, age at first full term pregnancy; OC use, oral contraceptive use; HRT use, hormone replacement therapy use.

are too cumbersome and expensive to be used in large epidemiological risk factor studies and explain their absence in literature.

CONCLUSION AND PROPOSAL

The characteristics and number of menstrual cycles before the first full-term pregnancy, LMA, and menstrual regularity require further investigation as part of epidemiological studies of breast cancer, as other risk factors such as age at menarche and menopause, parity, and nursing are only surrogates for cyclic hormonal exposure. Menstrual cycle characteristics should be included in risk factor models of breast cancer. Current models such as Gail, Tyrer-Cusick, Rosner Colditz BCRAT, BCPRO, and BOADICEA only include family history, germline mutation status, breast density, polygenic risk scores, and surrogates of cycle activity such as age at menarche, age at first full-term pregnancy (AFFP), parity, nursing, and age at menopause (33–39). The BOADICEA and Tyrer-Cusick models appear to be the most informative (39). Parity and AFFP may exert independent effects on differentiation of the breast epithelium, and are indirectly related to menstrual cycle activity. However, cyclic hormonal stimulation of the breast tissue, which is probably the most important hormonal factor contributing to breast cancer, is not directly investigated in such models. Proposed revised risk factor models for pre- and postmenopausal breast cancer are listed in **Table 2**. Only surrogates such as age at menarche, AFFP, parity, and nursing have been included in previous studies. Prospective life-time studies on menstrual cycle activity are encouraged, as current studies primarily include retrospective data collected at one time-point and often use average measures of menstrual factors. Studies covering longer time periods should include other factors of importance for the menstrual cycle such as physical activity, obesity, psychological stress, and intercurrent diseases such as osteoporosis (29).

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 grants from the Swedish Cancer Society, the Berta Kamprad Foundation, and local hospital funds.

REFERENCES

1. Olsson H, Landin-Olsson M, Gullberg B. Retrospective assessment of menstrual cycle length in patients with breast cancer, in patients with benign breast disease, and in women without breast disease. *J Natl Cancer Inst*. (1983) 70:17–20.
2. Olsson H, Ranstam J, Landin-Olsson M. The number of menstrual cycles prior to the first full-term pregnancy: an important risk factor of breast cancer? *Acta Radiol Oncol*. (1987) 26:387–9. doi: 10.3109/02841868709104365
3. Atashgaran V, Wrin J, Barry SC, Dasari P, Ingman WV. Dissecting the biology of menstrual cycle-associated breast cancer risk. *Front Oncol*. (2016) 6:267. doi: 10.3389/fonc.2016.00267
4. Ferguson D, Anderson T. Morphological evaluation of cell turnover in relation to the menstrual cycle in the "resting" human breast. *Br J Cancer*. (1981) 44:177. doi: 10.1038/bjc.1981.168
5. Olsson H, Jernstrom H, Alm P, Kreipe H, Ingvar C, Jonsson PE, et al. Proliferation of the breast epithelium in relation to menstrual cycle phase, hormonal use, and reproductive factors. *Breast Cancer Res Treat*. (1996) 40:187–96. doi: 10.1007/BF01806214
6. Huh SJ, Oh H, Peterson MA, Almendro V, Hu R, Bowden M, et al. The proliferative activity of mammary epithelial cells in normal tissue predicts breast cancer risk in premenopausal women. *Cancer Res*. (2016) 76:1926–34. doi: 10.1158/0008-5472.CAN-15-1927
7. Brisken C. Progesterone signalling in breast cancer: a neglected hormone coming into the limelight. *Nat Rev Cancer*. (2013) 13:385–96. doi: 10.1038/nrc3518
8. Hu H, Wang J, Gupta A, Shidfar A, Branstetter D, Lee O, et al. RANKL expression in normal and malignant breast tissue responds to progesterone and is up-regulated during the luteal phase. *Breast Cancer Res Treat*. (2014) 146:515–23. doi: 10.1007/s10549-014-3049-9
9. Tanos T, Sflomos G, Echeverria PC, Ayyanan A, Gutierrez M, Delaloye J-F, et al. Progesterone/RANKL is a major regulatory axis in the human breast. *Sci Transl Med*. (2013) 5:182. doi: 10.1126/scitranslmed.3005654
10. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *J Am Med Assoc*. (2002) 288:321–33. doi: 10.1001/jama.288.3.321
11. Olsson HL, Ingvar C, Bladstrom A. Hormone replacement therapy containing progestins and given continuously increases breast carcinoma risk in Sweden. *Cancer*. (2003) 97:1387–92. doi: 10.1002/cncr.11205
12. Beral V, Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet*. (2003) 362:419–27. doi: 10.1016/S0140-6736(03)14596-5
13. Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, et al. Women's health initiative steering committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *J Am Med Assoc*. (2004) 291:701–12. doi: 10.1001/jama.291.14.1701
14. Henningson M, Johansson U, Borg A, Olsson H, Jernstrom H. CYP17 genotype is associated with short menstrual cycles, early oral contraceptive

use and BRCA mutation status in young healthy women. *Mol Hum Reprod*. (2007) 13:231–6. doi: 10.1093/molehr/gam004
15. Bernstein L, Ross RK, Lobo RA, Hanisch R, Krailo MD, Henderson BE. The effects of moderate physical activity on menstrual cycle patterns in adolescence: implications for breast cancer prevention. *Br J Cancer*. (1987) 55:681–5. doi: 10.1038/bjc.1987.139
16. Yuan JM, Yu MC, Ross RK, Gao YT, Henderson BE. Risk factors for breast cancer in Chinese women in Shanghai. *Cancer Res*. (1988) 49:1949–53.
17. Rautalahti M, Albanes D, Virtamo J, Palmgren J, Haukka J, Heinonen OP. Lifetime menstrual activity – Indicator of breast cancer risk. *Eur J Epidemiol*. (1993) 9:17–25. doi: 10.1007/BF00463085
18. Whelan EA, Sandler DP, Root JL, Smith KR, Weinberg CR. Menstrual cycle patterns and risk of breast cancer. *Am J Epidemiol*. (1994) 140:1081–90. doi: 10.1093/oxfordjournals.aje.a117208
19. den Tonkelaar I, de Waard F. Regularity and length of menstrual cycles in women aged 41-46 in relation to breast cancer risk: results from the DOM-project. *Breast Cancer Res Treat*. (1996) 38:253–8. doi: 10.1007/BF01806143
20. Chie W, Fu C, Lee W, Li C, Huang C, Chang K, et al. Ages at different reproductive events, numbers of menstrual cycles in between and breast cancer risk. *Oncol Rep*. (1997) 4:1039–43. doi: 10.3892/or.4.5.1039
21. Titus-Ernztoff L, Lonenecker MP, Newcomb PA, Dain B, Greenberg ER, Mittendorf R, et al. Menstrual factors in relation to breast cancer risk. *Cancer Epidemiol Biomark Prev*. (1998) 7:783–9.
22. Garland M, Hunter DJ, Colditz GA, Manson JE, Stampfer MJ, Spiegelman D, et al. Menstrual cycle characteristics and history of ovulatory infertility in relation in breast cancer risk in a large cohort of US women. *Am J Epidemiol*. (1998) 147:636–43. doi: 10.1093/oxfordjournals.aje.a009504
23. Clavel-Chapelon F, E3N Group. Cumulative number of menstrual cycles and breast cancer risk: results from the E3N cohort study of French women. *Cancer Cause Control*. (2002) 13:831–8. doi: 10.1023/A:1020684821837
24. Beiler JS, Zhu K, Hunter S, Payne-Wilks K, Roland CL, Chinchilli VM. A case-control study of menstrual factors in relation to breast cancer risk in African-American women. *J Natl Med Assoc*. (2003) 95:930–8.
25. Chavez-MacGregor M, Elias SG, Onland-Moret NC, van der Schouw YT, Van Gils CH, Monninkhof E, et al. Postmenopausal breast cancer risk and cumulative number of menstrual cycles. *Cancer Epidemiol Biomark Prev*. (2005) 14:799–804. doi: 10.1158/1055-9965.EPI-04-0465
26. Butler LM, Potischman NA, Newman B, Millikan RC, Brogan D, Gammon MD, et al. Menstrual risk factors and early-onset breast cancer. *Cancer Cause Control*. (2000) 11:451–8. doi: 10.1023/A:1008956524669
27. Gorrindo T, Lu Y, Pincus S, Riley A, Simon JA, Singer BH, et al. Lifelong menstrual histories are typically erratic and trending: a taxonomy. *Menopause*. (2007) 14:74–88. doi: 10.1097/01.gme.0000227853.19979.7f
28. Small CM, Manatunga AK, Marcus M. Validity of self-reported menstrual cycle length. *Ann Epidemiol*. (2007) 17:163–70. doi: 10.1016/j.annepidem.2006.05.005
29. Harlow SD, Ephross SA. Epidemiology of menstruation and its relevance to women's health. *Epidemiol Rev*. (1995) 17:265–86. doi: 10.1093/oxfordjournals.epirev.a036193
30. Bosetti C, Scotti L, Negri E, Talamini R, Levi F, Franceschi S, et al. Benign ovarian cysts and breast cancer risk. *Int J Cancer*. (2006) 119:1679–82. doi: 10.1002/ijc.22016
31. Olsson H. Risk for malignant tumors after oral contraceptive use: is it related to organ size while taking the pill? *Med Oncol Tumor Pharmacother*. (1990) 7:61–4.
32. Grubstein A, Rapson Y, Benzaquen O, Rozenblatt S, Gadiel I, Atar E, et al. Comparison of background parenchymal enhancement and fibroglandular density at breast magnetic resonance imaging between BRCA gene mutation carriers and non-carriers. *Clin Imaging*. (2018) 51:347–51. doi: 10.1016/j.clinimag.2018.06.010
33. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst*. (1989) 81:1879–86. doi: 10.1093/jnci/81.24.1879
34. Tyrer J, Duffy SW, Cuzick JA. Breast cancer prediction model incorporating familial and personal risk factors. *Stat Med*. (2004) 23:1111–30. doi: 10.1002/sim.1668
35. Rosner B, Colditz GA. Nurses' Health Study: log-incidence mathematical model of breast cancer incidence. *J Natl Cancer Inst*. (1996) 88:359–64. doi: 10.1093/jnci/88.6.359
36. Pharoah PD, Antoniou AC, Easton DF, Ponder BA. Polygenes, risk prediction, and targeted prevention of breast cancer. *N Engl J Med*. (2008) 358:2796–803. doi: 10.1056/NEJMsa0708739
37. Brentnall AR, Cuzick J, Buist DSM, Bowles EJA. Long-term accuracy of breast cancer risk assessment combining classic risk factors and breast density. *JAMA Oncol*. (2018) 4:e180174. doi: 10.1001/jamaoncol.2018.0174
38. Lee A, Mavaddat N, Wilcox AN, Cunningham AP, Carver T, Hartley S, et al. BOADICEA: a comprehensive breast cancer risk prediction model incorporating genetic and nongenetic risk factors. *Genet Med*. (2019) 21:1708–18. doi: 10.1038/s41436-018-0406-9
39. Terry MB, Liao Y, Whittemore AS, Leoce N, Buchsbaum R, Zeinomar N, et al. 10-year performance of four models of breast cancer risk: a validation study. *Lancet Oncol*. (2019) 20:504–17. doi: 10.1016/S1470-2045(18)30902-1

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 in Medicine

MINI REVIEW
published: 05 February 2020
doi: 10.3389/fmed.2019.00325

Cost-Effectiveness of Lifestyle-Related Interventions for the Primary Prevention of Breast Cancer: A Rapid Review

Martine Bellanger[1,2,3]*, *Katharine Barry*[1], *Juwel Rana*[1,4] *and Jean-Philippe Regnaux*[1,5]

[1] MOS Research Unit, Department of Social Sciences, Ecole des Hautes Etudes en Sante Publique, Rennes, France, [2] Institut de Cancerologie de l'Ouest, Nantes, France, [3] International Breast Cancer and Nutrition Project, Lafayette, LA, United States, [4] Department of Biostatistics and Epidemiology, University of Massachusetts Amherst, Amherst, MA, United States, [5] Center CRESS - INSERM U1153, EpiAgeing Team, Paris, France

Background: In 2018, the global estimate of newly diagnosed breast cancer cases among women totaled 2.1 million. The economic and social burden that breast cancer places on societies has propelled research that analyzes the role of modifiable risk factors as the primary prevention methods. Healthy behavior changes, moderated alcohol intake, healthy body weight, and regular physical activity may decrease the risk of breast cancer among women. This review aimed to synthesize evidence on the cost-effectiveness of lifestyle-related interventions for the primary prevention of breast cancer in order to answer the question on whether implementing interventions focused on behavior changes are worth the value for money.

Methods: A rapid review was performed using search terms developed by the research team. The articles were retrieved from MEDLINE and the Tufts Medical Center Cost-Effectiveness Analysis Registry, with an additional web search in Google and Google Scholar. Comparisons were performed on the cost-effectiveness ratio per quality-adjusted life-year between the interventions using a league table, and the likelihood of cost-effective interventions for breast cancer primary prevention was analyzed.

Results: Six studies were selected. The median cost-effectiveness ratio (in 2018 USD) was $24,973, and 80% of the interventions had a ratio below the $50,000 threshold. The low-fat-diet program for postmenopausal women was cost-effective at a societal level, and the physical activity interventions, such as the Be Active Program in the UK, had the best cost saving results. A total of 11 of the 25 interventions ranked either as highly or very highly likely to be cost-effective for breast cancer primary preventions.

Conclusion: Although the review had some limitations due to using only a few studies, it showed evidence that diet-related and physical-activity-related interventions for the primary prevention of breast cancer were cost-effective. Many of the cost-effective interventions aimed to reduce the risk of non-communicable diseases alongside breast cancer.

Keywords: breast cancer, primary prevention, cost-effectiveness, lifestyle, behavior

OPEN ACCESS

Edited by:
Isabelle Durand-Zaleski, Assistance Publique Hopitaux De Paris (AP-HP), France

Reviewed by:
Gillian Bartlett, McGill University, Canada
Salah Ghabri, University of Rennes 1, France
Giovanni Fattore, Bocconi University, Italy

***Correspondence:**
Martine Bellanger
martine.bellanger@ehesp.fr

Specialty section:
This article was submitted to Family Medicine and Primary Care, a section of the journal Frontiers in Medicine

Received: *24 March 2019*
Accepted: *19 December 2019*
Published: *05 February 2020*

Citation:
Bellanger M, Barry K, Rana J and Regnaux J-P (2020) Cost-Effectiveness of Lifestyle-Related Interventions for the Primary Prevention of Breast Cancer: A Rapid Review. Front. Med. 6:325. doi: 10.3389/fmed.2019.00325

INTRODUCTION

Breast cancer has been ranked as the leading cause of cancer deaths in over 100 countries, accounting for 11.6% of all cancer deaths worldwide (1, 2). In 2018, 2.1 million women were newly diagnosed with breast cancer, and an estimated 626,679 women died due to breast cancer (2). Economically, breast cancer has been associated with increased healthcare costs and productivity losses (1–5). Among 27 European Union countries, breast cancer had the second largest share of overall cancer costs (12%), after lung cancer (15%) (€126 billion in 2009) (3). Low- and middle-income countries have experienced disproportionately high amounts of productivity loss, incidence, and mortality of women due to breast cancer (1, 3, 4). In 2012, breast cancer was found to contribute to the highest productivity loss among women in all but one BRICS countries (Brazil, Russia, India, China, and South Africa), representing 0.33% of their gross domestic product (4).

In recent years, the role of modifiable health behaviors in cancer prevention has been extensively studied (5–9). Associations were found between an increased risk in breast cancer and various lifestyle factors such as alcohol consumption, physical inactivity, exogenous hormone use, and excessive exposure to ionizing radiation (2). A research study which combined over 53 analyses on the links between alcohol and breast cancer onset found that with each increase of 10 g of daily alcohol consumption, women increased their risk for developing breast cancer by 7% (10). Over 100 studies which observed the association between weight and fat distribution and the development of breast cancer have found that women who are overweight or obese have 30–50% higher risk of developing postmenopausal breast cancer compared to women with a normal body mass index (BMI) (1, 5). An estimated 2.7 billion US dollars (USD) was spent on healthcare costs worldwide due to breast cancer that is attributed to physical inactivity (1, 3, 4).

To reduce the risk of breast cancer, primary prevention measures can focus on women who adopt healthy behaviors such as maintaining a normal weight, breastfeeding, minimizing alcohol consumption, eating a balanced diet, reducing stress, and decreasing the use of long-term hormone replacement therapy (11–14). Over 20 weight loss support programs have shown success in reducing the risk of breast cancer among postmenopausal participants by helping these women reach a normal BMI (8, 12).

The control of breast cancer through both early detection and primary prevention is of high priority in order to decrease the incidence and the premature mortality among women and to reduce the economic losses worldwide (11, 15). It is important to shed light on the benefits of investing in the primary prevention for breast cancer. Cost-effectiveness analysis can help in showing how to get the most of the available resources. A few published reviews on the cost-effectiveness of cancer interventions include the prevention strategies for breast cancer such as screening and chemoprevention, but lifestyle-related interventions were not included (16–19).

Our study aimed to review and synthesize the evidence on the cost-effectiveness of lifestyle-related interventions for the primary prevention of breast cancer. The objective of this review was to provide up-to-date evidence on the cost-effectiveness of the breast cancer prevention interventions focused on healthy weight programs, balanced diet interventions, physical activity (PA) programs, limited alcohol consumption interventions, and tobacco cessation programs. A rapid review approach, which aims to systematically synthesize the available evidence within a "limited time and resource framework," was adopted to summarize the relevant information (20–23).

METHODS

Rationale for a Rapid Review

Systematic reviews provide a rigorous and reproducible method to collect and summarize the available current evidence in the literature. They require very intensive resources and time to be conducted. They often fail to answer the research question when no or little relevant evidence is available. Rapid reviews have emerged as an alternative to address this issue. They are a novel form of systematic review which aim to produce faster and relevant evidence following the same methodological steps of a systematic review (24). They are useful to synthetize evidence for new or emerging research topics as well as to update previous reviews. Different approaches to conduct rapid reviews have been described (20–23). However, there is no recommendation on which shortcuts to use to conduct a rapid review faster than a systematic review. These may include: (1) more targeted research questions, (2) limited set of data sources searched, and (3) the use of only one reviewer for the study selection and/or the data extraction process. The finding synthesis is made of a descriptive/narrative summary instead of a qualitative summary plus meta-analysis (20–23).

Protocol and Registration

A pre-specified review protocol was developed and followed for all of the methods (MB, JPR, and KB). The Preferred Reporting Items for Systematic Review (PRISMA) guidelines were used to report our findings (25).

Information Sources and Search Strategy

The studies were identified using electronic databases. We searched MEDLINE via PubMed from its database inception until January 2019. A second database, the Tufts Medical Center Cost-Effectiveness Analysis Registry (www.cearegistry.org), was searched from 2014 to 2017 since a systematic review performed by Winn et al. summarized evidence on the cost-utility analysis of cancer prevention and treatment with studies dated up to 2013 (19). That systematic review was identified in the studies retrieved from the Medline search. We hand-searched reference lists from all of the studies and review articles included. Additional literature was searched using Google and Google Scholar.

The search terms were developed by the research team in collaboration with a faculty librarian. We used the following Population, Intervention, Comparison, Outcome (PICO) framework to identify the relevant terms: P: breast

cancer, I: primary prevention, and O: cost-benefit analysis. The complete MEDLINE search strategy is presented in **Supplementary Table 1**. The search query was developed using index vocabulary (MESH) and free-text words. To test the search equation, we manually identified four relevant studies, and then based on the results of the testing search, we modified the final strategy to ensure that the relevant titles were included.

Inclusion and Exclusion Criteria

To be included, the studies had to fulfill the PICO framework:

(1) Populations: Adult women aged 16 years and older with no diagnosed breast cancer.
(2) Interventions: Studies considering lifestyle-related primary prevention interventions such as dietary interventions, weight-loss-related interventions, PA interventions or physical exercise programs, alcohol consumption reduction interventions, and/or tobacco use reduction programs. The interventions were identified and informed based on international literature and previous studies (26–29). Studies related to early detection and diagnosis testing, chemoprevention (such as raloxifene or tamoxifen), surgical interventions (such as mastectomy), and ionizing radiation were excluded since the review focused on the lifestyle-related interventions. All interventions conducted on women diagnosed with breast cancer (i.e., tertiary prevention) were also excluded.
(3) Comparators: Women without interventions, women with standard care or status quo, such as usual diet or current practice for PA, also called "usual care."
(4) Outcomes: The primary outcomes of the cost-effectiveness analysis were the costs and the quality-adjusted life-years (QALYs) or the disability-adjusted life-years (DALYs) and the incremental cost-effectiveness ratio (ICER) that considers the change in the costs and the effects of interventions on breast cancer, including other non-communicable diseases (NCDs) or not, compared to the status quo.
(5) Study design: We applied no restriction on the type of study eligible for this review. We excluded any reports without results. We did not consider published letters or comments to be included.

Only the articles published in English were considered for this review.

Selection of Sources of Evidence

All search results were imported and de-duplicated using Covidence Software (https://www.covidence.org). The title/abstracts and the full text were screened by two reviewers (JPR and MB). One reviewer (MB) screened all of the abstracts and the full text of the relevant references. A second reviewer (JPR) double-checked 15% (200/2,944) of the abstracts and inspected all of the full text of the rejected articles (185/191) to ensure that no relevant study was excluded. Disagreements were resolved after discussion.

Data Items and Data Extraction Process

Two reviewers (MB JR) extracted data from the studies included. The data extraction form was piloted and modified as required based on the feedback from the team. The data were extracted from all of the studies included using a standardized template to capture optimal information. The extracted data about the general information of the published studies was collected in an EXCEL spreadsheet.

Critical Appraisal of Individual Sources of Evidence

The quality of the selected studies was assessed (MB, JR, and JPR) using the guidelines recommended by Drummond and Jefferson for cost-effectiveness analysis studies (30). The quality of the study was determined by analyzing three categories: (1) study design, (2) data collection methods (e.g., model input such as outcome measures, cost components, and estimates), and (3) interpretation of results (e.g., time horizon, discount rates, sensitivity analysis, including probabilistic sensitivity analysis, and relevance of alternatives compared). To rate the quality of the evidence, we used a three-point scale for each item, as suggested in previous studies by Gerard et al. and Zelle and Baltussen. The final percentage ranges were thus expressed, and the overall quality of the study was set as in Zelle and Baltussen (31, 32). Lastly, review commentaries from the Center for Reviews and Dissemination (CRD) of the University of York were also used to match our quality assessment (https://www.crd.york.ac.uk/). Of note is the fact that since there is no standardized method to critically appraise the quality of the studies included in a systematic review, we considered the guidelines recommended in the health economic evaluation as the most appropriate for our rapid review.

Synthesis of Results

We used a narrative synthesis to present the main findings of the studies and the different primary interventions selected. To compare the findings between studies, the non-USD cost-effectiveness ratios were converted into USD using the exchange rate factors for the price-year given in the studies. All ICERs were then inflated to 2018 USD based on the consumer price index from the Bureau of Labor Statistics (https://www.bls.gov/cpi/data.htm), as was done in previous studies (33). Median ICERs were estimated after inflation adjustment. A cost-effectiveness league table was constructed to present the ICER of the primary health interventions evaluated (34). The likelihood level of the cost-effectiveness of the intervention for breast cancer alone was estimated by extrapolating the incremental QALY required to get an ICER equal to $50,000, the most common WTP threshold used for the cost-effective strategies. Reductions in breast cancer incidence and breast cancer risk as well as the utilities associated with health states were analyzed. The interventions selected were those with high or very high likelihood levels of cost-effectiveness.

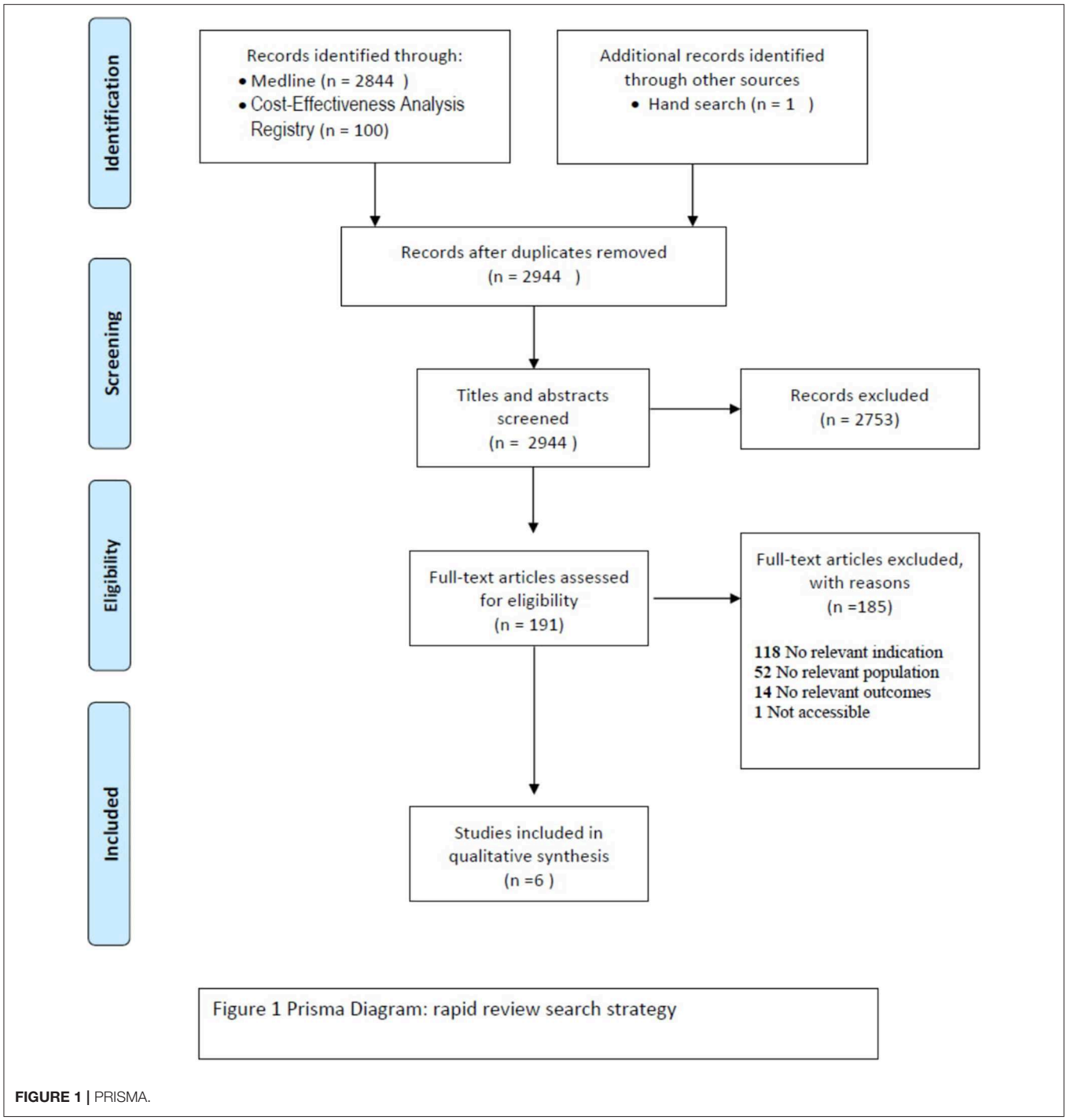


FIGURE 1 | PRISMA.

RESULTS

Search Strategy and Study Identification

The first step of the literature search for the primary prevention of breast cancer identified 2,955 references according to the outlined criteria above (**Figure 1**). The screening of titles and abstracts left 191 full texts to be examined. Further selection resulted in the exclusion of 185 studies that were ineligible for different reasons, such as irrelevant indication to our research question ($n = 118$), irrelevant population ($n = 52$), and irrelevant outcome measure ($n = 14$). One full text was not accessible. Six studies were considered for the qualitative analysis. Also, we found one protocol which analyzes the impact and the cost-effectiveness of the lifestyle interventions for breast cancer, but the results of this study will not be published until the end of 2019 (35).

Characteristics of the Studies Included

The six studies included were published between 2007 and 2014. All of the studies were conducted in high-income countries (HICs): two studies were from the USA, and one study each was from Australia, Belgium, Netherlands, and UK (**Supplementary Table 2**). Two types of primary prevention-related interventions were evaluated: PA ($n = 5$) and diet ($n = 2$) (36–41).

Breast cancer was the primary focus of prevention, along with ovarian cancer only, in Bós et al. who analyzed the cost-effectiveness of a low-fat diet on these two cancers (37). In five of the studies, breast cancer was among other non-communicable diseases (NCDs), such as coronary heart disease, diabetes, stroke, and colorectal cancer, targeted by the primary prevention interventions, and it was included in the cost-effectiveness model (**Supplementary Table 2**).

All of the PA-related studies were carried out in a community setting, except for one study which combined PA and diet in a secondary care setting. There were three types of study designs: hypothetical cohorts, closed cohorts of a given population, and randomized control trials (RCTs). The adult populations with ages from 16 to 30, as well as the populations aged 50 and above, were the most commonly targeted groups (36, 38, 39, 41). However, menopausal women were targeted for the primary prevention of breast and ovarian cancer (**Supplementary Table 2**) (37, 40). The PA strategies compared no intervention or "usual care" to one or up to six strategies in one study (41). The inter-strategy comparison was made by Peels et al. (40).

All studies were either cost-effectiveness analyses ($n = 5$) or cost-utility analyses ($n = 1$) based on Markov models ($n = 6$). The model inputs (i.e., outcomes, utility values, and costs) were derived from RCTs ($n = 3$), from literature ($n = 4$), and from national databases ($n = 3$). A natural experiment was used in Frew et al. (39) (**Supplementary Table 2**).

In all studies, the reported costs and benefits were combined in an ICER ($n = 5$) or an incremental cost per utility ratio (ICUR) ($n = 1$). The additional costs per QALY gained were estimated in most studies. Only (38) estimated the ICER per DALY for the diet and exercise interventions. Final estimates were available in the country currency and price-year ($n = 5$). The time horizon used in the studies varied from 5 years to the lifetime horizon of the population studied. Different time horizons were used in the sensitivity analysis. In all studies but one, the cost-effectiveness analysis was presented from the perspective of the society, and in half of the studies, both the society and the healthcare payer perspectives were included. Society WTP thresholds are presented (**Supplementary Table 2**).

Study Quality

Table 1 presents the quality of the six studies included, ranging from 74 to 89%. Bós et al. ranked the highest score for very good quality, followed by Frew et al. and Peels et al. (37, 39, 40), while the lowest score was found for Annemans et al. (36). All studies underperformed in category 2 ("data collection"). For instance, information on some model parameter sources was insufficient or not easily accessible, and total resource estimates were not reported separately from their unit costs and quantities for indirect costs. For domain 3 ("result analysis and interpretation"), the full score was not reached, mostly due to insufficient relevant alternative comparisons, except in Peels et al. (40). The price-year was not available only in one study, which hampers any inflation-adjusted estimation and comparison with the other interventions (36).

Lastly, our quality assessment for the four studies published between 2007 and 2011 fit the assessment published by the CRD from the National Institute for Health Research. For the two studies published in 2014, our assessment fit the expected findings based on the available positive pre-review.

Cost-Effectiveness Findings

The median cost-effectiveness (in 2018 USD) reported in the four studies, of which ICER/QALY was estimated and for which the price-year was available, was $24,973 (37, 39–41). From a societal perspective, 80% of the interventions had a ratio below $50,000 WTP threshold (as shown in **Table 2**). When the distribution across all of the interventions was assessed (i.e., including healthcare payer and society perspectives), 75% of the cost-effective ratios were below $50,000, 18% were between $50,000 and $100,000, and 7% were above $100,000.

The low-fat-diet program for postmenopausal women, which is the sole study focusing only on breast cancer and ovarian cancer, was cost-effective from a societal perspective (37). When looking at the age of the program start, women who enrolled at age 70 vs. age 50 with a high fat intake at baseline and a high risk of breast cancer had over three times higher cost-effectiveness ratio.

PA interventions targeting five major NCDs, including breast cancer, were ranked first in terms of their cost-effectiveness (39). Specifically, the Be Active Program in the UK had the best value for money or was cost-saving (39). The computer-tailored PA interventions implemented in Netherlands, as well as some community-based PA in the US, were also among the most cost-effective (**Table 2**) (40, 41).

A total of 11 out of 25 interventions were assessed as likely to be cost-effective for the primary prevention of breast cancer, and their likelihood levels of cost-effectiveness were ranked as very high or high (**Table 2**). The incremental QALYs required for the current incremental costs of the intervention related to breast and ovarian cancer to make the ICER at $50,000 were three to five times lower than the actual incremental QALYs (37). The same order of magnitude was found in Roux et al. and Peels et al. (40, 41). In the study of Frew et al., the "Be Active" program was shown to produce societal positive net benefit and also exhibited the highest chance for the PA program to be deemed cost-effective for breast cancer (39) (**Supplementary Table 3**).

DISCUSSION

Main Findings

This rapid review shows evidence of the cost-effectiveness of the diet-related interventions on breast cancer and ovarian cancer as well as the PA-related programs on breast cancer and other major NCDs. Our review also included interventions that addressed

TABLE 1 | Summary of quality assessment in percentage range[a].

References	Study design (14 points): research question, form of economic evaluation	Data collection (28 points): outcomes, costs, model, currency, and price	Result analysis and interpretation (26 points): time horizon, discount rate, sensitivity analysis, conclusions	Overall quality score	Final qualitative assessment[b]
Annemans et al. (36)	100	68–73	81–88	74–78	Good
Foster et al. (38)	100	68–73	88–96	82–88	Good
Roux et al. (41)	100	68–73	88–96	82–88	Good
Frew et al. (39)	100	68–73	92–100	84–89	Very good
Peels et al. (40)	100	68–73	92–100	84–89	Very good
Bós et al. (37)	100	54–58	92–100	84–89	Very good

[a] *The score was reduced with two points when a non-appropriate item in a domain was observed as done by Zelle and Balthussen (32).*
[b] *Final quality scoring adapted from Zelle and Balthussen as "poor quality (scoring 40–55%), good quality (scoring 55–70%), very good quality (scoring 71–85%), and excellent quality (scoring 86% or higher)" (32). The lowest bound of the score range gives the final quality level.*

breast cancer alongside other NCDs, such as coronary heart disease, stroke, diabetes, and colorectal cancer. Only one study differed from that approach, focusing only on two gynecological cancers (37). The benefits and value of primary prevention interventions in reducing the disease risk other than cancer and improving the overall quality of life have been documented (36, 38–41). The cost-effectiveness ratio for all of the studies included was estimated by calculating the overall cost-effectiveness of these multi-factorial interventions.

Estimating the cost-effectiveness of the lifestyle-related interventions only for breast cancer vs. the cost-effectiveness of these interventions for all NCDs would likely result in higher ICERs since, for the same change in costs, the differences in QALYs for breast cancer alone, in the denominator of the ICER, might be smaller. However, the favorable cost-effectiveness ratios of diet and PA-related interventions for all NCDs would remain below $50,000 per QALY for breast cancer alone. Despite our communication with the authors of these studies, we were not able to get the ICERs for breast cancer alone. For the low-fat-diet interventions, based on personal communication from Bós, favorable ICERs were found for breast cancer alone, and all were below the $50,000 threshold (37). The primary prevention strategies assessed in this analysis were congruent with other well-accepted public health strategies published in 2016 (19). These well-accepted interventions had a median cost-effectiveness ratio of $48,000 in 2014, which solely focused on drug therapy and mastectomy for breast cancer prevention. Some experts considered these therapies to be cost-effective, and societies incorporated them as one of the main strategies for breast cancer prevention (19, 33, 40).

The long-term effects of PA interventions have been shown to make the primary prevention interventions cost-effective, which is very sensitive to the time horizon in the economic evaluation. The longer the time, the lower the cost-effectiveness ratio will be. Time is needed to observe the potential outcomes of a primary prevention. Overall, the benefits would be greater in the long term than in the short term. Of the seven interventions assessed in the USA by Roux et al., six of them were cost-effective over a 40-years time horizon (41). Some interventions would be unlikely to be cost-effective due to the short time horizon of 10 years. For instance, the cost-effectiveness ratio for the walking education program would increase from $27,000 per QALY to $147,000 per QALY (41). Peels et al. showed that the computer-tailored PA interventions, with advice three times over 4 months and targeting Dutch community-dwelling adults, achieved cost-effectiveness on a long time horizon (40). ICERs below the $27,800 WTP threshold were used for prevention interventions in The Netherlands. On a 5-years horizon, only the web-based tailored intervention was borderline cost-effective. The impacts of primary prevention may take years to be noticeable. Hence, investment in primary prevention programs may be limited due to the decision-makers' desire for higher impacts in a shorter time frame (42, 43).

To our knowledge, this rapid review is the first review of its kind that focused on the lifestyle prevention interventions such as healthy weight programs, nutrition and balanced diet interventions, PA programs, limited alcohol consumption interventions, and tobacco cessation programs, excluding a previous study based on breast cancer preventions that found limited evidence of the effectiveness of primary prevention interventions (40). A benefit of performing a rapid review was that such evidence of the cost-effective interventions on breast cancer, for which limited research is available, might have not been possible to be synthesized from a traditional systematic review. Despite the observations and recommendations over the last two decades, few cost-effectiveness analyses have targeted healthy people, although some evidences are available for breast cancer (19, 33). Winn et al. showed in their systematic review on the "cost-utility analysis of cancer prevention, treatment, and control" that breast cancer was ranked first in terms of cost-utility-analysis-related studies (29% of all studies in the review) (19). However, tertiary prevention (treatment) and secondary prevention represented the majority of all studies (i.e., 77 and 15%, respectively), while the remainder (8%) was for primary prevention. Within the primary prevention interventions of breast cancer, the majority of studies focused on chemoprevention therapy and mastectomy procedures (88%). Based on current publications, the study shared the same conclusion that "researchers have

TABLE 2 | League table of incremental cost-effectiveness ratio by intervention, from a societal perspective and extrapolated likelihood of cost-effectiveness level for breast cancer (BC) for four studies included.

References	Intervention type and comparator	2018 US$/QALY	Likelihood cost-effectiveness level for BC
Frew et al. (39)	Base case analysis Be Active vs. no scheme, 5-years time horizon	721	Very high
Frew et al. (39)	Be active vs. no scheme, 2-years time horizon	3,374	Very high
Frew et al. (39)	Reduction physical activity over time Be Active vs. no scheme	3,850	Very high
Peels et al. (40)	Computer-tailored PA intervention: basic printed vs. usual care, lifetime horizon	11,606	Very high
Bós et al. (37)	Low-fat-diet-intervention women with high risk of breast cancer with fat intake ≥32% vs. usual diet, starting at age 50 years; lifetime horizon	12,600	Very high
Bós et al. (37)	Low-fat-dieta-intervention women with high fat intake at baseline >36.8% vs. usual diet, starting at age 50 years; lifetime horizon	15,468	High
Peels et al. (40)	Computer-tailored PA intervention: web-based basic vs. usual care, lifetime horizon	15,629	High
Roux et al. (41)	An 8-weeks community intervention for walking/NO; lifetime horizon	19,475	High
Bós et al. (37)	Low-fat-diet-intervention women with high risk of breast cancer with fat intake ≥32% vs. usual diet, starting at age 55 years; lifetime horizon	17,752	High
Bós et al. (37)	Low-fat-diet-intervention women with high fat intake at baseline >36.8% vs. usual diet, starting at age 55 years; lifetime horizon	18,583	High
Bós et al. (37)	Low-fat-diet-intervention women with high risk of breast cancer with fat intake ≥32% vs. usual diet, starting at age 60 years; lifetime horizon	18,647	High
Bós et al. (37)	Low-fat-diet-intervention women with high fat intake at baseline >36.8% vs. usual diet, starting at age 60 years; lifetime horizon	23,911	Medium high
Bós et al. (37)	Low-fat-diet-intervention women with high with high risk of breast cancer with fat intake ≥32% vs. usual diet, starting at age 65 years; lifetime horizon	24,451	Medium high
Roux et al. (41)	Exposure to an environment favoring a more active lifestyle/NO; lifetime horizon	34,827	Medium
Bós et al. (37)	Low-fat-diet-intervention women with high fat intake at baseline >36.8% vs. usual diet, starting at age 65 years; lifetime horizon	31,443	Medim low
Roux et al. (41)	Initial training session for walking program/NO; lifetime horizon	37,315	Medium low
Peels et al. (40)	Computer-tailored PA intervention: web-based environment vs. printed; 5-years time horizon	31,723	Medium low
Roux et al. (41)	Personal trainer intervention and financial incentives for PA/NO; lifetime horizon	40,657	Medium low
Bós et al. (37)	Low-fat-diet-intervention women with high risk of breast cancer with fat intake ≥32% vs. usual diet, starting at age 70 years; lifetime horizon	41,168	Low
Roux et al. (41)	Organized walking groups, social events for promoting PA/N; lifetime horizon	54,105	Very low
Peels et al. (40)	Computer-tailored PA intervention: printed environment vs. basic, 5-years time horizon	45,959	Very low
Bós et al. (37)	Low-fat-diet-intervention women with high fat intake at baseline >36.8% vs. usual diet, starting at age 70 years; lifetime horizon	51,197	Very low
Peels et al. (40)	Computer-tailored PA intervention: vs. basic web-based; 5-years time horizon	49,967	Very low
Roux et al. (41)	Intensive lifestyle modification program, for high risk diabetes 2 adults/NO; lifetime horizon	63,953	Very low
Roux et al. (41)	A 6-years community health education intervention (Stanford 5 City Project) vs. no intervention (/NO); lifetime horizon	93,457	Null

ICER values or value ranges were ≤12,499 for very high likelihood, 12,500–17,499 for high, 17,500–22,499 for medium high, 22,500–27,499 for medium, 27,500–32,499 for medium low, 32,500–37,499 for low, 37,500–50,000 for very low and null for ICER > 50,000. The study of Annemans et al. (36) is not included since no price-year was available, and Foster et al. (38) was not included since ICER/DALY was estimated. In Bós et al. estimates are presented from intervention start; estimates from the start of randomization as well as ICERs for the payer perspective were available in the publication, but not presented here, for purpose of comparison with the other three studies (37). Source: league table adapted from Greenberg et al. and likelihood extrapolation made by co-authors of the review (34).

devoted relatively little attention" to the cost-effectiveness of primary prevention (33). In contrast, an estimated 40% of cancers could be prevented if time and resources were invested to identify the protective factors which individuals can take to avoid the onset of cancer (8, 12, 44). Moreover, several studies on NCDs including breast cancer and their lifestyle-related risk factors, such as physical inactivity and excess weight, recommended conducting cost-effectiveness analyses of these interventions (45–48).

Limitations

Our study had several limitations. Firstly, the number of studies that could be included was limited. Only two types of interventions were identified: physical activity (in five studies)

and diet (in two studies). The small number of interventions did not permit the differentiation of the primary-prevention-related impact of intervention on breast cancer. More studies might be required to reach such an impact of public health interventions. The lack of sufficient evidence on the primary prevention interventions in reducing breast cancer might hinder the economic evaluations of lifestyle-related interventions. Also, it might be a result of our rapid review strategy and the limited number of databases searched. However, similar limitations were observed in previous systematic reviews in a number of studies retrieved (19). Secondly, the review included some studies in which the interventions were targeted not only for breast cancer but also for other NCDs. This may limit the implications of our findings. However, we believe that the inclusion of those NCDs still made our findings comprehensive and inclusive for lifestyle-related interventions for breast cancer that could not have been selected otherwise. Thirdly, the study quality assessment of the breast cancer primary-prevention-related cost-effectiveness rapid review had some limitations. The specific challenges of public health economic modeling require particular attention, notably related to uncertainty, which we checked in the quality assessment of the studies selected. However, additional items required to be assessed especially when different study designs are used. Natural experiment studies increasingly used in the evaluation of public health interventions may provide high "real-world setting" relevance and higher external validity than the RCTs at the expense of internal validity, unless the authors of the study select the optimal control group. Additionally, the authors' conflicts of interest were omitted from the quality assessment. This might have resulted in a "publication bias" as observed in a previous systematic review (34). Including those items in the quality assessment grid in future systematic reviews will improve the comparison between the interventions.

There are further limitations. While physical inactivity, excess weight, and unhealthy diet are significant threats to worldwide populations, our cost-effectiveness estimates were limited to HICs only (15, 47, 48). Thus, it is difficult to extrapolate or generalize the findings of the study to other countries and settings. Finally, the policy interventions related to lifestyle behaviors were not included in our study, which might hamper some complementary health benefits of selected taxation policies (49–51).

CONCLUSIONS

The rapid review of the six primary prevention studies highlighted that the use of PA programs and low-fat-diet interventions among particular subgroups of women had high cost-effectiveness. Many of the cost-effective interventions aimed to reduce the risk of NCDs alongside breast cancer, allowing public health professionals to use a holistic program addressing multiple aspects of a woman's health. Societies have invested in primary prevention drug therapies and surgical procedures for breast cancer, and the same investment can be made in the lifestyle interventions targeting breast cancer. We intend that a future systematic review will help in identifying the additional cost-effectiveness of lifestyle-related primary prevention of breast cancer.

AUTHOR CONTRIBUTIONS

MB, J-PR, and KB contributed to conceptualization and design. MB and JR collected and assembled information. All authors contributed to data analysis and interpretation, contributed to manuscript writing, and agree to be accountable of all aspects of the work.

FUNDING

Publication fees are covered by IBCN headquarters at Purdue University.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of the International Breast Cancer and Nutrition (IBCN) project at Purdue University. MB was a member of IBCN.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2019.00325/full#supplementary-material

REFERENCES

1. Beaglehole R, Bonita R, Magnusson R. Global cancer prevention: an important pathway to global health and development. *Public Health.* (2011) 125:821–31. doi: 10.1016/j.puhe.2011.09.029
2. 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
3. Luengo-Fernandez R, Leal J, Gray A, Sullivan R. Economic burden of cancer across the European Union: a population-based cost analysis. *Lancet Oncol.* (2013) 14:1165–74. doi: 10.1016/S1470-2045(13)70442-X
4. Pearce A, Sharp L, Hanly P, Barchuk A, Bray F, de Camargo Cancela M, et al. Productivity losses due to premature mortality from cancer in Brazil, Russia, India, China, South Africa (BRICS): a population-based comparison. *Cancer Epidemiol.* (2018) 53:27–34. doi: 10.1016/j.canep.2017.12.013
5. Ding D, Lawson KD, Kolbe-Alexander TL, Finkelstein EA, Katzmarzyk PT, van Mechelen W, et al. The economic burden of physical inactivity: a global analysis of major non-communicable diseases. *Lancet.* (2016) 388:1311–24. doi: 10.1016/S0140-6736(16)30383-X
6. Baade PD, Meng X, Sinclair C, Youl P. Estimating the future burden of cancers preventable by better diet and physical acivity in Australia. *Med J.* (2012) 196:337–40. doi: 10.5694/mja11.11082
7. LoConte NK, Gershenwald JE, Thomson CA, Crane TE, Harmon GE, Rechis R. Lifestyle modifications and policy implications for primary and secondary cancer prevention: diet, exercise, sun safety, and alcohol reduction. *Am Soc Clin Oncol Educ Book.* (2018) 38:88–100. doi: 10.1200/EDBK_200093

8. McTiernan A, Porter P, Potter, JD. Breast cancer prevention in countries with diverse resources. *Cancer*. (2008). 113:2325–30. doi: 10.1002/cncr.23829
9. Parkin DM, Boyd L, Walker LC. The fraction of cancer attributable to lifesytle and environmental factors in the UK in 2010. *BR J Cancer*. (2011) 105 (Suppl. 2):S77–81. doi: 10.1038/bjc.2011.489
10. Ekwueme DU, Allaire BT, Parish WJ, Thomas CC, Poehler D, Guy GP, et al. Estimation of breast cancer incident cases and medical care costs attributable to alcohol consumption among insured women aged <45 years in the U.S. *Am J Prev Med.* (2017) 53(3S1):S47–S54. doi: 10.1016/j.amepre.2017.05.023
11. Bray F, Jemal A, Torre LA, Forman D, Vineis P. Long-term realism and cost-effectiveness: primary prevention in combatting cancer and associated inequalities worldwide. *J Natl Cancer Inst.* (2015) 107:djv273. doi: 10.1093/jnci/djv273
12. Colditz GA, Emmons KM. Accelerating the pace of cancer prevention- right now. *Cancer Prevent. Res.* (2018) 11:171–84. doi: 10.1158/1940-6207.CAPR-17-0282
13. Kruk J. Lifestyle components and primary breast cancer prevention. *Asian Pac J Cancer Prev.* (2014) 15:10543–55. doi: 10.7314/apjcp.2014.15.24.10543
14. Sauter ER. Breast cancer prevention: current approaches and future directions. *Eur J Breast Health.* (2018) 14:64–71. doi: 10.5152/ejbh.2018.3978
15. Fitzmaurice C, Akinyemiju TF, Al Lami FH, Alam T, Alizadeh-Navaei R, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016: a systematic analysis for the global burden of disease study. *JAMA Oncol.* (2018) 4:1553–68. doi: 10.1200/JCO.2018.36.15_suppl.1568
16. Crew KD, Coe AM. Trends in the cost-effectiveness of chemoprevention for breast cancer −2001 to 2015. *Oncol Hematol Rev.* (2015) 11:104. doi: 10.17925/OHR.2015.11.02.104
17. Melnikow J, Tancredi DJ, Yang Z, Ritley D, Jiang Y, Slee C, et al. Program-specific cost-effectiveness analysis: breast cancer screening policies for a safety-net program. *Value Health*. (2013) 16:932–41. doi: 10.1016/j.jval.2013.06.013
18. Ulloa-Pérez E, Mohar-Betancourt A, Reynoso-Noverón N. Estimation of the cost-effectiveness of breast cancer screening using mammography in Mexico through a simulation. *Rev Invest Clin.* (2016) 68:184–91.
19. Winn AN, Ekwueme DU, Guy GP, Neumann PJ. Cost–utility analysis of cancer prevention, treatment, and control: a systematic review. *Am J Prev Med.* (2016) 50:241–8. doi: 10.1016/j.amepre.2015.08.009
20. Featherstone RM, Dryden DM, Foisy M, Guise JM, Mitchell MD, Paynter RA, et al. Advancing knowledge of rapid reviews: an analysis of results, conclusions and recommendations from published review articles examining rapid reviews. *Syst Rev.* (2015) 4:50. doi: 10.1186/s13643-015-0040-4
21. Haby MM, Chapman E, Clark R, Barreto J, Reveiz L, Lavis JN, et al. What are the best methodologies for rapid reviews of the research evidence for evidence-informed decision making in health policy and practice: a rapid review. *Health Res Policy Syst*. (2016) 14:83. doi: 10.1186/s12961-016-0155-7
22. Khangura S, Konnyu K, Cushman R, Grimshaw J, Moher D. Evidence summaries: the evolution of a rapid review approach. *Syst Rev*. (2012) 1:10. doi: 10.1186/2046-4053-1-10
23. Tricco AC, Antony J, Zarin W, Strifler L, Ghassemi M, Ivory J, et al. A scoping review of rapid review methods. *BMC Med*. (2015) 13:224. doi: 10.1186/s12916-015-0465-6
24. Hartling L, Guise J-M, Kato E, Anderson J, Aronson N, Belionson S, et al. *EPC Methods: An Exploration of Methods and Context for the Production of Rapid Reviews*. Rockville, MD: Agency for Healthcare Research and Quality (2015).
25. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation. *Ann Intern Med.* (2018) 169:467–73. doi: 10.7326/M18-0850
26. Colditz GA, Bohlke K. Priorities for the primary prevention of breast cancer. *CA Cancer J Clin*. (2014) 64:186–94. doi: 10.3322/caac.21225
27. Colditz GA, Bohlke K. Preventing breast cancer now by acting on what we already know. *NPJ Breast Cancer*. (2015) 1:15009. doi: 10.1038/npjbcancer.2015.9
28. Kolak A, Kaminska M, Sygit K, Budny A, Surdyka D, Kukiełka-Budny B, et al. Primary and secondary prevention of breast cancer. *Ann Agric Environ Med*. (2017) 24:549–53. doi: 10.26444/aaem/75943
29. WHO. *Preventing Noncommunicable Diseases*. (2019). Available online at: https://www.who.int/actiities/preventing-noncommunicable-diseases
30. Drummond MF, Jefferson TO. Guidelines for authors and peer reviewers of economic submissions to the BMJ. *BMJ*. (1996) 313:275–83. doi: 10.1136/bmj.313.7052.275
31. Gerard K, Seymour J, Smoker I. A tool to improve quality of reporting published economic analyses. *Int J Technol Assess Health Care*. (2000) 16:100–10. doi: 10.1017/S0266462300016196
32. Zelle SG, Baltussen RM. Economic analyses of breast cancer control in low- and middle-income countries: a systematic review. *Syst Rev*. (2013) 2:20. doi: 10.1186/2046-4053-2-20
33. Neumann PJ, Rosen AB, Greenberg D, Olchanski NV, Pande R, Chapman RH, et al. Can we better prioritize resources for cost–utility research? *Med Decis Making*. (2005) 25:429–36. doi: 10.1177/0272989X05276853
34. Greenberg D, Earle C, Fang CH, Eldar-Lissai A, Neumann PJ. When is cancer care cost-effective? A systematic overview of cost-utility analyses in oncology. *J Natl Cancer Inst.* (2010) 102:82–8. doi: 10.1093/jnci/djp472
35. Anderson AC, Craigie AM, Gallant S, McAdam C, Macaskill E, Mutrie N, et al. Randomised controlled trial to assess the impact of a lifesytle intervention (ActWELL) in women invited to NHS breast screening. *BMJ Open.* (2018) 8:e024136. doi: 10.1136/bmjopen-2018-024136
36. Annemans L, Lamotte M, Clarys P, Van den Abeele E. Health economic evaluation of controlled and maintained physical exercise in the prevention of cardiovascular and other prosperity diseases. *Eur J Cardiovasc Prev Rehabil.* (2007) 14:815–24. doi: 10.1097/HJR.0b013e3282ef514f
37. Bós AM, Howard BV, Beresford SA, Urban N, Tinker LF, Waters H, et al. Cost-effectiveness analysis of a low-fat diet in the prevention of breast and ovarian cancer. *J Am Diet Assoc.* (2011) 111:56–66. doi: 10.1016/j.jada.2010.10.011
38. Forster M, Veerman JL, Barendregt JJ, Vos T. Cost-effectiveness of diet and exercise interventions to reduce overweight and obesity. *Int J Obes.* (2011) 35:1071–8. doi: 10.1038/ijo.2010.246
39. Frew EJ, Bhatti M, Win K, Sitch A, Lyon A, Pallan M, et al. Cost-effectiveness of a community-based physical activity programme for adults (Be Active) in the UK: an economic analysis within a natural experiment. *Br J Sports Med.* (2014) 48:207–12. doi: 10.1136/bjsports-2012-091202
40. Peels DA, Hoogenveen RR, Feenstra TL, Golsteijn RH, Bolman C, Mudde AN, et al. Long-term health outcomes and cost-effectiveness of a computer-tailored physical activity intervention among people aged over fifty: modelling the results of a randomized controlled trial. *BMC Public Health.* (2014) 14:1099. doi: 10.1186/1471-2458-14-1099
41. Roux L, Pratt M, Tengs TO, Yore MM, Yanagawa TL, Van Den Bos J, et al. Cost effectiveness of community-based physical activity interventions. *Am J Prev Med.* (2008) 35:578–88. doi: 10.1016/j.amepre.2008.06.040
42. Richardson AK. Investing in public health: barriers and possible solutions. *J Public Health.* (2012) 34:322–7. doi: 10.1093/pubmed/fds039
43. Wild CP, Espina C, Bauld L, Bonanni B, Brenner H, Brown K, et al. Cancer prevention Europe. *Mol Oncol*. (2019) 13:528–34. doi: 10.1002/1878-0261.12455
44. Bielemann RM, Silva BG, Coll Cde V, Xavier MO, Silva SG. Burden of physical inactivity and hospitalization costs due to chronic diseases. *Rev Saude Publica.* (2015) 49:75. doi: 10.1590/S0034-8910.2015049005650
45. Chalkidou K, Marquez P, Dhillon PK, Teerawattananon Y, Anothaisintawee T, Gadelha CA, et al. Evidence-informed frameworks for cost-effective cancer care and prevention in low, middle, and high-income countries. *Lancet Oncol.* (2014) 15: e119–31. doi: 10.1016/S1470-2045(13)70547-3
46. Krueger H, Andres EN, Koot JM, Reilly BD. The economic burden of cancers attributable to tobacco smoking, excess weight, alcohol use, and physical inactivity in Canada. *Curr Oncol.* (2016) 23:241–9. doi: 10.3747/co.23.2952
47. Lee I, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Lancet physical activity series working group: effect of physical inactivity on major non-communicable diseases worldwide: analysis of burden of disease and life expectancy. *Lancet*. (2012) 380:219–29. doi: 10.1016/S0140-6736(12)61031-9
48. WHO. *Global Action Plan for the Prevention and Control of NCDs 2013–2020*. (2019). Available online at: https://www.who.int/nmh/events/ncd_action_plan/en/
49. Burton R, Henn C, Lavoie D, O'Connor R, Perkins C, Sweeney K, et al. A rapid evidence review of the effectiveness and cost-effectiveness of

alcohol control policies: an English perspective. *Lancet*. (2017) 389:1558–80. doi: 10.1016/S0140-6736(16)32420-5

50. Holm AL, Laursen MB, Koch M, Jensen JD, Diderichsen F. The health benefits of selective taxation as an economic instrument in relation to IHD and nutrition-related cancers. *Public Health Nutr*. (2013) 16:2124–31. doi: 10.1017/S1368980013000153
51. Islami F, Torre LA, Drope JM, Ward EM, Jemal A. Global cancer in women: cancer control priorities. *Cancer Epidemiol Biomarkers Prev*. (2017) 26:458–70. doi: 10.1158/1055-9965.EPI-16-0871

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 in Medicine

MINI REVIEW
published: 11 February 2020
doi: 10.3389/fmed.2020.00028

Peripheral Blood-Based Biopsy for Breast Cancer Risk Prediction and Early Detection

Farah J. Nassar[1], Ghada Chamandi[2], Mohamad Ali Tfaily[2], Nathalie Khoueiry Zgheib[3] and Rihab Nasr[2]**

[1] Department of Internal Medicine, Faculty of Medicine, American University of Beirut, Beirut, Lebanon, [2] Department of Anatomy, Cell Biology and Physiological Sciences, Faculty of Medicine, American University of Beirut, Beirut, Lebanon, [3] Department of Pharmacology and Toxicology, Faculty of Medicine, American University of Beirut, Beirut, Lebanon

Among women, breast cancer (BC) is not only the most common cancer worldwide but also the leading cause of cancer death. Only 5–10% of breast cancer cases are attributed to inherited mutations (BRCA1, BRCA2, and other breast cancer susceptibility genes). Breast cancer incidence has been rising particularly in young women who are not eligible for mammography, and it has been acting as a burden especially in developing countries that lack screening and awareness programs. For this reason, research has shifted to use minimally invasive liquid biopsies especially blood-based biomarkers with potential value for breast cancer risk prediction and early detection. This mini-review will tackle the different blood-based biomarkers focusing mainly on circulating miRNA, circulating proteins, cell-free nucleic acids, methylation patterns, and exosomes. It also introduces the potential opportunities for the clinical use of these blood-based biomarkers for breast cancer risk prediction.

Keywords: breast cancer, liquid biopsy, early detection, risk prediction, microRNA, cfDNA, exosome, methylation patterns

OPEN ACCESS

Edited by:
Victoria Seewaldt, Beckman Research Institute, City of Hope, United States

Reviewed by:
Eron Grant Manusov, University of Texas Rio Grande Valley Brownsville, United States
Eric C. Dietze, City of Hope National Medical Center, United States

****Correspondence:***
Nathalie Khoueiry Zgheib
nk16@aub.edu.lb
Rihab Nasr
rn03@aub.edu.lb

Specialty section:
This article was submitted to Family Medicine and Primary Care, a section of the journal Frontiers in Medicine

Received: *04 April 2019*
Accepted: *20 January 2020*
Published: *11 February 2020*

Citation:
Nassar FJ, Chamandi G, Tfaily MA, Zgheib NK and Nasr R (2020) Peripheral Blood-Based Biopsy for Breast Cancer Risk Prediction and Early Detection. Front. Med. 7:28. doi: 10.3389/fmed.2020.00028

INTRODUCTION

Breast cancer (BC) is the second most common cancer worldwide, with an incidence and mortality of 2,088,849 and 626,679, respectively in 2018. These alarming numbers are expected to continue rising by the year 2040 (1), hence the need to develop newer strategies for early detection and predisposition to BC. Predisposition to BC is not solely dependent on one risk factor; thus several BC risk assessment models were developed for that purpose. Regarding early detection, several randomized trials showed that screening can decrease BC burden and mortality, with a 0.74 relative risk of mortality among women who underwent mammography compared to those who did not, particularly for the age groups between 50 and 74 years (2–4). The selection of screening age depends on the age of BC onset in each population as well as the poor sensitivity of this screening method before the age of 40 (5, 6). Notably, the median age of BC diagnosis in developing countries remains a decade lower than that of Western Europe and the United States, which is 62 years (7, 8). For example, 70% of BC patients in Sub-Saharan Africa present with BC before the age of 50 years, making mammography a poor screening tool for the majority of this population (8–10). In addition to that, mammography can cause discomfort, overdiagnosis, and false-positive results accompanied by patient distress and anxiety (6). Imaging based diagnostic tools are also expensive and may not have the same performance and quality everywhere as well as may not be available equally for all populations especially people residing in rural areas.

Therefore, investigators shifted their scientific focus toward developing novel minimally invasive methods for early BC detection and risk prediction. Recently, liquid biopsy is the measurement of markers from easily accessible biologic fluids such as saliva, urine, and peripheral blood has become an attractive and increasingly investigated field of research. It was first introduced by Diaz et al. (11) in 2014 for the detection and examination of circulating tumor DNA in the blood. Then, its use was extended for the analysis of other circulating biomarkers such as microRNAs, exosomes, cell-free DNA, proteins, and methylated genes. There has been accumulating evidence for the potential clinical value of peripheral blood-based biopsy for cancer risk prediction and diagnosis, tracking of disease relapse and resistance, and stratification of patients for targeted therapy.

In this mini-review, we introduce the novel circulating blood-based biomarkers that are being investigated for either early BC detection or risk prediction. We focus on circulating microRNAs, proteins, cell-free nucleic acids, DNA methylation patterns, and exosomes (**Figure 1**).

Methodology

The research strategy for this review was guided by the main objective of reviewing the role of peripheral blood-based biopsy for BC risk prediction and early detection. The guiding specific question was: what empirical research is available on specific blood-based biomarkers in BC? This comprehensive research strategy targeted mainly journal articles published in English with no year specification. Only PubMed database was used with the following MeSH (Medical Subjects Headings) key terms.

(1) ***breast neoplasms*** *AND* ***microRNA***
(2) ***breast neoplasms*** *AND* ***circulating*** *AND* ***protein***
(3) ***breast neoplasms*** *AND* ***circulating*** *AND* ***DNA/RNA/lnRNA***
(4) ***breast neoplasms*** *AND* ***circulating*** *AND* ***DNA*** *AND* ***methylation***
(5) ***breast neoplasms*** *AND* ***exosomes***.

Following this, the compiled abstracts were discussed among the research team. Only articles that were on human samples and concerned with BC risk prediction and early detection were exported to EndNote software.

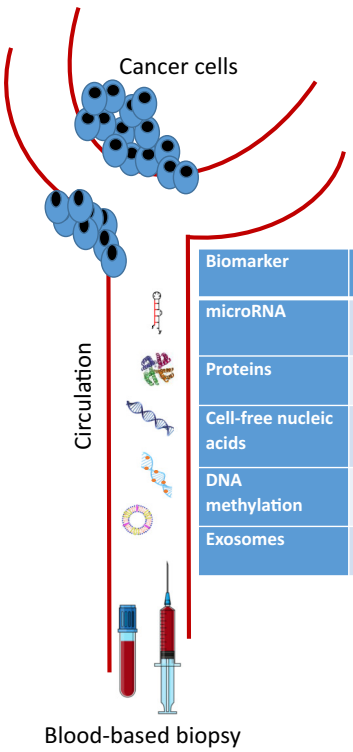
CIRCULATING microRNA

microRNAs (miRNA) are small non-coding RNA that regulate gene expression at the post-transcriptional level (12). miRNAs can act as oncogenes or tumor suppressors; thus playing an important role in tumor pathogenesis (13). As such, different miRNAs were shown to be dysregulated in cancer tissues, especially in BC as compared to normal tissues (14, 15). Moreover, miRNA dysregulation may be reflected in the biological fluids of BC patients including serum, plasma, and whole blood. miRNA are easily quantifiable, stable and resistant to degradation in the extracellular environment, hence supporting their potential role as biomarkers for BC screening and diagnosis (16, 17).

Dysregulation of circulating miRNA was noted in women who were at risk of developing BC. miR-144-3p, miR-451a, and miR-144-5p were found to be upregulated, while miR-708-5p was found to be downregulated in prospectively collected PBMC of 20 women who were unaffected at the time of recruitment and later diagnosed with breast cancer, as compared to 20 unaffected control women. However, these results failed to be confirmed using quantitative reverse transcription polymerase chain reaction (RT-PCR) in a validation set (18). Another study worth noting found that miR-195-5p and miR-495 are downregulated in PBMC of BC patients compared to healthy subjects, with a 77.8 and 100% sensitivity and 100 and 66.7% specificity, respectively, enabling them to be valuable diagnostic tools (19). It was not until 2009 when Zhu et al. (20) demonstrated that miRNA deregulation can also be detected in the serum of BC patients. In a following prospective cohort study on 205 cases of BC matched with 205 controls from the Sister Study Cohort with all recruited women BC free at the time of enrollment, global miRNA expression patterns revealed 21 differentially expressed miRNAs in the serum of BC patients when compared to healthy subjects (21). Several of these dysregulated miRNA such as the downregulated miR-99a-5p (22), miR-4634, miR-6875-5p (23), miR-18a, and miR-139-5p (24) or the upregulated miR-1246, miR-1307-3p, miR-6861-5p (23), and miR-21 (22) were later validated to be promising serum biomarkers for BC detection. In a meta-analysis by Li et al. (25), diagnosing BC by measuring serum miR-21 levels were found to be associated with high sensitivity and specificity of 0.79 and 0.85, respectively.

Even though several candidate miRNAs were individually studied as potential biomarkers for BC detection, they all failed to replace currently available detection models. This is due to the absence of standardization in the pre-analytical variables such as sample processing, storage, and handling, as well as data normalization strategy for miRNA quantification. This led several investigators to assess early detection with combinations of different miRNAs in the body fluids, an endeavor that translated into promising results in terms of sensitivity and specificity. For example, a study showed that selected miRNA signatures (such as in miR-21-3p, miR-21-5p, and miR-99a-5p) from miRNA profiles of 409 early breast cancer patients and 87 healthy controls from The Cancer Genome Atlas database were successfully validated as serum miRNA signatures with a diagnostic sensitivity and specificity of 97.9 and 73.5%, respectively (22). Also, a large cohort study investigated a combination of five miRNAs (miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, and miR-6875-5p) in sera of 1,206 BC patients using microarray for expression analysis and quantitative RT-PCR for validation. This combination was shown to have a sensitivity of 97.3%, a specificity of 82.9%, and an accuracy of 89.7%, with the potential to detect early BC and to differentiate it from other possible tumors (23). As for plasma, other combinations of miRNAs were also able to detect BC with high sensitivity (26). These combinations included miR-192-5p/miR-382-5p and miR-192-5p/miR-574-5p (26).

Besides their growing role in early detection of BC, miRNAs have been evaluated as potential circulating biomarkers to predict BC risk. As such, a study measured serum miRNA deregulation in 48 patients at high risk of developing BC, 24 of whom eventually



Biomarker	Source	Blood content	Measurement	Breast cancer risk prediction	Breast cancer early detection
microRNA	Tumor cells, other cells such as blood and immune cells	Whole blood, Serum, Plasma, White blood cells	Levels	+	+
Proteins	Not clear	Serum, Plasma	Levels	+	+
Cell-free nucleic acids	Apoptosis and necrosis of degenerating tumor cells	Serum, Plasma	Mutations, transposable elements	-	+
DNA methylation	Apoptosis and necrosis of degenerating tumor cells	Serum, Plasma	Methylation percentage	-	+
Exosomes	Tumor cells	Serum, Plasma	Content: Lipids, Proteins, miRNA	-	+

FIGURE 1 | Peripheral blood-based biopsy for breast cancer risk prediction and early detection.

developed the disease. A panel of 6 miRNA showed an ability to predict the risk of BC with high accuracy and precision (27). Nevertheless, and despite these encouraging results, more studies are needed to investigate circulating miRNA's role in BC risk prediction.

CIRCULATING PROTEINS

Several tumor proteins are detected in circulation though their origin is not known; however, only a few of them were shown to be clinically useful biomarkers in BC. The most currently measured circulating tumor protein markers are Carcinoembryonic antigen (CEA) and Cancer antigen (CA) 15-3 (also known as MUC1). These are however more useful for assessment of BC prognosis and recurrence rather than diagnosis since they lack specificity and sensitivity for low-volume disease (28, 29).

Recently, 8-hydroxy-2'-deoxyguanosine (8-OHdG), a nucleic acid damage marker due to oxidative stress, was reported to be a potential circulating biomarker for early detection of BC by two studies conducted on two different ethnic groups (Spain and Saudi Arabia). For instance, blood levels of 8-OHdG were significantly higher in women with BC group as compared to healthy women. The same pattern of 8-OHdG was observed with another diagnostic marker, which is cancer antigen CA 15-3 (30, 31). Moreover, in a prospective study including 2,835 cases and 3,122 matched controls from 10 cohorts, circulating anti-Müllerian hormone that is usually produced by ovaries also correlated with BC risk, particularly with ER+/PR+ tumors, with a 60% higher risk for women in the top quartile as compared to the bottom quartile of anti-Müllerian blood concentration (32).

Other circulating proteins under active investigation include the circulating adipose fatty acid-binding protein (A-FABP) that was recently shown to promote the development of BC in obese patients (33). Also, adipose metabolism has been linked to BC risk as plasma concentrations of adipose-derived fatty acid-binding protein 4 (FABP4) were found to be higher in 98 BC patients when compared to 96 healthy controls (34). Other protein regulators involved in bone resorption such as the Receptor Activator of NF-kB Ligand (RANKL), its receptor RANK, and the natural antagonist osteoprotegerin (OPG) were also found to be involved in BC (35–37). For instance, high serum levels of RANKL and RANKL/OPG ratios were reported in postmenopausal women at high risk for BC (38). Another study identified high serum OPG levels to be mainly associated with increased risk for ER- BC (39). On the other hand, a large scale investigation with a cohort of 1,976 incident invasive BC cases, of which 1,598 were ER+, showed limited evidence for correlating circulating RANKL levels with BC risk (40). Notably, and despite the availability of a myriad of BC studies in the proteomics literature (41, 42), the field is still lacking invalidated protein markers for both BC risk prediction and early detection.

CELL-FREE NUCLEIC ACIDS

In 1977, cell-free DNA (cfDNA) was first reported in the serum of cancer patients after surgery and/or chemotherapy, and its concentration varied depending on the response to therapy (43).

Later in 1989, a detectable amount of cfDNA was found in the plasma of cancer patients as compared to that of normal subjects (44). The origin of this extracellular DNA was shown to be mainly from the apoptosis and necrosis of degenerating cells in tumor tissue (45). cfDNA could be analyzed for specific genetic alterations including microsatellite alterations, allelic imbalance, translocations, mutations, and presence of viral genes (46, 47).

PIK3CA is the most commonly evaluated mutation detected in BC and occurring at a frequency of 20–45%. For instance, a prospective study assessed cfDNA *PIK3CA* mutations in the plasma of early BC patients before and after breast surgery and detected *PIK3CA* mutations preoperatively with 93.3% sensitivity and 100% specificity (48). Also, a meta-analysis that evaluated the overall diagnostic performance of cfDNA for *PIK3CA* mutation detection in BC from five different studies concluded that cfDNA *PIK3CA* mutation has a pooled sensitivity and specificity of 86 and 98%, respectively, with highest diagnostic accuracy in metastatic BC (49). More recently, next-generation sequencing of cfDNA in plasma of 100 women pretreated for advanced BC revealed the presence of a landscape of somatic mutations in different genes, such as *TP53*, *PIK3CA*, *ESR1*, and *NOTCH1*, in different subtypes of advanced BC (50). These results underscore the fact that BC is a heterogeneous disease, hence several mutations could be present, and researchers ought to analyze combinations of multiple cDNA targets.

Other recently studied cfDNA biomarkers for early BC detection are *LINE1* and *ALU*. These are transposable elements that were referred to as "junk DNA" in the past. A pilot study showed that *LINE1* copy number is significantly higher in the serum of 36 BC patients as compared to 29 healthy subjects (51). Similarly, serum *ALU115* levels and *ALU247/115* index or ratio were significantly higher in 40 patients newly diagnosed with BC patients as compared to 40 healthy controls. Serum *ALU247/115* index or ratio was the best in terms of sensitivity, specificity, positive and negative prediction values, and total efficiency of BC diagnosis when compared to *ALU115* levels and serum concentration of CEA and CA15 proteins. Notably, that improved sensitivity (97.5%) and negative prediction values (96.4%) were attained when all of the latter biomarkers were combined (52). Another study identified plasma cfDNA *ALU-247*, *ALU-115*, and DNA integrity (ratio between *ALU 247* and *115*) as potential biomarkers for BC diagnosis upon evaluating them in 40 BC patients and 10 healthy volunteers (53).

In addition to DNA, cfRNA can be found in the circulation. For example, long non-coding RNA (lncRNA), has also been examined as a potential biomarker for BC early detection. As such, large intergenic non-coding RNA-ROR (lncROR) measured in 96 plasma samples from BC patients had a high sensitivity (80.0%) and specificity (73.3%) for BC detection, and these values were greater than those of CEA and CA15-3 measured from the same patients (54). Similarly, two other lncRNA, H19, and HOX transcript antisense intergenic ribonucleic acid (HOTAIR), were identified as promising markers for BC detection in plasma (55, 56).

CIRCULATING DNA METHYLATION PATTERNS

DNA methylation is one of the hallmarks of epigenetic modifications associated with cancer. Several studies on DNA methylation in cancer have utilized cell-free DNA from plasma and serum to assess differences in methylation levels between BC patients and healthy controls (57). For example, significant DNA hypermethylation of *APC* and $RAR\beta_2$ were detected in the serum of patients with malignant BC as compared to serum from subjects with benign lesions and healthy controls, with both sensitivities and specificities of these two methylated genes being superior to traditional tumor markers (CEA and CA 15-3) for BC detection (58). Another study revealed that the hypermethylation of at least one of these genes (*APC, GSTP1, RASSF1A*, and $RAR\beta 2$) can be detected with a sensitivity of 62% and a specificity of 87% in BC (59). Another study examined the promoter methylation of six genes, *SFN*, *P16, hMLH1, HOXD13, PCDHGB7*, and *RASSF1a* in the serum of 749 subjects including patients with BC, patients with benign breast diseases, and healthy women. Results indicated that methylation analysis of the six-gene panel had significantly high sensitivities of 82.4 and 79.6% and specificities of 78.1 and 72.4% in the diagnosis of BC when compared to subjects with benign disease and healthy controls, respectively (60). In contrast, a recent paper showed that there were no significant differences in the levels of methylation of *RASSF1a* and *ATM* in peripheral blood DNA of 229 sporadic BC patients compared to that of 151 healthy controls (61). Other investigators evaluated DNA methylation of *14-3-3* σ promoter in circulation and produced controversial results (62, 63). Results from all of the above-described studies highlight the fact that the measurement of circulating DNA methylation patterns requires further investigation before being translated to clinical practice in BC (57).

CIRCULATING EXOSOMES

Exosomes are membrane-derived nanoscale vesicles that are actively released by most cells into the circulation (64). The content of these tiny particles, which are also shed by cancer cells, includes DNA, lipids, messenger RNA, microRNA, and other small regulatory RNA. Relevant molecular information can be obtained by analyzing exosomes' content. Exosomes and their cargo have been shown to play an important role in cell-cell communication between the tumor and the stroma, and in establishing the pre-metastatic niche. They demonstrate a promising blood-based biomarker for early cancer detection (65–67), as well as for BC since much higher levels of exosomes with altered cargo were found in sera of BC patients relative to healthy subjects (68).

It was also reported that exosomes released by BC cells into biological fluids contain important information about the primary tumor (69). For example, miRNA-containing exosomes (Exo-miR), an important and abundant exosomal cargo, were shown to potentially represent an ideal biomarker of disease onset (70, 71). As such, the diagnostic value of serum exosomal

TABLE 1 | Sensitivities and specificities of different breast cancer detection methods (Imaging and blood-based biomarkers).

Detection method	Biomarker	Sensitivity %	Specificity %	Meta-analysis Y/N	References
Imaging	Mammography	89	84	Y	(80)
	MRI	90	72	Y	(81)
	Ultrasound	80.1	88.4	Y	(82)
microRNA	miR-21	79	85	Y	(25)
	miR-195-5p	77.8	100	N	(19)
	miR-495	100	66.7	N	(19)
	miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, and miR-6875-5p	97.3	82.9	N	(23)
	miR-21-3p, miR-21-5p, and miR-99a-5p	97.9	73.5	N	(22)
	miR-21-3, miR-192-5p, miR-221-3p, miR-451a, miR-574-5p, miR-1273g-3p, hsa-miR-152, miR-22-3p, miR-222-3p, miR-30a-5p, miR-30e-5p, miR-324-3p, and miR-382-5p	88.1	77.5	N	(26)
Proteins	8-OHdG	82	80	N	(31)
Cell free nucleic acids	cfDNA concentration	87	87	Y	(83)
	PIK3CA	86	98	Y	(49)
	ALU115, ALU247/115, CEA, and CA15-3	97.5	67.5	N	(52)
	lncROR	80	73.3	N	(54)
	H19	56.7	86.7	N	(56)
	HOTAIR	80	68.3	N	(55)
DNA methylation	*APC*	93.4	95.4	N	(58)
	RARβ$_2$	95.5	92.4	N	(58)
	APC, GSTP1, RASSF1A, and *RARβ2*	62	87	N	(59)
	SFN, P16, hMLH1, HOXD13, PCDHGB7, and RASSF1a	79.6	72.4	N	(60)
	14-3-3 σ promoter	69	99	Y	(63)
Exosomes	Del-1	94.7	86.36	N	(77)
	FN	69.2	73.3	N	(76)

miRNA in BC was studied (72), nevertheless, no exosomal analysis was reported in subjects with a high risk of developing cancers. However, Exo-miR-233-3p was able to discriminate between ductal carcinoma *in situ* and infiltrating ductal cancer, suggesting its potential role for the early detection of invasive BC (73). Moreover, exosomal miR-21 and miR-1246 were found to be higher in plasma of BC patients or mice transplanted with patients derived breast tumors as compared to healthy controls (74). Furthermore, there exists a differential expression of exosomal miR based on the tumor molecular subtypes. For instance, higher levels of exosomal miR-373 were indicative of triple-negative BC (75). In addition to miRNAs, the exosomal proteins fibronectin and developmental endothelial Locus-1 (Del-1) are promising biomarkers for early-stage BC (76, 77). Although circulating exosomes have emerged as potential candidates for a non-invasive biomarker for BC, recent efforts have focused on the detection of metastasis and assessment of disease prognosis as well as on optimizing their isolation. Few promising exo-miR candidates for early detection were reported (71). However, until now, there is no compelling evidence for the potential clinical utility of exosomes for BC risk assessment.

CONCLUSIONS

In order to identify BC predisposition of healthy subjects, numerous BC risk prediction tools that take into consideration multiple risk factors are available (78, 79). Yet, only a few examples of peripheral blood-based biopsy have been evaluated for BC risk assessment. As for BC screening and early detection, several blood-based biomarkers are likely to be clinically used as easily accessible and minimally invasive substitutes or supplements to routine screening tests such as mammography. A comparison in the sensitivity and specificity of various blood- and serum-based biomarkers to imaging methods in the diagnosing and screening for breast cancer is required. Based on the literature (**Table 1**), several biomarkers have better sensitivity and specificity than imaging-based methods. For instance, miR-495 alone, a miRNA panel of miR-21-3p, miR-21-5p, and miR-99a-5p, a miRNA panel of miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, and miR-6875-5p, PIK3CA proteins, ALU115 combined with ALU247/115 cfDNA, CEA, and CA15-3, methylated APC and RARβ2, as well as Del-1 exosomes, appear to have the highest sensitivities, even as compared to the current imaging screening standards, making them potential screening tool for early breast cancer. On the other hand, miR-195-5p, mutated PIK3CA, and methylated APC and RARβ2 and 14-3-3 σ promoter have the highest specificities. This makes them powerful diagnostic tools for breast cancer, especially for PIK3CA protein, and methylated 14-3-3 σ promoter as the evidence is based on meta-analyses. Further studies and meta-analyses are needed to provide stronger evidence for these data before

adopting these biomarkers for screening and early detection of breast cancer.

The field of liquid biopsy research is still in its infancy but it is evolving rapidly and providing a rich space for discovery. To speed up the process of discovery and clinical translation, research should resolve some of the overarching challenges. Most of the studies on blood based biomarkers are retrospective case-control with a small sample size and with variable methodologies of sample handling and storage. Hence, studies should examine biomarkers in large ethnically diverse populations as well as prospectively measuring levels in healthy subjects especially those with a high risk of developing cancer well before the appearance of symptoms. Furthermore, the deficiency of standardized and robust methods for sample isolation, quantification and analysis, and the lack of benchmarking the sensitivity and specificity of biomarkers in large and ethnically diverse BC cohorts in comparison not only to healthy subjects but also to other cancer patients (84). By overcoming these drawbacks, the clinical application of these small molecules will surely amaze the world and save lives due to more accurate risk prediction and earlier detection of BC.

AUTHOR CONTRIBUTIONS

All authors contributed to the writing and critical reviewing of the article.

ACKNOWLEDGMENTS

We acknowledge the Medical Plan Practice (MPP) for the financial support. RN is a member of the International Breast Cancer and Nutrition.

REFERENCES

1. 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
2. Tabár L, Gad A, Holmberg LH, Ljungquist U, Fagerberg CJG, Baldetorp L, et al. Reduction in mortality from breast cancer after mass screening with mammography: randomised trial from the Breast Cancer Screening Working Group of the Swedish National Board of Health and Welfare. *Lancet.* (1985) 325:829–32. doi: 10.1016/S0140-6736(85)92204-4
3. Kerlikowske K, Grady D, Rubin SM, Sandrock C, Ernster VL. Efficacy of screening mammography: a meta-analysis. *JAMA.* (1995) 273:149–54. doi: 10.1001/jama.1995.03520260071035
4. Ajai A. 16-year mortality from breast cancer in the UK trial of early detection of breast cancer. *Lancet.* (1999) 353:1909–14. doi: 10.1016/S0140-6736(98)07412-1
5. Saslow D, Boetes C, Burke W, Harms S, Leach MO, Lehman CD, et al. American Cancer Society Guidelines for breast screening with MRI as an adjunct to mammography. *CA Cancer J Clin.* (2007) 57:75–89. doi: 10.3322/canjclin.57.2.75
6. Lauby-Secretan B, Scoccianti C, Loomis D, Benbrahim-Tallaa L, Bouvard V, Bianchini F, et al. Breast-cancer screening — viewpoint of the IARC Working Group. *N Engl J Med.* (2015) 372:2353–8. doi: 10.1056/NEJMsr1504363
7. Najjar H, Easson A. Age at diagnosis of breast cancer in Arab nations. *Int J Surg.* (2010) 8:448–52. doi: 10.1016/j.ijsu.2010.05.012
8. 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) 22:828–35. doi: 10.1016/j.breast.2013.02.011
9. Sighoko D, Bah E, Haukka J, McCormack VA, Aka EP, Bourgeois D, et al. Population-based breast (female) and cervix cancer rates in the Gambia: evidence of ethnicity-related variations. *Int J Cancer.* (2010) 127:2248–56. doi: 10.1002/ijc.25244
10. Jedy-Agba E, Curado MP, Ogunbiyi O, Oga E, Fabowale T, Igbinoba F, et al. Cancer incidence in Nigeria: a report from population-based cancer registries. *Cancer Epidemiol.* (2012) 36:e271–8. doi: 10.1016/j.canep.2012.04.007
11. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol.* (2014) 32:579–86. doi: 10.1200/JCO.2012.45.2011
12. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell.* (1993) 75:843–54. doi: 10.1016/0092-8674(93)90529-Y
13. Peng Y, Croce CM. The role of microRNAs in human cancer. *Signal Transduct Target Ther.* (2016) 1:15004. doi: 10.1038/sigtrans.2015.4
14. Iorio MV, Ferracin M, Liu C-G, Veronese A, Spizzo R, Sabbioni S, et al. microRNA gene expression deregulation in human breast cancer. *Cancer Res.* (2005) 65:7065–70. doi: 10.1158/0008-5472.CAN-05-1783
15. Pigati L, Yaddanapudi SC, Iyengar R, Kim DJ, Hearn SA, Danforth D, et al. Selective release of microRNA species from normal and malignant mammary epithelial cells. *PLoS ONE.* (2010) 5:e13515. doi: 10.1371/journal.pone.0013515
16. Weber JA, Baxter DH, Zhang S, Huang DY, How Huang K, Jen Lee M, et al. The microRNA spectrum in 12 body fluids. *Clin Chem.* (2010) 56:1733–41. doi: 10.1373/clinchem.2010.147405
17. Sohel MH. Extracellular/circulating microRNAs: release mechanisms, functions, and challenges. *Achiev Life Sci.* (2016) 10:175–86. doi: 10.1016/j.als.2016.11.007
18. Chang CW, Wu HC, Terry MB, Santella RM. microRNA expression in prospectively collected blood as a potential biomarker of breast cancer risk in the BCFR. *Anticancer Res.* (2015) 35:3969–77.
19. Mishra S, Srivastava AK, Suman S, Kumar V, Shukla Y. Circulating miRNAs revealed as surrogate molecular signatures for the early detection of breast cancer. *Cancer Lett.* (2015) 369:67–75. doi: 10.1016/j.canlet.2015.07.045
20. Zhu W, Qin W, Atasoy U, Sauter ER. Circulating microRNAs in breast cancer and healthy subjects. *BMC Res Notes.* (2009) 2:89. doi: 10.1186/1756-0500-2-89
21. Godfrey AC, Xu Z, Weinberg CR, Getts RC, Wade PA, DeRoo LA, et al. Serum microRNA expression as an early marker for breast cancer risk in prospectively collected samples from the Sister Study cohort. *Breast Cancer Res.* (2013) 15:R42. doi: 10.1186/bcr3428
22. Yu X, Liang J, Xu J, Li X, Xing S, Li H, et al. Identification and validation of circulating microRNA signatures for breast cancer early detection based on large scale tissue-derived data. *J Breast Cancer.* (2018) 21:363–70. doi: 10.4048/jbc.2018.21.e56
23. Shimomura A, Shiino S, Kawauchi J, Takizawa S, Sakamoto H, Matsuzaki J, et al. Novel combination of serum microRNA for detecting breast cancer in the early stage. *Cancer Sci.* (2016) 107:326–34. doi: 10.1111/cas.12880
24. Kodahl AR, Lyng MB, Binder H, Cold S, Gravgaard K, Knoop AS, et al. Novel circulating microRNA signature as a potential non-invasive multi-marker test in ER-positive early-stage breast cancer: a case control study. *Mol Oncol.* (2014) 8:874–83. doi: 10.1016/j.molonc.2014.03.002
25. Li S, Yang X, Yang J, Zhen J, Zhang D. Serum microRNA-21 as a potential diagnostic biomarker for breast cancer: a systematic review and meta-analysis. *Clin Exp Med.* (2016) 16:29–35. doi: 10.1007/s10238-014-0332-3
26. Fang R, Zhu Y, Hu L, Khadka VS, Ai J, Zou H, et al. Plasma microRNA pair panels as novel biomarkers for detection of early stage breast cancer. *Front Physiol.* (2019) 9:1879. doi: 10.3389/fphys.2018.01879
27. Farina NH, Ramsey JE, Cuke ME, Ahern TP, Shirley DJ, Stein JL, et al. Development of a predictive miRNA signature for breast

cancer risk among high-risk women. *Oncotarget.* (2017) 8:112170–83. doi: 10.18632/oncotarget.22750
28. Sławicki S, Mroczko B, Szmitkowski M. Tumor markers of breast cancer. *Postepy Higieny I Medycyny Doswiadczalnej.* (2004) 58:292–300.
29. Kabel AM. Tumor markers of breast cancer: new prospectives. *J Oncol Sci.* (2017) 3:5–11. doi: 10.1016/j.jons.2017.01.001
30. Bayo J, Castano MA, Rivera F, Navarro F. Analysis of blood markers for early breast cancer diagnosis. *Clin Transl Oncol.* (2018) 20:467–75. doi: 10.1007/s12094-017-1731-1
31. Nour Eldin EEM, El-Readi MZ, Nour Eldein MM, Alfalki AA, Althubiti MA, Mohamed Kamel HF, et al. 8-hydroxy-2'-deoxyguanosine as a discriminatory biomarker for early detection of breast cancer. *Clin Breast Cancer.* (2019) 19:e385–93. doi: 10.1016/j.clbc.2018.12.013
32. Ge W, Clendenen TV, Afanasyeva Y, Koenig KL, Agnoli C, Brinton LA, et al. Circulating anti-Mullerian hormone and breast cancer risk: a study in ten prospective cohorts. *Int J Cancer.* (2018) 142:2215–26. doi: 10.1002/ijc.31249
33. Hao J, Zhang Y, Yan X, Yan F, Sun Y, Zeng J, et al. Circulating adipose fatty acid binding protein is a new link underlying obesity-associated breast/mammary tumor development. *Cell Metab.* (2018) 28:689–705.e685. doi: 10.1016/j.cmet.2018.07.006
34. Guaita-Esteruelas S, Saavedra-Garcia P, Bosquet A, Borras J, Girona J, Amiliano K, et al. Adipose-derived fatty acid-binding proteins plasma concentrations are increased in breast cancer patients. *Oncologist.* (2017) 22:1309–15. doi: 10.1634/theoncologist.2016-0483
35. Schramek D, Leibbrandt A, Sigl V, Kenner L, Pospisilik JA, Lee HJ, et al. Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. *Nature.* (2010) 468:98–102. doi: 10.1038/nature09387
36. Nolan E, Vaillant F, Branstetter D, Pal B, Giner G, Whitehead L, et al. RANK ligand as a potential target for breast cancer prevention in BRCA1-mutation carriers. *Nat Med.* (2016) 22:933–9. doi: 10.1038/nm.4118
37. Sigl V, Owusu-Boaitey K, Joshi PA, Kavirayani A, Wirnsberger G, Novatchkova M, et al. RANKL/RANK control Brca1 mutation. *Cell Res.* (2016) 26:761–74. doi: 10.1038/cr.2016.69
38. Kiechl S, Schramek D, Widschwendter M, Fourkala EO, Zaikin A, Jones A, et al. Aberrant regulation of RANKL/OPG in women at high risk of developing breast cancer. *Oncotarget.* (2017) 8:3811–25. doi: 10.18632/oncotarget.14013
39. Fortner RT, Sarink D, Schock H, Johnson T, Tjonneland A, Olsen A, et al. Osteoprotegerin and breast cancer risk by hormone receptor subtype: a nested case-control study in the EPIC cohort. *BMC Med.* (2017) 15:26. doi: 10.1186/s12916-017-0786-8
40. Sarink D, Schock H, Johnson T, Chang-Claude J, Overvad K, Olsen A, et al. Receptor activator of nuclear factor kB ligand, osteoprotegerin, and risk of death following a breast cancer diagnosis: results from the EPIC cohort. *BMC Cancer.* (2018) 18:1010. doi: 10.1186/s12885-018-4887-3
41. Hathout Y. Proteomic methods for biomarker discovery and validation. Are we there yet? *Exp Rev Proteom.* (2015) 12:329–31. doi: 10.1586/14789450.2015.1064771
42. Boschetti E, D'Amato A, Candiano G, Righetti PG. Protein biomarkers for early detection of diseases: the decisive contribution of combinatorial peptide ligand libraries. *J Proteom.* (2018) 188:1–14. doi: 10.1016/j.jprot.2017.08.009
43. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. *Cancer Res.* (1977) 37:646–50.
44. Stroun M, Anker P, Maurice P, Lyautey J, Lederrey C, Beljanski M. Neoplastic characteristics of the DNA found in the plasma of cancer patients. *Oncology.* (1989) 46:318–22. doi: 10.1159/000226740
45. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch R-D, et al. DNA Fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. *Cancer Res.* (2001) 61:1659–65.
46. Chen XQ, Stroun M, Magnenat JL, Nicod LP, Kurt AM, Lyautey J, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. *Nat Med.* (1996) 2:1033–5. doi: 10.1038/nm0996-1033
47. Chang HW, Lee SM, Goodman SN, Singer G, Cho SK, Sokoll LJ, et al. Assessment of plasma DNA levels, allelic imbalance, and CA 125 as diagnostic tests for cancer. *J Natl Cancer Inst.* (2002) 94:1697–703. doi: 10.1093/jnci/94.22.1697
48. Beaver JA, Jelovac D, Balukrishna S, Cochran R, Croessmann S, Zabransky DJ, et al. Detection of cancer DNA in plasma of patients with early-stage breast cancer. *Clin Cancer Res.* (2014) 20:2643–50. doi: 10.1158/1078-0432.CCR-13-2933
49. Zhou Y, Wang C, Zhu H, Lin Y, Pan B, Zhang X, et al. Diagnostic Accuracy of PIK3CA mutation detection by circulating free DNA in breast cancer: a meta-analysis of diagnostic test accuracy. *PLoS ONE.* (2016) 11:e0158143. doi: 10.1371/journal.pone.0158143
50. Yi Z, Ma F, Li C, Chen R, Yuan L, Sun X, et al. Landscape of somatic mutations in different subtypes of advanced breast cancer with circulating tumor DNA analysis. *Sci Rep.* (2017) 7:5995. doi: 10.1038/s41598-017-06327-4
51. Sunami E, Vu AT, Nguyen SL, Giuliano AE, Hoon DS. Quantification of LINE1 in circulating DNA as a molecular biomarker of breast cancer. *Ann N Y Acad Sci.* (2008) 1137:171–4. doi: 10.1196/annals.1448.011
52. Tang Z, Li L, Shen L, Shen X, Ju S, Cong H. Diagnostic value of serum concentration and integrity of circulating cell-free DNA in breast cancer: a comparative study with CEA and CA15–3. *Lab Med.* (2018) 49:323–8. doi: 10.1093/labmed/lmy019
53. Hussein NA, Mohamed SN, Ahmed MA. Plasma ALU-247, ALU-115, and cfDNA integrity as diagnostic and prognostic biomarkers for breast cancer. *Appl Biochem Biotechnol.* (2018) 187:1028–45. doi: 10.1007/s12010-018-2858-4
54. Zhao T, Wu L, Li X, Dai H, Zhang Z. Large intergenic non-coding RNA-ROR as a potential biomarker for the diagnosis and dynamic monitoring of breast cancer. *Cancer Biomark.* (2017) 20:165–73. doi: 10.3233/CBM-170064
55. Zhang L, Song X, Wang X, Xie Y, Wang Z, Xu Y, et al. Circulating DNA of HOTAIR in serum is a novel biomarker for breast cancer. *Breast Cancer Res Treat.* (2015) 152:199–208. doi: 10.1007/s10549-015-3431-2
56. Zhang K, Luo Z, Zhang Y, Zhang L, Wu L, Liu L, et al. Circulating lncRNA H19 in plasma as a novel biomarker for breast cancer. *Cancer Biomark.* (2016) 17:187–94. doi: 10.3233/CBM-160630
57. Vu TL, Nguyen TT, Van Thi Hong Doan LT, Vo T. Methylation profiles of BRCA1, RASSF1A and GSTP1 in Vietnamese women with breast cancer. *Asian Pac J Cancer Prev.* (2018) 19:1887–93. doi: 10.22034/APJCP.2018.19.7.1887
58. Swellam M, Abdelmaksoud MD, Mahmoud MS, Ramadan A, Abdel-Moneem W, Hefny MM. Aberrant methylation of APC and RARβ2 genes in breast cancer patients. *Iubmb Life.* (2015) 67:61–8. doi: 10.1002/iub.1346
59. Hoque MO, Feng Q, Toure P, Dem A, Critchlow CW, Hawes SE, et al. Detection of aberrant methylation of four genes in plasma DNA for the detection of breast cancer. *J Clin Oncol.* (2006) 24:4262–9. doi: 10.1200/JCO.2005.01.3516
60. Shan M, Yin H, Li J, Li X, Wang D, Su Y, et al. Detection of aberrant methylation of a six-gene panel in serum DNA for diagnosis of breast cancer. *Oncotarget.* (2016) 7:18485. doi: 10.18632/oncotarget.7608
61. Cao X, Tang Q, Holland-Letz T, Gündert M, Cuk K, Schott S, et al. Evaluation of promoter methylation of RASSF1A and ATM in peripheral blood of breast cancer patients and healthy control individuals. *Int J Mol Sci.* (2018) 19:900. doi: 10.3390/ijms19030900
62. Jing F, Zhang J, Tao J, Zhou Y, Jun L, Tang X, et al. Hypermethylation of tumor suppressor genes BRCA1, p16 and 14–3-3sigma in serum of sporadic breast cancer patients. *Onkologie.* (2007) 30:14–9. doi: 10.1159/000096892
63. Ye M, Huang T, Ying Y, Li J, Yang P, Ni C, et al. Detection of 14–3-3 sigma (σ) promoter methylation as a noninvasive biomarker using blood samples for breast cancer diagnosis. *Oncotarget.* (2016) 8:9230–42. doi: 10.18632/oncotarget.13992
64. Boyiadzis M, Whiteside TL. Information transfer by exosomes: a new frontier in hematologic malignancies. *Blood Rev.* (2015) 29:281–90. doi: 10.1016/j.blre.2015.01.004
65. Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol.* (2015) 17:816–26. doi: 10.1038/ncb3169
66. 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) 523:177–82. doi: 10.1038/nature14581
67. Wendler F, Favicchio R, Simon T, Alifrangis C, Stebbing J, Giamas G. Extracellular vesicles swarm the cancer microenvironment: from

tumor-stroma communication to drug intervention. *Oncogene.* (2017) 36:877–84. doi: 10.1038/onc.2016.253
68. Melo SA, Sugimoto H, O'Connell JT, Kato N, Villanueva A, Vidal A, et al. Cancer exosomes perform cell-independent microRNA biogenesis and promote tumorigenesis. *Cancer Cell.* (2014) 26:707–21. doi: 10.1016/j.ccell.2014.09.005
69. Guzman N, Agarwal K, Asthagiri D, Yu L, Saji M, Ringel MD, et al. Breast cancer-specific miR signature unique to extracellular vesicles includes "microRNA-like" tRNA fragments. *Mol Cancer Res.* (2015) 13:891–901. doi: 10.1158/1541-7786.MCR-14-0533
70. Ciesla M, Skrzypek K, Kozakowska M, Loboda A, Jozkowicz A, Dulak J. microRNAs as biomarkers of disease onset. *Anal Bioanal Chem.* (2011) 401:2051–61. doi: 10.1007/s00216-011-5001-8
71. Sempere LF, Keto J, Fabbri M. Exosomal microRNAs in breast cancer towards diagnostic and therapeutic applications. *Cancers.* (2017) 9:e71. doi: 10.3390/cancers9070071
72. Joyce DP, Kerin MJ, Dwyer RM. Exosome-encapsulated microRNAs as circulating biomarkers for breast cancer. *Int J Cancer.* (2016) 139:1443–8. doi: 10.1002/ijc.30179
73. Yoshikawa M, Iinuma H, Umemoto Y, Yanagisawa T, Matsumoto A, Jinno H. Exosome-encapsulated microRNA-223–3p as a minimally invasive biomarker for the early detection of invasive breast cancer. *Oncol Lett.* (2018) 15:9584–92. doi: 10.3892/ol.2018.8457
74. Hannafon BN, Trigoso YD, Calloway CL, Zhao YD, Lum DH, Welm AL, et al. Plasma exosome microRNAs are indicative of breast cancer. *Breast Cancer Res.* (2016) 18:90. doi: 10.1186/s13058-016-0753-x
75. Eichelser C, Stuckrath I, Muller 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) 5:9650–63. doi: 10.18632/oncotarget.2520
76. Moon PG, Lee JE, Cho YE, Lee SJ, Chae YS, Jung JH, et al. Fibronectin on circulating extracellular vesicles as a liquid biopsy to detect breast cancer. *Oncotarget.* (2016) 7:40189–99. doi: 10.18632/oncotarget.9561
77. Moon PG, Lee JE, Cho YE, Lee SJ, Jung JH, Chae YS, et al. Identification of developmental endothelial locus-1 on circulating extracellular vesicles as a novel biomarker for early breast cancer detection. *Clin Cancer Res.* (2016) 22:1757–66. doi: 10.1158/1078-0432.CCR-15-0654
78. Cintolo-Gonzalez JA, Braun D, Blackford AL, Mazzola E, Acar A, Plichta JK, et al. Breast cancer risk models: a comprehensive overview of existing models, validation, and clinical applications. *Breast Cancer Res Treat.* (2017) 164:263–84. doi: 10.1007/s10549-017-4247-z
79. Wood ME, Farina NH, Ahern TP, Cuke ME, Stein JL, Stein GS, et al. Towards a more precise and individualized assessment of breast cancer risk. *Aging.* (2019) 11:1305–16. doi: 10.18632/aging.101803
80. Zhu X, Huang JM, Zhang K, Xia LJ, Feng L, Yang P, et al. Diagnostic value of contrast-enhanced spectral mammography for screening breast cancer: systematic review and meta-analysis. *Clin Breast Cancer.* (2018) 18:e985–95. doi: 10.1016/j.clbc.2018.06.003
81. Peters NHGM, Rinkes IHMB, Zuithoff NPA, Mali WPTM, Moons KGM, Peeters PHM. Meta-analysis of MR imaging in the diagnosis of breast lesions. *Radiology.* (2008) 246:116–24. doi: 10.1148/radiol.24610 61298
82. Sood R, Rositch AF, Shakoor D, Ambinder E, Pool KL, Pollack E, et al. Ultrasound for breast cancer detection globally: a systematic review and meta-analysis. *J Glob Oncol.* (2019) 5:1–17. doi: 10.1200/JGO.19.00127
83. Yu D, Tong Y, Guo X, Feng L, Jiang Z, Ying S, et al. Diagnostic value of concentration of circulating cell-free DNA in breast cancer: a meta-analysis. *Front Oncol.* (2019) 9:95. doi: 10.3389/fonc.2019.00095
84. Nassar FJ, Nasr R, Talhouk R. microRNAs as biomarkers for early breast cancer diagnosis, prognosis and therapy prediction. *Pharmacol Therapeut.* (2017) 172:34–49. doi: 10.1016/j.pharmthera.2016. 11.012

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 in Oncology

HYPOTHESIS AND THEORY
published: 18 February 2020
doi: 10.3389/fonc.2020.00129

Breast Cancer and Nutrition: A Paradigm for Prevention in 3D Across the Life Course

*Michele R. Forman**

Department of Nutrition Science, Purdue University Cancer Center, Center for Aging and the Life Course, Purdue University, West Lafayette, IN, United States

Breast cancer, the most common cancer in women worldwide, has recognized reproductive and anthropometric risk factors including age at menarche and adult height. Yet the age *when* a woman attains her adult height or experiences menarche for example is simply the timing of the major life event at the end of a long trail of exposures that began *in utero*. The objective of this article is to investigate through a review of the literature the role of nutrition in breast cancer prevention through three dimensions (D). Each D offers a different lens. The First D identifies *windows/ages* of exposures or conditions that convey vulnerability or protection from breast cancer. The Second *D* addresses the *intensity and duration* of the exposure; and the (*Third* D) examines the pace, i.e., how rapid or slow the young woman experiences her growth and development. Birthweight illustrative of the First D reveals a strong signal across the life course on BC risk, but the risk group varies from low to high birthweight. Stressful life events like being a pubertal aged girl living in a household with an unemployed father during the Great Depression or high levels of environmental contaminants exposure are representative of the Second D. Height velocity at specific ages and weight loss in postmenopausal years are illustrative of anthropometric trajectories that reveal an adaptive biosystem that provides a contextual state to interact with the other two Ds. This article presents a new paradigm of nutrition and breast cancer prevention through the lens of three very different dimensions. It is the premise of this article that all three dimensions are essential tasks to tease apart the life course and identify windows for preventive strategies.

Keywords: nutrition, prevention, life course, paradigm, breast cancer

OPEN ACCESS

Edited by:
Mary Beth Terry,
Columbia University, United States

Reviewed by:
Hamidullah Khan,
University of Wisconsin-Madison, United States
Abdelbaset Mohamed Elasbali,
Al Jouf University, Saudi Arabia

***Correspondence:**
Michele R. Forman
mforman@purdue.edu

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

Received: *31 October 2019*
Accepted: *24 January 2020*
Published: *18 February 2020*

Citation:
Forman MR (2020) Breast Cancer and Nutrition: A Paradigm for Prevention in 3D Across the Life Course. Front. Oncol. 10:129. doi: 10.3389/fonc.2020.00129

Breast cancer is the most common cancer in women across the world (1). A family history of breast cancer (BC), high breast density, reproductive risk factors including early age at menarche, late age at menopause, older age at first birth, and nulliparity, as well as being tall, moderate to high alcohol consumption, being physically inactive and menopausal status specific-body mass index are a constellation of recognized risk factors influencing BC risk (2, 3). Yet the age *when* a woman attains her adult height or experiences menarche for example is simply the timing of the major life event at the end of a long trail of exposures that began *in utero*. The tempo of height velocity and the peak height velocity that end in a woman's adult height, and the age of first birth and pace of occurrence (i.e., time interval between first and last births) are essential components to understanding the cumulative risk from adult height and parity on BC risk (4). Indeed *profiling* a woman's linear growth trajectory *from birth across her life course* may likely be key to identifying and understanding strategies for BC prevention.

Hormonal exposures begin *in utero*. Proxy markers including the maternal pregnancy comorbidity of preeclampsia and an infant's birthweight are indicators of the hormonal milieu in fetal life. Estrogen, progesterone and insulin-like growth factor 1 (IGF-1) levels in cord blood vary by birthweight and preeclampsia exposure; they may set the baseline concentrations of hormones for breast cancer (5, 6). Each hormone has proliferative effects on the breast and concentrations vary dramatically by race-ethnicity, phase of the menstrual cycle, and parity (7–9). Haiman's ethnic- specific investigation of hormones by phase of the menstrual cycle in ovulatory Latina, non-Hispanic whites (NHW) and non-Hispanic Black (NHB) women revealed higher follicular and luteal phase estradiol concentrations in NHB women than Latinas and NHW; and in turn, Latinas had higher levels than NHW (10). In the multi-ethnic cohort of postmenopausal women, Japanese American and NHB women had higher estrogen levels than NHW (11). The absolute concentration of and timing of a hormone trajectory may be due to genetic and environmental influences as illustrated by ethnic-group specific differences above that have implications for BC risk. Understanding hormone trajectories and the timing of changes in the trajectory by life stage may help in capturing the *cumulative load of hormonal insults* related to the incidence of premenopausal BC.

To achieve the goal of breast cancer prevention, we need to examine the arsenal of exposures (both preventive and adverse), the window of the life course for the exposure (or its proxy indicator like hunger or an economic depression), and the trajectory of growth and the hormonal tone in a woman. Nutrition is fundamental to BC prevention because a woman's body mass and height for example are the result of diet, physical activity, metabolism, hormones, and reproductive life events that are underlying her body mass index, linear growth and attained adult height. The four indicators of nutritional status–anthropometric, biochemical, clinical and diet- are typically measured at one point in time in research rather than repeated measures that capture trajectories and change over the life course. It is the intent of this article to focus on life course approaches to research in nutrition and BC. The objective of this article is to investigate the role of nutrition in breast cancer prevention through three dimensions (D). Each D offers a different lens. The First D identifies *windows/ages* of exposures or conditions that convey vulnerability or protection from breast cancer. The Second *D* addresses the *intensity and duration* of the exposure; and the (*Third* D) examines the pace i.e., how rapid or slow the young woman experiences her growth and development. Growth occurs with damage to DNA repair and other components like radical oxygen species in carcinogenesis. Examination of the growth trajectory may provide context for biosystemic aging and interact with the influence of an exposure through prolonging or shortening it or modifying its intensity of effect as evidenced in the other 2Ds. Pregnancy has commonalities to carcinogenesis, because growth factors, hormones, and molecular pathways are up- and down-regulated with gestation but in a "controlled sense." Pregnancy is a hyperinsulinemic state, with hormones at the highest concentrations experienced by a women in her life. Therefore, growth and pregnancy have always been risk factors but not placed into the context of their trajectory in a life course approach. Encapsulating a life course approach to breast cancer through nutrition can offer a unique lens into prevention and provide strategies for intervention and further research.

THE FIRST D: WINDOWS OF EXPOSURE ACROSS THE LIFE COURSE (FIGURE 1)

The hormonal milieu in pregnancy/*in utero* offers a window of exposure for breast cancer. Hormone levels in pregnancy vary by race-ethnicity, birthweight and parity. Concentrations of free estradiol and percent free estradiol are higher in the first than subsequent pregnancies (12). Non-hispanic Black women have higher testosterone levels in pregnancy than Non-Hispanic whites or Asians (9). Estriol and sex-hormone binding globulin protein levels increase with each standard unit (112 and 75 g increase) of birthweight (13). Furthermore, cord blood insulin like growth factor-1 levels are significantly higher amongst the high birthweight than normal or low birthweight newborns (5).

Birthweight of the offspring is a proxy indicator for the fetal hormonal milieu and the nutritional status of the mother in pregnancy. Weighing 8.8 pounds or more at birth is associated with a 3.2-fold higher risk of early breast development (Tanner Stage 4–5) by 9–10 years among girls in the U.S. (14). Higher birthweight as illustrated by each 500 g increment is associated with a seven percent (95% CI; 1.02–1.13) risk for premenopausal breast cancer amongst Scandinavian women (15). A meta-analysis of birthweight and postmenopausal breast cancer revealed a 20% higher risk (95% CI 1.08–1.34) amongst those who weighed 4,000 grams or more at birth (16). Conversely low birthweight was associated with reduced risk (of a hazard ratio (HR) = 0.66; 95% CI: 0.47–93) of premenopausal breast cancer in the Nurses' Health Cohort Studies I and II (17). Birthweight reveals its signal through its effects on timing of breast development through to BC risk across the life course. In contrast, maternal pre-pregnancy body mass index and gestation weight gain were not associated with breast mammographic density in daughters of the index pregnancy in one study (18).

Evidence for *infancy* as a period of vulnerability for breast cancer arises in conjunction with *the third D* notably the trajectory of weight gain. Specifically, risk for breast development by 10.8 years in Norway varies by timing of peak weight gain in infancy and by maternal preeclampsia status. In a nested case-cohort study of preeclampsia, we report that peak weight gain during the third through 6th months of infancy in a daughter of a women with a normotensive pregnancy incurs a 1.87 risk for early breast development by 10.8 years. In contrast peak weight gain in the last 6 months of infancy in daughters of preeclamptic pregnancy has a 3.19-fold increased risk for early breast development (Thelus-Jean R 2009). Rapid weight gain in the first 4 months of infancy is associated with a 60% or higher risk for a diagnosis of benign breast disease (19). In contrast, other exposures during infancy such as infant feeding practices are not associated with risk for breast benign breast disease (20) or breast cancer (21).

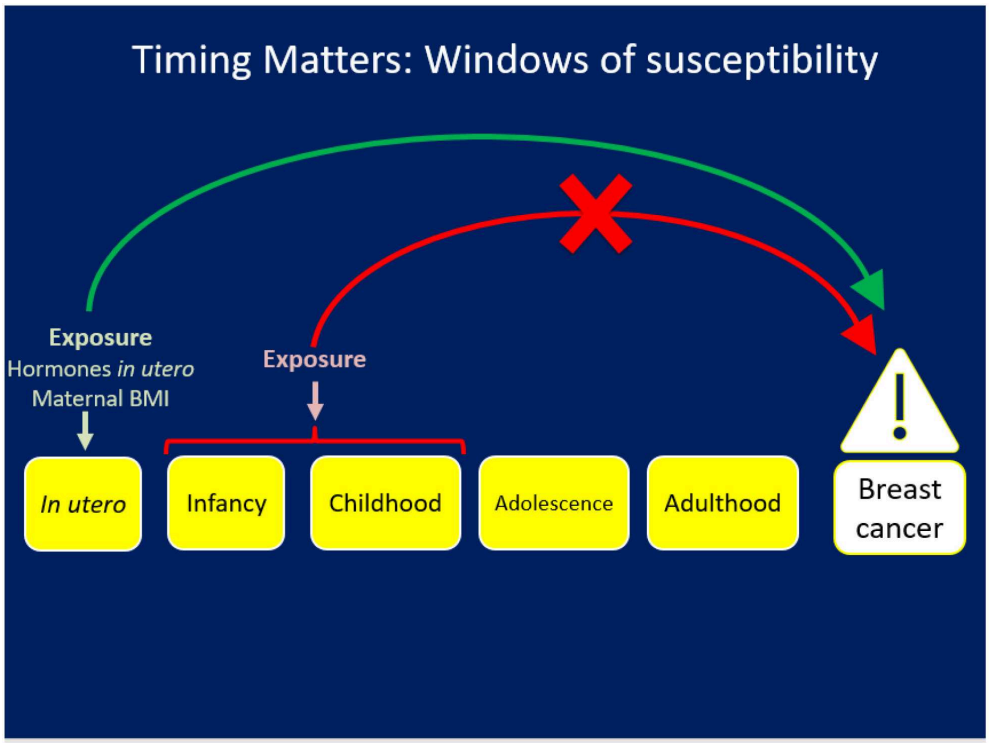


FIGURE 1 | The First D: Exposures occurring during a specific window like *in utero* may have an impact on risk for chronic disease/breast cancer but the same exposure at a different life stage will not have the same impact.

Diet and body size in *childhood* are related to early breast development in Norway and percent breast density in the U.S. Specifically milk, butter and ice cream consumption at 3–5 years was inversely associated with early breast development in Norwegian girls aged 10.8 years (OR = 0.97, 95% CI: 0.95–1.00) after adjustment for birthweight, preeclampsia, weight, and height and other covariates (22). A recent systematic review concluded there was a likely association between childhood animal protein intake and *earlier puberty* assessed by age at menarche and age at peak height velocity (23). Finally the heaviest body size at age 10 as illustrated using the Stunkard images vs. the leanest body sized girls had a 5.9 fold (95% CI: −9.2–2.3) lower percent breast density when they reached ages 40–64 years, with 7.69 cm^2 (95% CI: −13.9–0.63) smaller dense breast area, and 26.17 cm^2 (95% CI: 9.42–43.58) larger non-dense area (24).

*The Second D (**Figure 2**) addresses the intensity and duration of the exposure* and offers a different lens into breast cancer prevention. Cohn et al. reported that women who were exposed to the middle and highest tertiles of DDT before 14 years of age had a 2.80 (95% 1.10–6.80) and 5.14 (95% CI 1.70–17.1) fold increased risk for breast cancer, respectively, compared to women in the lowest tertile of exposure at the same age. Those women exposed at or after 14 years had no risk of BC by tertile of exposure to DDT (25). Thus, early to late childhood when the breast is developing comprised the window of vulnerability for BC risk due to DDT exposure. Being in the middle and highest tertile of exposure to DDT during puberty was the marker for the intensity of exposure to confer BC risk.

Stressful life events in the family also offer a perspective on the timing of and intensity with which these events may have a role in breast cancer. For example, the Netherlands Cohort Study covered the era of the Great Depression 1929–32 through the hunger winter of 1944–45 that was rampant in certain regions of the Netherlands. In this cohort, if the father was unemployed during the Great Depression (1929–32) the daughter had a marginally reduced risk by 18% (95% CI 0.66–1.02) of breast cancer (26) Living in a city during World War II when a girl was experiencing a growth spurt was associated with a 28% (95% CI 0.54–0.97) lower risk of BC (26). Further living in a city during the hunger winter of 1944–45 was associated with a 51% (95% CI 1.06–2.17) higher risk of BC if the girl had completed her growth spurt. Therefore, the Netherlands cohort study reveals that the type, timing, and intensity of life stress events (the first and second D) can be associated with higher or lower risk of BC.

*The third D (**Figure 3**) examines the effects of how rapidly or slowly a girl/woman experiences her linear growth and weight trajectory and/or hormonal and pubertal development and their implications for BC risk*. This D is revealed in a life stage-specific lens for BC risk with a strength that can be manifest across life stages (27–29). The first study appeared in the work by Ahlgren et al. amongst 117,415 Danish women with 3,340 BC cases that demonstrated the independent effects of a 10–17% range in higher BC risk for: the high birthweight, those with

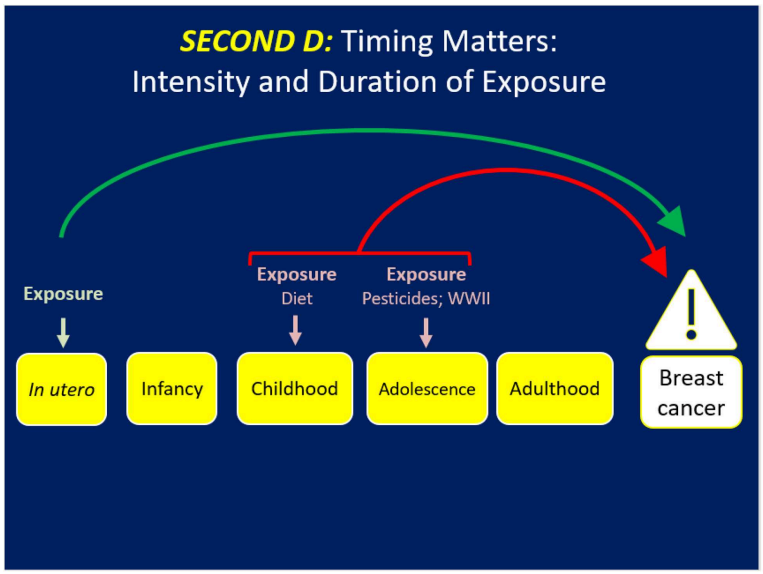


FIGURE 2 | Timing matters but the intensity or concentration and duration of the exposure may dramatically influence risk of BC.

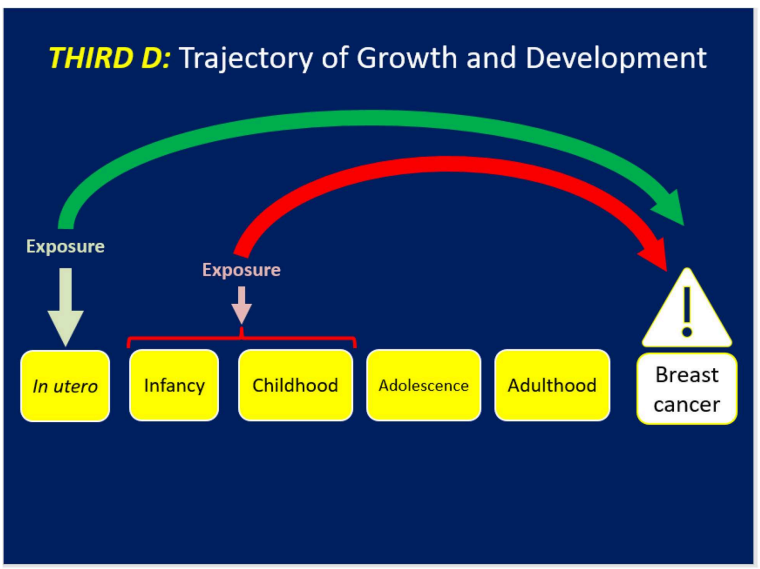


FIGURE 3 | Trajectories of growth and development may reveal how the biosystem has adapted to cumulative hormones and growth factors that may influence BC risk. These trajectories may also set the stage for exposures identified in the first D and/or stressful life events in the second D to have an impact on BC.

peak linear growth from 8 to 14 years i.e., puberty and attained adult height on BC risk (30). This landmark research introduced linear growth trajectory as a key component of BC risk. Berkey et al. investigated in the Growing Up Today Study (GUTS) that height at age 10 and peak height velocity were associated with risk for benign breast disease (31). Li et al. reported in the Vitamin and Lifestyle study that reaching the age of maximum height by 12 years conferred a 50% (95% CI 1.10–1.90) higher risk of BC than those who reached maximum height by age 17 years after adjustment for covariates (32). Rosner examined weight and weight changes in early adulthood and later BC risk using the NHSII (33). Weight at age 18 was inversely associated with pre and postmenopausal BC (HR per 30 Kg = 0.52, 95% CI: 0.39–0.71; HR = 0.82 95% CI: 0.72–0.92). In contrast, weight gain since age 18 was positively associated with ER+/PR+ postmenopausal BC (HR per 30 kg = 1.50 (95% CI: 1.36–1.65) but not with ER+/PR- or ER-/PR- BC. Overall 17% of ER+/PR+ BC was attributable to weight gain of >5 kg since age 18. In a multi-center analysis of pooled cohort studies, premenopausal BC risk was inversely associated with BMI at ages 18–24 years (HR per

5 kg/m^2 difference 0.77 95% CI 0.73–0.80) (34). Associations were strongest for ER+/PR+ subtype of BC but the HR did not vary by other BC risk factors nor for BMI later in adulthood. Chlebowski et al. recently reported that among a cohort of 61,335 healthy postmenopausal women without breast cancer, those who experienced a weight loss of five percent or more over 3 years had a HR of 0.88 (95% CI: 0.78–0.98) for BC compared to those whose weight remained stable, revealing how weight loss in the postmenopausal years can prevent BC (35). Another recent work by Luo et al. demonstrated in the Women's Health Initiative (WHI) that being low birthweight conferred a lower risk of postmenopausal BC by 22% (95% CI: 0.79–0.99). The effect of birthweight on postmenopausal BC risk was appreciably mediated by adult height (40% proportion mediated) and weight at baseline ages of 50–79 years (21% proportion mediated). Obesity in late adulthood (>50 years) was associated with higher risk of BC. Furthermore, weight gain in adulthood over a 25 years period was also positively associated with BC risk regardless of the age/life stage (36).

SUMMARY AND CONCLUSIONS

This paper presents a life course approach to nutrition and breast cancer in three dimensions. The evidence base for each D and the picture puzzle that appears by addressing all three Ds offers a unique lens into nutrition and BC. The first D focuses on windows of vulnerability for indicators of nutritional and hormonal status. Birthweight reveals a strong signal across the life course on BC risk, but the direction of the associations are not consistent. Specifically the signal for high birthweight on BC appeared in some (15, 16) but no other studies (17, 36) thereby casting a doubt whether high birthweight can be a proxy indicator for fetal hormonal milieu (5). Self-reported birth weight data in Xu et al. (16), Michels et al. (17) and Luo et al. (36) and enrollment of different birth cohorts influence the overall distribution of birthweight (and concomitant percent low or high birthweight) in each cohort study that may contribute to the inconsistency of the findings. The appreciable proportion of the birthweight effect on BC risk that is mediated my adult height and weight lends credence to the need for repeated measures of anthropometrics to recognize the trajectory and strength of the signal from birthweight across the life course (36).

Weight gain (and the pace of weight gain) during specific months in infancy influences breast development, and the risk for benign breast disease. The turning point for weight and its direct influence on BC risk arises from the data on the independent effect of weight at age 18 and of weight gain over the adult years on BC risk. Stunning evidence now appears that BC can be prevented by weight loss over a 25 years period capturing peri- and postmenopausal intervals; these data are primarily based on NHW in the U.S. and need further research in other race-ethnic groups and countries. How much weight is sufficient to prevent BC and how long the weight loss needs to be sustained to reduce risk are other elements that need flushing out.

Height in the absolute sense and in multiple manifestations of the linear growth trajectory has a strong signal for BC. Height velocity, age of peak height velocity, and attained height directly influence BC risk. Illuminating what these markers of BC risk mean is a challenge. The insulin-like growth factor 1 signaling pathway and genes are contributors to height but different ages have different patterns of linear growth. For example, infants typically gain weight before a linear growth spurt, however this pattern is not so evident in adolescence, when leptin and IGF-1 work in tandem during puberty. What are the underlying pathways at these stages lending themselves to different phenotypic hormonal precursors to linear growth? How do they relate to BC risk?

The timing and intensity of exposure to pesticides and stressful life events influence BC risk. DDT exposure at a certain level and before 14 years, i.e., puberty exhibited a signal for BC risk; any exposure at 14 years or later let alone exposure to a lower level had no effect. Likewise being in a household with an unemployed father during the Great Depression or experiencing hunger in an urban area during World War II was sufficient to be an indicator of risk for BC. It appears that three parameters–age, the intensity of the exposure and the timing during development– are key to identifying the components in the life course that are related to BC risk later in life.

This article presents a new paradigm of nutrition and breast cancer prevention through the lens of three very different dimensions. It is the premise of this article that all three dimensions are essential tasks to tease apart the life course and identify windows for preventive strategies. The picture puzzle has the potential for enrichment by examination of the gene-environment interactions in diverse populations and the examination of the epigenetic influences from diet, pesticides, and other environmental exposures.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

REFERENCES

1. Ferlay JEM, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, et al. *Global Cancer Observatory: Cancer Today*. Lyon: International Agency for Research on Cancer (2018).
2. Kelsey JL. Breast cancer epidemiology: summary and future directions. *Epidemiol Rev*. (1993) 15:256–63. doi: 10.1093/oxfordjournals.epirev.a036112
3. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. *Epidemiol Rev*. (1993) 15:36–47. doi: 10.1093/oxfordjournals.epirev.a036115
4. Forman MR, Cantwell MM, Ronckers C, Zhang Y. Through the looking glass at early-life exposures and breast cancer risk. *Cancer Invest*. (2005) 23:609–24. doi: 10.1080/07357900500283093
5. Ross JA, Perentesis JP, Robison LL, Davies SM. Big babies and infant leukemia: a role for insulin-like growth factor-1? *Cancer Causes Control*. (1996) 7:553–9. doi: 10.1007/BF00051889
6. Odegard RA, Vatten LJ, Nilsen ST, Salvesen KA, Vefring H, Austgulen R. Umbilical cord plasma interleukin-6 and fetal growth restriction in preeclampsia: a prospective study in Norway. *Obstet Gynecol*. (2001) 98:289–94. doi: 10.1097/00006250-200108000-00019
7. Olson BR, Forman MR, Lanza E, McAdam PA, Beecher G, Kimzey LM, et al. Relation between sodium balance and menstrual cycle symptoms in normal women. *Ann Intern Med*. (1996) 125:564–7. doi: 10.7326/0003-4819-125-7-199610010-00005
8. Forman MR, Beecher GR, Muesing R, Lanza E, Olson B, Campbell WS, et al. The fluctuation of plasma carotenoid concentrations by phase of the menstrual cycle: a controlled diet study. *Am J Clin Nutr*. (1996) 64:559–65. doi: 10.1093/ajcn/64.4.559
9. Bernstein L, Pike MC, Depue RH, Ross RK, Moore JW, Henderson BE. Maternal hormone levels in early gestation of cryptorchid males: a case-control study. *Br J Cancer*. (1988) 58:379–81. doi: 10.1038/bjc.1988.223
10. Haiman CA, Pike MC, Bernstein L, Jaque SV, Stanczyk FZ, Afghani A, et al. Ethnic differences in ovulatory function in nulliparous women. *Br J Cancer*. (2002) 86:367–71. doi: 10.1038/sj.bjc.6600098
11. Setiawan VW, Haiman CA, Stanczyk FZ, Le Marchand L, Henderson BE. Racial/ethnic differences in postmenopausal endogenous hormones: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev*. (2006) 15:1849–55. doi: 10.1158/1055-9965.EPI-06-0307
12. Bernstein L, Depue RH, Ross RK, Judd HL, Pike MC, Henderson BE. Higher maternal levels of free estradiol in first compared to second pregnancy: early gestational differences. *J Natl Cancer Inst*. (1986) 76:1035–9.
13. Mucci LA, Lagiou P, Tamimi RM, Hsieh C-C, Adami H-O, Trichopoulos D. Pregnancy estriol, estradiol, progesterone, and prolactin in relation to birth weight and other birth size variables (United States). *Cancer Causes Control*. (2003) 14:311–8. doi: 10.1023/A:1023966813330
14. Olivo-Marston SE, Mechanic LE, Mollerup S, Bowman ED, Remaley AT, Forman MR, et al. Serum estrogen and tumor-positive estrogen receptor-alpha are strong prognostic classifiers of non-small-cell lung cancer survival in both men and women. *Carcinogenesis*. (2010) 31:1778–86. doi: 10.1093/carcin/bgq156
15. Troisi R, Grotmol T, Jacobsen J, Tretli S, Toft-Sørensen H, Gissler M, et al. Perinatal characteristics and breast cancer risk in daughters: a Scandinavian population-based study. *J Dev Origin Health Dis*. (2013) 4:35–41. doi: 10.1017/S2040174412000645
16. Xu X, Dailey AB, Peoples-Sheps M, Talbott EO, Li N, Roth J. Birth weight as a risk factor for breast cancer: a meta-analysis of 18 epidemiological studies. *J Womens Health*. (2009) 18:1169–78. doi: 10.1089/jwh.2008.1034
17. Michels KB, Xue F, Terry KL, Willett WC. Longitudinal study of birthweight and the incidence of breast cancer in adulthood. *Carcinogenesis*. (2006) 27:2464–8. doi: 10.1093/carcin/bgl105
18. Michels KB, Cohn BA, Goldberg M, Flom JD, Dougan M, Terry MB. Maternal anthropometry and mammographic density in adult daughters. *Pediatrics*. (2016) 138(Suppl. 1):S34–41. doi: 10.1542/peds.2015-4268F
19. Goldberg M, Cohn BA, Houghton LC, Flom JD, Wei Y, Cirillo P, et al. Early-life growth and benign breast disease. *Am J Epidemiol*. (2019) 188:1646–54. doi: 10.1093/aje/kwz126
20. Berkey CS, Rosner B, Willett WC, Tamimi RM, Frazier AL, Colditz GA. Prenatal factors and infant feeding in relation to risk of benign breast disease in young women. *Breast Cancer Res Treat*. (2015) 154:573–82. doi: 10.1007/s10549-015-3637-3
21. Michels KB, Trichopoulos D, Rosner BA, Hunter DJ, Colditz GA, Hankinson SE, et al. Being breastfed in infancy and breast cancer incidence in adult life: results from the two nurses' health studies. *Am J Epidemiol*. (2001) 153:275–83. doi: 10.1093/aje/153.3.275
22. Schraw JM, Ogland B, Dong YQ, Nilsen ST, Forman MR. *In utero* preeclampsia exposure, milk intake, and pubertal development. *Reprod Toxicol*. (2015) 54:19–25. doi: 10.1016/j.reprotox.2014.12.004
23. Hornell A, Lagstrom H, Lande B, Thorsdottir I. Protein intake from 0 to 18 years of age and its relation to health: a systematic literature review for the 5th Nordic Nutrition Recommendations. *Food Nutr Res*. (2013) 57. doi: 10.3402/fnr.v57i0.21083
24. Athilat S, Joe C, Rodriguez CB, Terry MB, Tehranifar P. Childhood body size and midlife mammographic breast density in foreign-born and US-born women in New York City. *Ann Epidemiol*. (2018) 28:710–6. doi: 10.1016/j.annepidem.2018.08.002
25. Cohn BA, Wolff MS, Cirillo PM, Sholtz RI. DDT and breast cancer in young women: new data on the significance of age at exposure. *Environ Health Perspect*. (2007) 115:1406–14. doi: 10.1289/ehp.10260
26. Elands RJ, Offermans NS, Simons CCM, Schouten LJ, Verhage BA, van den Brandt PA, et al. Associations of adult-attained height and early life energy restriction with postmenopausal breast cancer risk according to estrogen and progesterone receptor status. *Int J Cancer*. (2019) 144:1844–57. doi: 10.1002/ijc.31890
27. Baer HJ, Colditz GA, Rosner B, Michels KB, Rich-Edwards JW, Hunter DJ, et al. Body fatness during childhood and adolescence and incidence of breast cancer in premenopausal women: a prospective cohort study. *Breast Cancer Res*. (2005) 7:R314–25. doi: 10.1186/bcr998
28. Baer HJ, Rich-Edwards JW, Colditz GA, Hunter DJ, Willett WC, Michels KB. Adult height, age at attained height, and incidence of breast cancer in premenopausal women. *Int J Cancer*. (2006) 119:2231–5. doi: 10.1002/ijc.22096
29. Xue F, Rosner B, Eliassen H, Michels KB. Body fatness throughout the life course and the incidence of premenopausal breast cancer. *Int J Epidemiol*. (2016) 45:1103–12. doi: 10.1093/ije/dyw149
30. Ahlgren M, Melbye M, Wohlfahrt J, Sorensen TI. Growth patterns and the risk of breast cancer in women. *N Engl J Med*. (2004) 351:1619–26. doi: 10.1056/NEJMoa040576
31. Berkey CS, Willett WC, Frazier AL, Rosner B, Tamimi RM, Colditz GA. Prospective study of growth and development in older girls and risk of benign breast disease in young women. *Cancer*. (2011) 117:1612–20. doi: 10.1002/cncr.25692
32. Li CI, Littman AJ, White E. Relationship between age maximum height is attained, age at menarche, and age at first full-term birth and breast cancer risk. *Cancer Epidemiol Prev Biomark*. (2007) 16:2144–9. doi: 10.1158/1055-9965.EPI-07-0242
33. Rosner B, Eliassen AH, Toriola AT, Chen WY, Hankinson SE, Willett WC, et al. Weight and weight changes in early adulthood and later breast cancer risk. *Int J Cancer*. (2017) 140:2003–14. doi: 10.1002/ijc.30627
34. Premenopausal Breast Cancer Collaborative G, Schoemaker MJ, Nichols HB, Wright LB, Brook MN, Jones ME, et al. Association of body mass index and age with subsequent breast cancer risk in premenopausal women. *JAMA Oncol*. (2018) 4:e181771. doi: 10.1001/jamaoncol.2018.1771
35. Chlebowski RT, Luo J, Anderson GL, Barrington W, Reding K, Simon MS, et al. Weight loss and breast cancer incidence in postmenopausal women. *Cancer*. (2019) 125:205–12. doi: 10.1002/cncr.31687
36. Luo J, Chen X, Manson JE, Shadyab AH, Wactawski-Wende J, Vitolins M, et al. Birth weight, weight over the adult life course and risk of breast cancer. *Int J Cancer*. (2019). doi: 10.1002/ijc.32710

Conflict of Interest: The author declares 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 in Medicine

REVIEW
published: 12 May 2020
doi: 10.3389/fmed.2020.00175

Current and Emerging Magnetic Resonance-Based Techniques for Breast Cancer

Apekshya Chhetri[1,2], *Xin Li*[1] *and Joseph V. Rispoli*[1,3,4*]

[1] *Magnetic Resonance Biomedical Engineering Laboratory, Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN, United States,* [2] *Basic Medical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN, United States,* [3] *Center for Cancer Research, Purdue University, West Lafayette, IN, United States,* [4] *School of Electrical & Computer Engineering, Purdue University, West Lafayette, IN, United States*

Breast cancer is the most commonly diagnosed cancer among women worldwide, and early detection remains a principal factor for improved patient outcomes and reduced mortality. Clinically, magnetic resonance imaging (MRI) techniques are routinely used in determining benign and malignant tumor phenotypes and for monitoring treatment outcomes. Static MRI techniques enable superior structural contrast between adipose and fibroglandular tissues, while dynamic MRI techniques can elucidate functional characteristics of malignant tumors. The preferred clinical procedure—dynamic contrast-enhanced MRI—illuminates the hypervascularity of breast tumors through a gadolinium-based contrast agent; however, accumulation of the potentially toxic contrast agent remains a major limitation of the technique, propelling MRI research toward finding an alternative, noninvasive method. Three such techniques are magnetic resonance spectroscopy, chemical exchange saturation transfer, and non-contrast diffusion weighted imaging. These methods shed light on underlying chemical composition, provide snapshots of tissue metabolism, and more pronouncedly characterize microstructural heterogeneity. This review article outlines the present state of clinical MRI for breast cancer and examines several research techniques that demonstrate capacity for clinical translation. Ultimately, multi-parametric MRI—incorporating one or more of these emerging methods—presently holds the best potential to afford improved specificity and deliver excellent accuracy to clinics for the prediction, detection, and monitoring of breast cancer.

Keywords: breast cancer, magnetic resonance, MRI, diffusion, spectroscopy, contrast

OPEN ACCESS

Edited by:
Rabih Shakib Talhouk, American University of Beirut, Lebanon

Reviewed by:
Alexander F. I. Osman, Al-Neelain University, Sudan
Raja R. Narayan, Stanford University, United States

***Correspondence:**
Joseph V. Rispoli
jrispoli@purdue.edu

Specialty section:
This article was submitted to Family Medicine and Primary Care, a section of the journal Frontiers in Medicine

Received: *15 April 2019*
Accepted: *15 April 2020*
Published: *12 May 2020*

Citation:
Chhetri A, Li X and Rispoli JV (2020) Current and Emerging Magnetic Resonance-Based Techniques for Breast Cancer. Front. Med. 7:175. doi: 10.3389/fmed.2020.00175

INTRODUCTION

The American Cancer Society has estimated that within the United States in 2020, a total of 276,480 females will be diagnosed with breast cancer and 42,170 are likely to die from the disease (1). While breast cancer treatment has advanced, early detection remains a principal factor for improved patient outcomes and reduced mortality. Although, mammography has been the standard method of breast cancer screening since the 1960s, magnetic resonance (MR) imaging (MRI) offers superior sensitivity, particularly within denser breasts, and an annual MRI exam is recommended for high-risk women (e.g., women with familial history, genetic predisposition, significant chest radiation history, or lobular cancer) (2).

Amongst the existing and routinely practiced modalities to screen breast cancer, MRI has the highest sensitivity. In a recent study conducted over a period of eight years, Kuhl et al. reported a 95% confidence interval of 96.5–97.6% for specificity with a positive predictive value of 35.7% in diagnosing high grade breast tumors of sizes as small as 8 mm (3). A major limitation of clinical MRI lies in its wide range of specificity (37–97%) manifested as failures in differentiating malignant breast tumors vs benign lesions (4–6). However, false positive results from MRI observed in high risk lesions differ significantly from the low risk lesions associated false positive results through radiographs (7). These inherent biological differences with significant prognostic implications cannot be overlooked as we compare the results between MRI and other radiographic screening modalities. The advancements in MRI techniques and future research summarized in this paper are aimed at overcoming the specificity associated limitation of MRI to differentiate benign lesions from aggressive breast tumors with improved accuracy.

At present, secondary breast cancer prevention for males is not emphasized as widely as in females owing to the low male breast cancer incidence rate of 1% (8, 9). Studies demonstrating the use of MRI in screening male breast cancer patients are few, yet not uncommon (10–12). Survival outcomes of male breast cancer patients have worsened in recent years (12–14). The present treatment options for male breast cancer patients are derived from the clinical outcomes on female patients, which could be a potential limiting factor (14). Thus, more studies highlighting the impact of secondary breast cancer prevention on males, particularly given improved risk assessment from genetic testing, e.g., BRCA2-associated phenotype (15), are needed.

Advances in MRI and MR spectroscopy (MRS) have enabled clinicians to detect numerous biomarkers of breast cancer and to monitor the patient's response to chemotherapy. Studies have shown a correlation between these MR-based biomarkers and histopathological features of tumors. This linkage could provide a powerful technique for monitoring the progression of the disease and the patient's response to chemotherapy (16–21).

Image contrast based on tissue T_1 and T_2 are common MRI sequences exploiting the differences in the relaxation times of protons within the tissue under examination. T_1 provides longitudinal relaxation time while T_2 provides transverse relaxation time for a set of protons. By exploiting the distinct T_1 and T_2 relaxation properties of various tissues, static MRI provides superior structural contrast between adipose and fibroglandular tissues and remains a mainstay for risk analysis, tumor detection, and treatment monitoring. Dynamic MRI techniques go one step further, elucidating functional characteristics of malignant tumors. Dynamic contrast enhanced (DCE) MRI detects T_1 changes in tissues over time immediately following bolus administration of a gadolinium-based contrast agent; the hypervascularity of breast tumors results in altered uptake and washout rates, and the unique time-intensity curve can distinguish malignant from benign tumors. Recent concerns regarding lasting gadolinium accumulation and toxicity, however, have impacted patient's assent to undergo techniques requiring gadolinium-based contrast agent, including DCE MRI, and research efforts have renewed to design alternative, noninvasive methods. One leading contender is diffusion weighted imaging (DWI), which already has proven valuable as an adjunct to DCE by improving combined sensitivity. DWI can elucidate tissue properties based on the Brownian motion of water. Since diffusivity differs inside and outside cells, the pattern of tissue morphology can be established based on the restriction of motion of water molecules in densely packed cells (22). Emerging techniques including MRS and chemical exchange saturation transfer can shed light on underlying chemical composition, providing snapshots of tissue metabolism and characterizing microstructural heterogeneity. Furthermore, non-compartmentalized, non-Gaussian diffusion models have the potential to derive micrometer-scale diffusion metrics that may reflect tumor heterogeneity and microstructural dimensions. This review article outlines the various MRI techniques currently used for breast cancer and examines several research techniques that demonstrate capacity for clinical translation or potential to facilitate discoveries in basic research.

CURRENT MR-BASED TECHNIQUES

Structural Imaging

Among the clinical imaging modalities, MRI yields superior sensitivity of breast tumors and, notably among dense breasts, provides excellent contrast between tumor, adipose, and fibroglandular tissues (23, 24). A typical structural breast imaging protocol includes a T_2-weighted sequence and a T_1-weighted sequence, with and without fat suppression (25). Bilateral imaging is performed in order to evaluate asymmetries. High breast density is a known risk factor of developing malignant breast tumors (26), and specialized sequences have been developed for breast density measurement (27). The American College of Radiology Breast Imaging Reporting and Data System (BI-RADS) provides guidance for the succinct classification of overall breast composition, with emphasis on the proportion of fibroglandular tissues (25). As illustrated in **Figure 1**, fibroglandular tissues are readily differentiated from adipose tissues when using a T_1-weighted sequence with fat suppression.

Contrast-Enhanced Perfusion MRI

Standard clinical breast MRI protocols also include a gadolinium dynamic contrast enhanced scan for distinguishing malignant from benign tumors. A fat-suppressed T_1-weighted sequence is run before and up to 15 minutes after an intravenous bolus injection of gadolinium-based contrast agent followed by a saline flush. The rate of gadolinium washout is indicative of the microvascular properties and hyperintensity within malignant tumors is very sensitive and specific to malignant tumors (5). Notably, hormonal fluctuations can affect the uptake of gadolinium in healthy breast tissue, so dynamic contrast enhancement is only recommended to be performed during the first half of the menstrual cycle (29, 30). Representative dynamic contrast enhanced MRI are shown in **Figure 2A**.

In contrast to conventional dynamic contrast enhancement techniques, whole breast area (normal parenchymal breast tissues) can be enhanced utilizing the background parenchymal

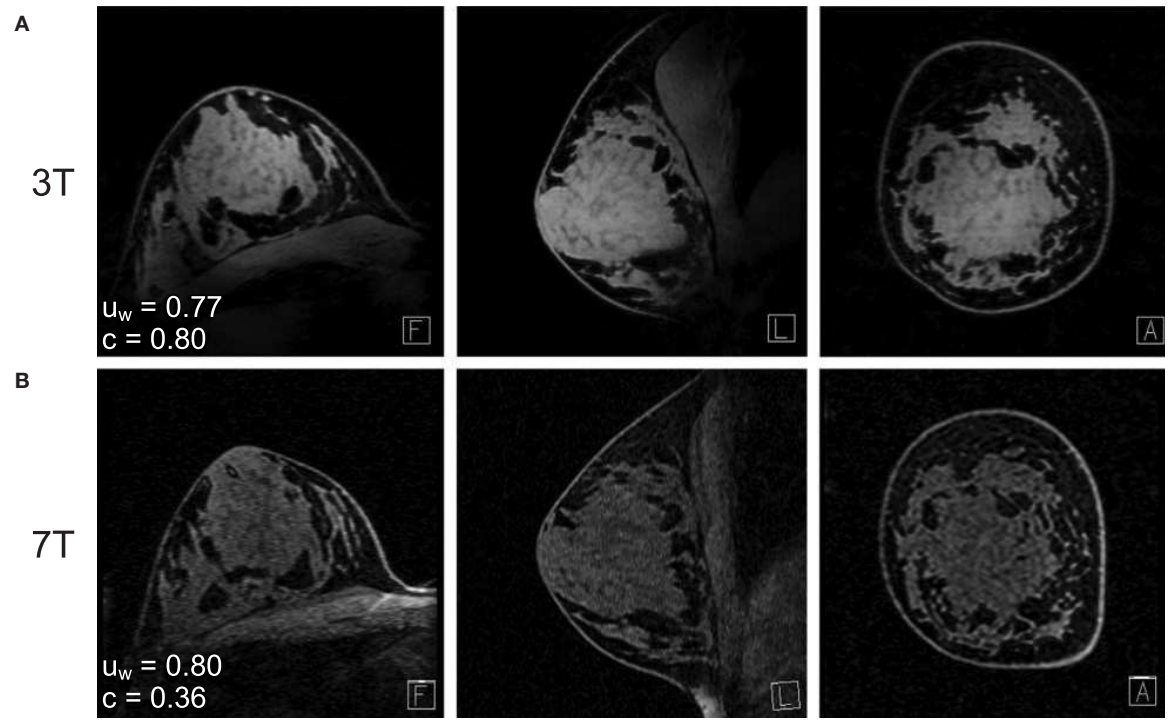


FIGURE 1 | Fat-suppressed T_1-weighted MRI of the same subject at **(A)** 7T and **(B)** 3T. The water signal uniformity (u_w) is similar across 3T and 7T, while the fat-water contrast (c) is markedly improved at 7T. Reprinted with permission from Brown et al. (28); ©2013 Wiley Periodicals, Inc.

enhancement (BPE) technique. This technique can identify specific regions of differences within normal mammary tissues over others which facilitates a wider prediction of the tumor microenvironment and its possible changes. These features augment the specificity and sensitivity of MRI and is advantageous in reducing false positive results. BPE is assessed by four qualitative BI-RADS categories: minimal (<25% of glandular tissue demonstrating enhancement), mild (25-50% enhancement), moderate (50-75% enhancement), or marked (> 75% enhancement). In 2011, King et al. concluded that increased BPE is strongly predictive of breast cancer odds (32), however more recent studies have found no correlation with positive biopsy rate, sensitivity, or specificity (33).

Clinical MR Scanners

Clinical 1.5 tesla (T) and 3T scanners typically include a built-in body coil for transmitting radiofrequency (RF) pulses, i.e., the B_1 field. Given the off-center positioning of the breasts within the body coil, and the asymmetric loading presented by the torso, transmit B_1 inhomogeneity is prone to worsen at higher magnetic fields. At 3T, the body coil has been reported to produce up to 50% error in tip angle (34), which significantly confounds the accuracy of quantitative image-derived measures including DCE enhancement ratio (35) and T_1 mapping (36). These issues may be mitigated using advanced quantification techniques and accompanying pulse sequences, e.g., saturation-recovery snapshot-fast low angle shot (37).

Irrespective of the scanner's magnetic field strength, receive array coils improve signal-to-noise ratio (SNR) throughout the breast compared to utilizing the body coil to receive the RF signal (38). A variety of commercial breast receive array coils are available (39, 40) and custom 3T array coils have been reported to further improve performance for specific applications (41, 42).

EMERGING MR-BASED TECHNIQUES

Diffusion-Weighted MRI

Gaussian Models

Diffusion weighted imaging

As a noninvasive MRI technique, diffusion weighted imaging (DWI) detects the bulk diffusion of water within tissue and offers substantial advantages in visualizing and differentiating tumors based on their vascularization patterns. The amount of diffusion weighting applied to the MRI signal is set by the operator-defined b-value, with zero indicating no diffusion weighting (**Figure 3A**) and commonly employed b-values for breast DWI being on the order of 1,000 s/mm^2. DWI encodes water diffusion in one to three orthogonal directions (each direction corresponding to a gradient direction) and assumes unrestricted isotropic diffusion. The resulting apparent diffusion coefficient (ADC) quantifies the mean bulk diffusion per pixel and is an established quantitative surrogate for tissue cellularity. While the cell membranes and vascularity within tumors preclude unrestricted water motion, the simple DWI model

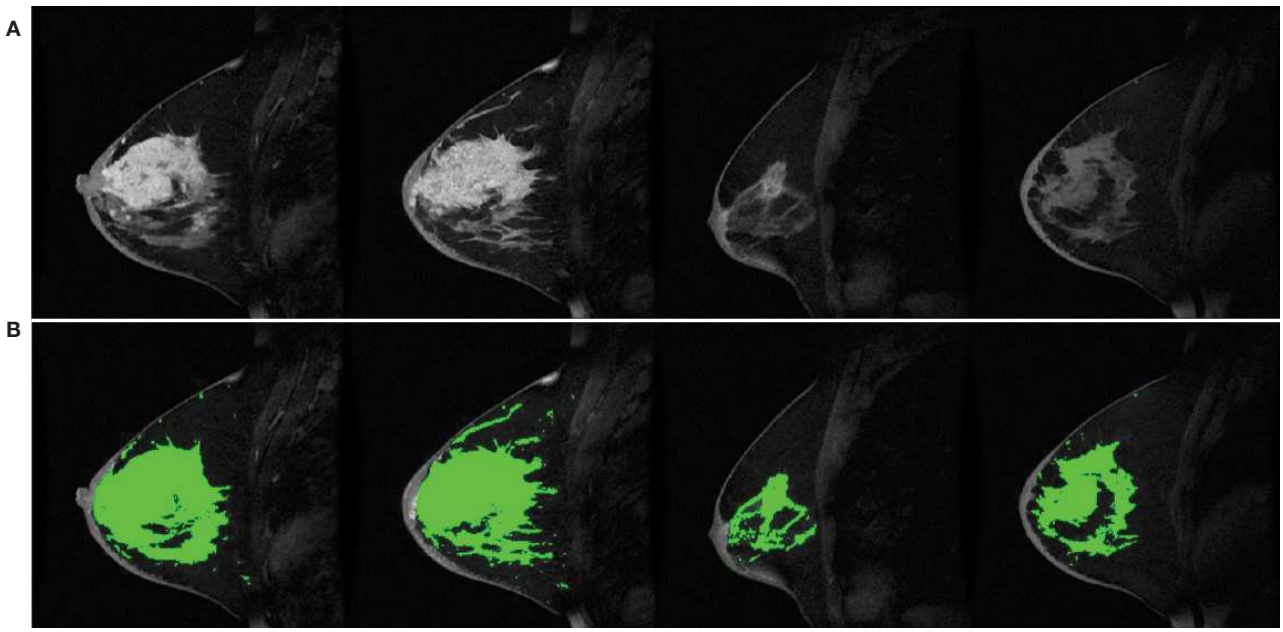


FIGURE 2 | High-resolution 1.5T DCE MRI of four subjects from the American College of Radiology Imaging Network (ACRIN) 6657 repository (31) **(A)** unmodified and **(B)** with the segmented breast fibroglandular tissue overlaid in green.

accurately represents voxels (single data-specific locations on a 3D tissue construct) with high water content and low cell density and the resulting hypo intensity within breast tumors remains informative. This effect is illustrated in **Figure 3B**. Moreover, a technique known as automated DWI, which retrospectively computes higher b-value images from the typical DWI acquisitions, has been shown to improve lesion detection, particularly when calculations are performed on a voxel-wise basis (44).

Traditional spin-echo DWI relies on a conventional single-shot echo planar imaging readout prone to produce ghosting artifacts that hinder image quality. Other readouts such as spatio-temporal encoding mitigate ghosting artifacts at the expense of added noise (45). Ultimately, readout-segmented (or multi-shot) echo planar imaging has been established as a robust solution with good sensitivity; ghosting artifacts are prevented since each shot acquires the full extent of k-space in the phase-encode direction but only traverses a segment in the readout direction (46). The readout-segmented DWI sequence is prevalent and frequently prescribed for bilateral breast DWI with 2-mm in-plane resolution.

Higher-resolution DWI may be attained by reducing the field of view, which focuses on a target region within the breast. With this technique, 0.8-mm in-plane resolution can be resolved at 3T, and the resulting ADC maps provide greater detail facilitating the assessment of tumor morphology (47). Imaging time can be reduced by combining the high-resolution reduced field of view approach with multiband RF excitation (48).

Obtaining consistently high-quality breast DWI is one of the challenges that current studies are targeting to overcome. The American College of Radiology Imaging Network (ACRIN) 6698 clinical trial has shown that ADC can be measured with excellent repeatability and reproducibility in a multi-institution setting using a standardized protocol and QA procedure (49). An MRI platform that can provide a clearer distinction between tumors delivers more deterministic results to the patients, thus restricting the number of unnecessary biopsies performed on patients largely due to false positive results. However, it is important to note DWI should not be used as a stand-alone clinical protocol; rather, DWI hold a compelling role within multi-parametric MRI (mpMRI) protocols. For example, DWI detects significantly fewer cancers compared to dynamic contrast enhancement technique, but when incorporated as an adjunct it will yield superior sensitivity (46). Similar improvements can be achieved when pairing DWI with other complementary techniques such as MRS, as discussed later.

Diffusion tensor imaging

Diffusion tensor imaging (DTI) builds on the DWI technique by increasing the number of diffusion-encoding directions, thus enabling the calculation of anisotropic diffusion. While DWI characterizes isotropic diffusion within each voxel as a sphere, DTI employs at least six gradient directions and geometrically represents anisotropic diffusion within each voxel as an ellipsoid. The diffusion tensor, a matrix of directional diffusion coefficients, is established for each voxel based on the diffusion rates detected concurrent with each gradient configuration. Given the directionality of resulting diffusion information, DTI can provide additional insight into tissue microstructure through mean diffusivity—the DTI analogue to the ADC in DWI—and various anisotropy measures which provide critical

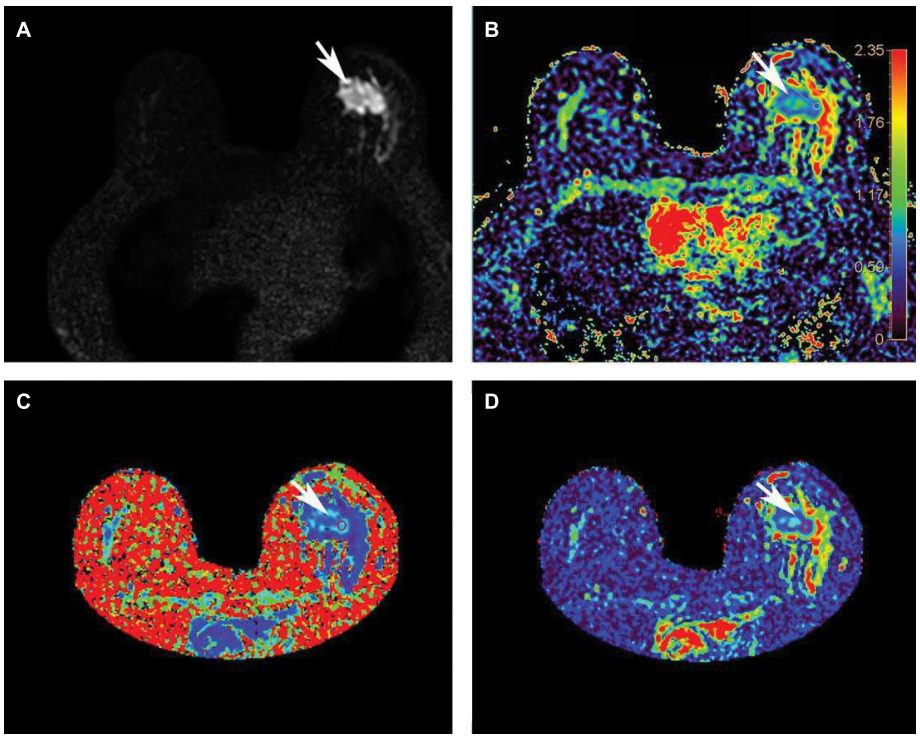


FIGURE 3 | A comparison of diffusion techniques and metrics from scanning a 57-year-old woman with left breast invasive ductal carcinoma (tumor indicated by the arrow) at 3T. **(A)** The baseline b = 0 image acquired without diffusion gradients; **(B)** conventional DWI: apparent diffusion coefficient (ADC) map (scale bar 0-2.35 mm^2/s), arrow indicating tumor ADC value of 1.090 mm^2/s; **(C)** diffusion kurtosis imaging: mean kurtosis map (scale bar 0-3 mm^2/s), arrow indicating tumor mean kurtosis value of 1.154 mm^2/s; **(D)** DTI: mean diffusivity map (scale bar 0-2.8 mm^2/s), arrow indicating tumor mean diffusivity value of 0.808 mm^2/s. Reprinted with permission from Li et al. (43); ©2018 International Society for Magnetic Resonance in Medicine.

information such as a tissue's vascularity, density, and cellular features. Such anisotropic features include fractional anisotropy, radial anisotropy, the individual diffusion coefficients, and the maximal anisotropy index. A mean diffusivity map is shown in **Figure 3D**.

While there is a consensus across studies that mean diffusivity is significantly lower in malignant tumors compared to benign lesions, there are conflicting results regarding the diagnostic utility of the anisotropy indices (50). Some reports suggest the standard DTI metrics of fractional anisotropy, radial anisotropy, and mean diffusivity cannot differentiate healthy tissue from cancer, while the diffusion coefficients and absolute maximal anisotropy index can assist in differentiating malignant tumors from both benign lesions and healthy tissue (51, 52). A recent approach suggests modifying the DTI model by compartmentalizing the diffusion signal as a combination of an anisotropic diffusion tensor (stroma cells) and a spectrum of highly-restricted (lymphocytes), restricted (cancer cells), and hindered (edema) isotropic-diffusion tensors; initial results with this modified diffusion basis spectrum imaging technique indicate greater diagnostic sensitivity and specificity distinguishing between malignant tumors and benign lesions (53).

Remarkably, DTI metrics have been shown to have distinctive correlations with breast cancer subtypes. Onaygil et al. found statistical significance between several anisotropy indices in estrogen receptor positive and negative (ER+ and ER-) breast cancers, and separate correlations with the levels of Ki-67, a biomarker for cellular proliferation, while Ozal et al. reported identifying distinct correlations between various DTI metrics and levels of breast cancer prognostic factors: ER, progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), Ki-67, and lymphatic invasion in invasive tumors (54, 55).

The challenge of achieving excellent repeatability and reproducibility across sites remains ongoing with breast DTI. Studies indicate the ADC can be reproduced with more accuracy compared to DTI anisotropy metrics such as fractional anisotropy (56, 57).

Notably, the technical development that drove substantial improvements into the DTI technique was largely motivated by the quest to map neuronal tracks of white matter in the brain. Preliminary studies reconsidering the utility of DTI for breast cancer have investigated utilizing DTI for breast tractography (58). Given the stark difference between the two-point connections of neuronal tracks and the branching ductal tree, Degani and colleagues proposed a novel computational methodology of post-processing DTI data using vector maps and clustering to infer the detailed structure of the mammary tree (59, 60).

Non-gaussian Models

Diffusion kurtosis imaging

While a Gaussian distribution of diffusion indeed applies to pure liquids and gels, barriers from complex tissue structures in effect modify the probability distribution of diffusion. Accordingly, the statistical metric for quantifying the actual probability distribution within tissue is designated as kurtosis. By acquiring additional, higher b-value images (where b value is an operator-defined parameter correlating with the strength and time for diffusion in imaged tissues), on the order of b = 1000–3000 s/mm^2, and at least 15 diffusion gradient directions, the diffusion kurtosis imaging technique can map multiple structures within a single voxel, e.g., crossing white matter fibers in the brain. In the context of breast imaging, diffusion kurtosis imaging is sensitive to intracellular structures such as membranes and organelles (61) and, in addition to a mean kurtosis map, can provide a diffusion heterogeneity index sensitive to the tumor microstructure (62). Importantly, diffusion kurtosis analysis of the breast improves with correction for unsuppressed fat signal (63). A mean kurtosis map is shown in **Figure 3C**.

Intravoxel incoherent motion

While technically also a perfusion imaging method, the intravoxel incoherent motion model adds additional quantitative terms to account for microvascularity. Accordingly, intravoxel incoherent motion has the potential to discern both tissue diffusivity and microcapillary perfusion without the need for contrast agents (64). Additional quantitative metrics include the perfusion fraction (or blood volume fraction of vasculature) and a pseudodiffusion coefficient corresponding to water movement within microvasculature. For breast cancer imaging, the intravoxel incoherent motion model is more often added to non-Gaussian diffusion methods (65). A combination of high perfusion fraction, high kurtosis, and low diffusion coefficient is often observed at the periphery of tumors, while the opposite pattern is apparent in the necrotic core as well as within fibroadenomas (66). Accordingly, the intravoxel incoherent motion model shows promise for differentiating between malignant and benign breast lesions (67, 68). Furthermore, a recent report also indicates histogram analysis can accurately predict neoadjuvant chemotherapy (NAC) response (69).

Other Diffusion Models

Many other advanced diffusion methods have been proposed with the goal of probing intravoxel heterogeneity and cellularity; a review of several such methods and their suitability for cancer imaging was recently published by Tang and Zhou (62). Generally, these methods require additional acquisitions with b-values up to 4000 s/mm^2, presenting a challenge given the lower SNR inherent with high b-value acquisition.

Magnetic Resonance Spectroscopy

Proton Spectroscopy

Magnetic resonance spectroscopy (MRS) provides a localized snapshot of the biochemical makeup of tissue (70). Proton (^{1}H) MRS offers the greatest sensitivity and simplest data acquisition. Elevated levels of choline-containing compounds indicate cell membrane turnover and are a biomarker for malignant breast tumors (71). All choline-containing compounds are quantified as total choline (tCho) and appear as a peak at 3.2 ppm on the ^{1}H MRS spectrum. A thorough 2013 meta-analysis of tCho studies ($n = 1193$ patients) suggests this biomarker offers 73% sensitivity and 88% specificity (72). Moreover, high levels of glutathione measured with ^{1}H MRS have been associated with increased resistance of cancer cells to radiation-induced cell death (73).

The recent ACRIN 6657 MRS clinical trial aimed to predict response to NAC with tCho single-voxel MRS; the results were inclusive, with only 29/119 subjects providing useable data (74). A primary limitation of the protocol was the manual placement of the MRS voxel within or encompassing the tumor, leading to issues with reproducibility across clinical sites. In the future this limitation can be addressed by running a full 3D magnetic resonance spectroscopic imaging sequence, allowing localized analysis to be performed retrospectively.

The high specificity of tCho studies suggests ^{1}H MRS could be an effective addition to a mpMRI protocol (75). For superior differentiation of benign tumors from normal physiology, ADC values from DWI in combination with tCho peaks can provide a comprehensive result (76).

Proton MRS also facilitates lipid analysis, i.e., proportions of mono- and poly-unsaturated fats, fatty acid chain length, and mean saturation, all measures that are sensitive to past dietary intake. Specific lipid signatures have been reported to be significantly lower in malignant versus benign tumors, and luminal cancers can be differentiated via lipid MRS (77–79). Acquisition issues stemming from water-lipid susceptibility boundaries can be avoided by running a zero-quantum-coherence 2D MRS sequence (80).

Multinuclear Spectroscopy

With ^{1}H MRS, many spectral peaks overlap and potentially mask lower-concentration metabolites. While multinuclear MRS suffers upfront from reduced sensitivity—an inherent deficit in SNR that is somewhat mitigated at higher fields—they provide a window into breast cancer metabolism with information inaccessible to ^{1}H MRS (81). Phosphorus-31 (^{31}P) MRS separates distinct choline compounds, specifically phosphorylcholine and glycerophosphocholine, otherwise overlapped as tCho on the ^{1}H spectrum. The role of phosphocholines in breast cancer metabolism is of broad interest (82–85), with the ratio of phosphocholine to glycerophosphocholine hypothesized to switch from low to high during malignant transformation (86), and to increase further with tumor progression (87). The ratio of phosphomonoesters to phosphodiesters has been shown to decrease after successful NAC (88). An example ^{31}P spectrum from an ER+, PR+, HER2- tumor is presented in **Figure 4**.

Carbon-13 (^{13}C) MRS can provide additional information such as the composition of breast fat and correlations that may predispose to cancer. Performing *in vivo* ^{13}C MRS is difficult for many reasons, including low natural abundance, low (in comparison to ^{1}H) sensitivity, J-coupling bonds between ^{1}H and ^{13}C atoms that obfuscate spectral peaks, and unique hardware instrumentation requirements. The preferred ^{13}C MRS experiment, applying broadband proton decoupling, requires

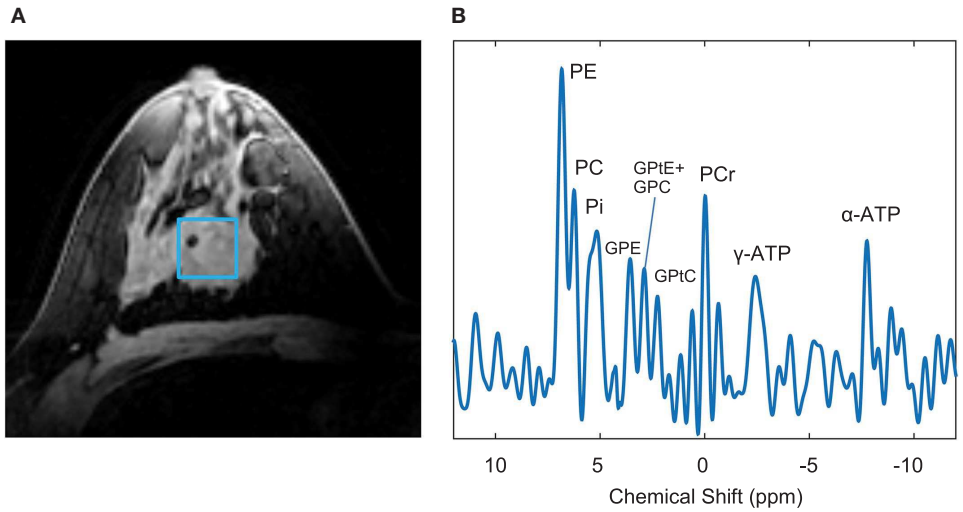


FIGURE 4 | Example 7T data of a patient with an ER+, PR+, HER2- tumor. **(A)** T_1-weighted image with indicated voxel selection (blue square), **(B)** ^{31}P MRS spectrum of nine fitted metabolites. Adapted from Krikken et al. (88), used under CC BY.

RF coils operating at both the ^{1}H and ^{13}C frequencies; the ^{1}H channel is used for scout imaging as well as to transmit proton-decoupling pulses across the J-coupled chemical shift band (89). By employing proton decoupling at 7T, natural abundance ^{13}C lipid analysis from the breast was demonstrated (90). Enriched or hyperpolarized ^{13}C studies boost the SNR and facilitate additional studies, including using ^{13}C-labeled choline to distinguish between catabolic and anabolic pathways in choline metabolism (91), and gauging glucose metabolism in the breast using [U-^{13}C] glucose bolus injection (92).

Magnetization Transfer

Magnetization transfer (MT) was first introduced by Wolff and Balaban (93); the MT image contrast reflects the exchange of magnetization between protons in free water and protons bound to macromolecules due to chemical exchange and dipole-dipole interactions. After image acquisition with a specialized off-resonance RF pulse, the MT effect among voxels of interest is quantified using either the so-called z-spectrum or a histogram of the MT ratio. The repeatability of quantitative breast MT measurements among cohorts of healthy volunteers has recently been demonstrated (94, 95). MT images can provide important information of tumor response to NAC (96). Chemical exchange saturation transfer extends the capabilities of MRS by indirectly detecting low-concentration chemicals through their proton exchange with water, including protein aggregates in malignant tumors. For example, amide proton transfer imaging detects the protein and peptide concentration by saturating the amide protons within peptide bonds. Dula et al. defined an integrated voxel-wise metric assumed to reflect the cellular protein and peptide content, designated amide proton transfer residual, and calculated this measure before and after neoadjuvant chemotherapy for two women with ER- breast cancer who experienced contradictory outcomes (95). As illustrated in **Figure 5**, they found a decrease in amide proton transfer residual from the woman with a complete response, while the metric from the woman with progressive response increased (95). Moreover, chemical exchange saturation transfer can discriminate between nonmalignant and aggressive human breast cancer cells, as it can characterize the metabolites altered by breast cancer cell aggressiveness and chemotherapy response (97). For example, the amide proton transfer signal in triple negative tumors is distinct and may result from the unique microenvironment of the tumor subtype (98). In addition, amide proton transfer asymmetry is observed in patients with breast cancer treatment-related lymphedema (99). Notably, high quality amide proton transfer images can be readily obtained at 7T, because both the chemical exchange saturation transfer effect and SNR are enhanced at higher field strengths (100).

Other Techniques

Sodium MRI

Sodium (^{23}Na) is abundant in the body and, unlike other non-proton nuclei that yield spectra for chemical quantification, sodium has no chemical shift dispersion and instead produces images (101). Malignant tumors are thought to increase sodium content due to disruption of the sodium-potassium pump in cell membranes. Elevated tissue sodium concentration has been confirmed in malignant lesions (102), and sodium concentration correlates well with the ADC of DWI (103).

Susceptibility Weighted Imaging

Historically recognized as the cause of frequent MRI artifacts, particularly near air-tissue interfaces or in the vicinity of metal implants, differences in magnetic susceptibility can also produce contrast between diamagnetic and paramagnetic tissues. Ductal carcinoma *in situ* (DCIS) is frequently missed by DCE MRI and has been shown to associate with certain patterns of breast calcifications (104). Calcium is more diamagnetic than tissue water, and the susceptibility effects are intensified

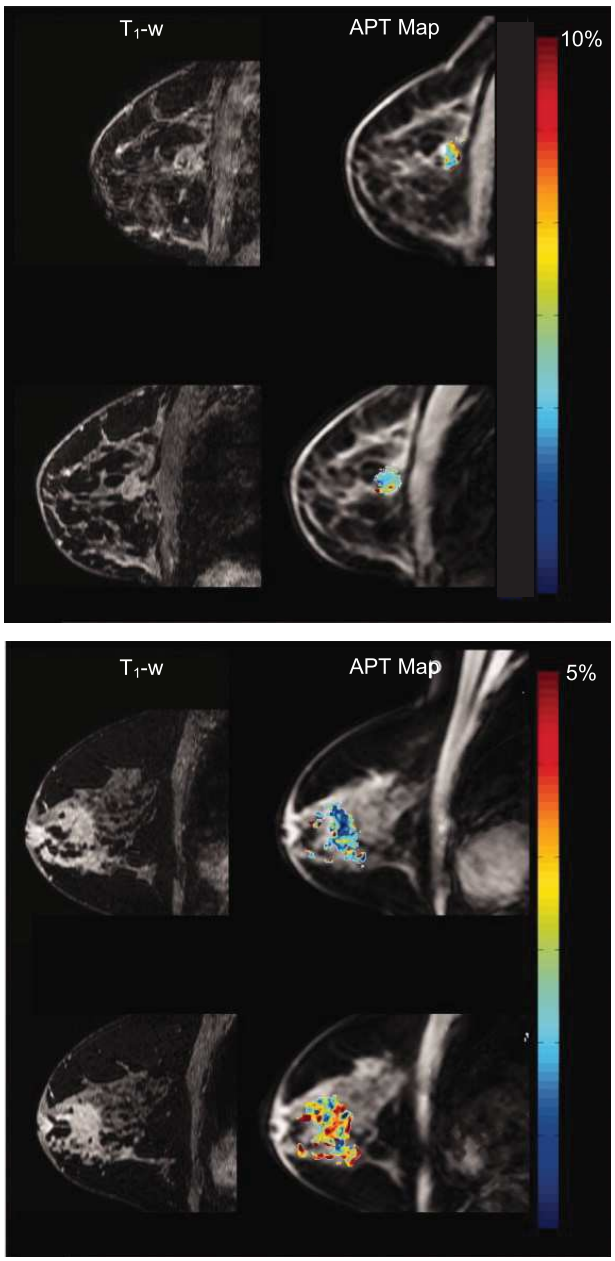


FIGURE 5 | Amide proton transfer maps overlaying anatomical T_1-weighted images acquired at 3T. The top row shows data acquired prior to neoadjuvant chemotherapy (NAC); the bottom row shows data acquired after one cycle of NAC. **(A)** Patient who had complete response (i.e., no residual tumor) and **(B)** patient who had progressive disease. Reprinted with permission from Chan et al. (95); ©2012 Wiley Periodicals, Inc.

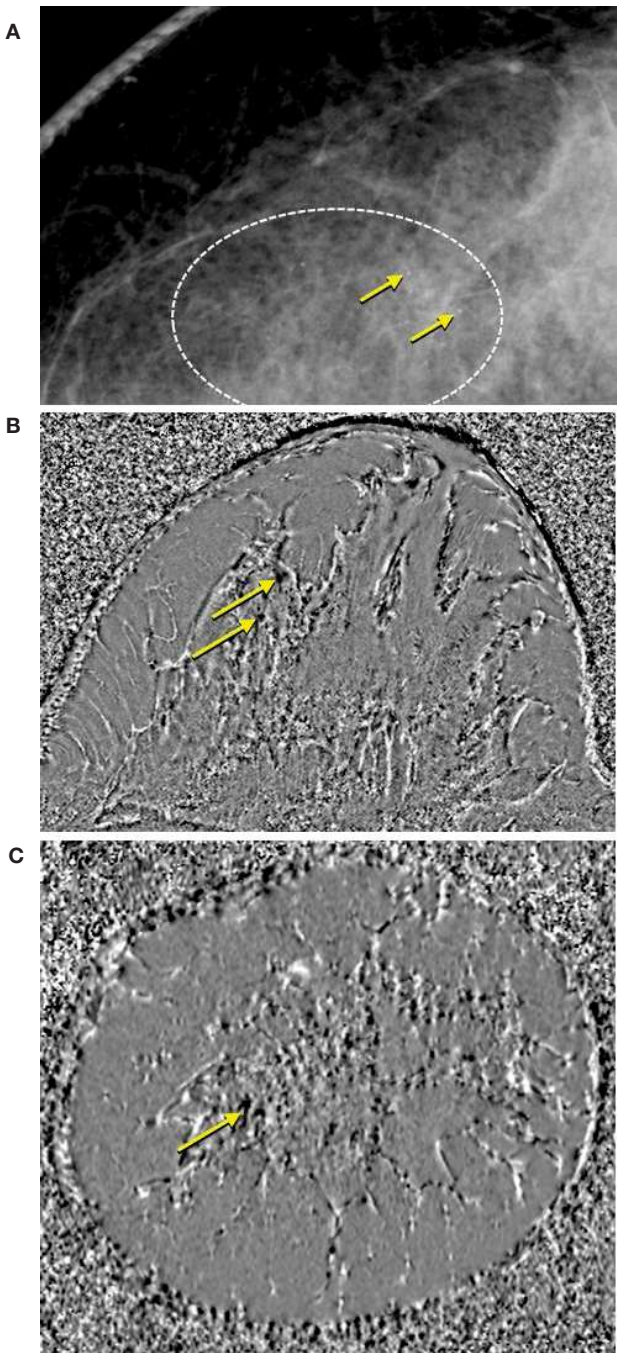


FIGURE 6 | Comparison of **(A)** mammogram and **(B,C)** susceptibility weighted phase images acquired at 7T with a 0.35-mm isotropic resolution T_2^*-weighted 3D gradient echo sequence (105). Diamagnetic microcalcifications are indicated by yellow arrows and are hypointense in the susceptibility weighted phase images.

at higher magnetic fields. **Figure 6** illustrates the ability of 7T susceptibility-weighted MRI to identify microcalcifications otherwise only visible using mammography (105).

MR Elastography

MR elastography (MRE) images a low-frequency acoustic wave as it propagates throughout tissue. By calculating the local complex sheer modulus, MRE can characterize biomechanical properties of breast tissue including differences in stiffness. The initial aim of employing MRE for breast cancer was to differentiate benign lesions from malignant tumors; the more liquid-like behavior of

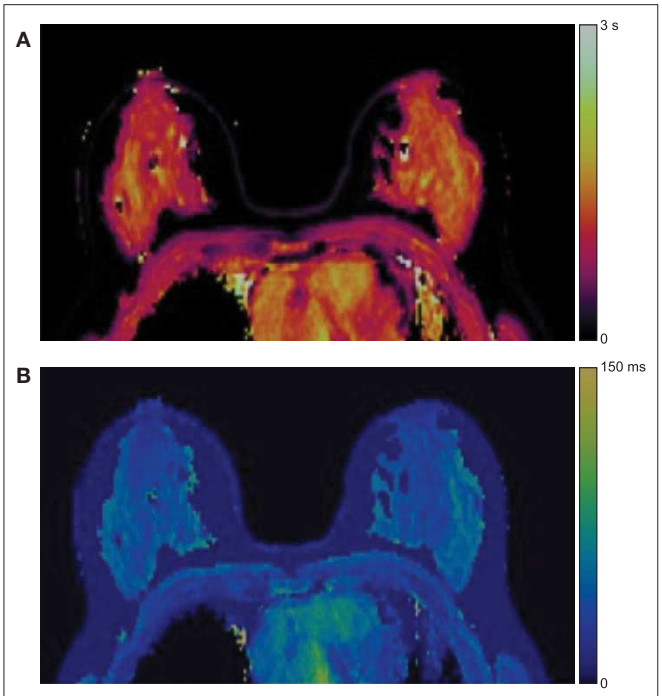


FIGURE 7 | Example MR fingerprinting of the breast. Representative **(A)** T_1 and **(B)** T_2 MR fingerprinting color maps from one subject. Reprinted with permission from Chen et al. (109); ©2019 International Society for Magnetic Resonance in Medicine.

malignant tumors provided sufficient MRE contrast to achieve this aim (106). More recently, MRE is being combined with 3D strain imaging, the latter altering the stress-load relation of tumors; ongoing studies are investigating the potential of MRE to determine mechanical forces to estimate the metastatic potential of tumors (107).

MR Fingerprinting

A relatively new technique known as MR fingerprinting utilizes a pseudorandom RF excitation and pattern recognition to produce quantitative maps of tissue properties (108). Results from preliminary breast MR fingerprinting studies illustrate the simultaneous quantitative mapping of T_1 and T_2 in a bilateral configuration (109, 110). Representative T_1 and T_2 MR fingerprinting maps are shown in **Figure 7**.

MR Electrical Properties Tomography

MR electrical properties tomography exploits typically undesirable distortions in the RF transmit field (B_1) to reconstruct the conductivity and electrical permittivity of tissue (111). A preliminary breast MR electrical property tomography study by Shin et al. found malignant cancers have higher conductivity than benign lesions, and invasive cancers showed higher conductivity compared to DCIS (112).

Novel Contrast Agents

Recent discoveries of gadolinium retention within the body have raised questions regarding the long-term toxicity of gadolinium-based contrast agents and propelled the quest for novel contrast agents that are both safe and equally effective (113). Recent studies have begun reevaluating alternative contrast agents for breast cancer, including manganese (114, 115) and iron chelates (116). Even so, research continues on gadolinium-based contrast agent's improvements, and agents can be designed to target specific molecular peptides. A preclinical study utilized one such contrast agent to bind to fibrin-fibronectin complexes abundant in malignant cancer, including micro metastases (117). While human trials have not commenced, these novel contrast agents have potential to improve the early detection and characterization of high-risk breast tumors.

Machine Learning

Machine learning is a branch of data science that "trains" computers to learn data without preprograming the computers to perform specific tasks. There are two types of machine learning models: unsupervised learning and supervised learning. Unsupervised learning aims to classify data that have not been assigned labels or categories; examples include neural networks and clustering to map input data (e.g., breast images) into output categories that share similar contents (e.g., tumor assessments). On the other hand, supervised learning aims to classify data that have been assigned with ground truth labels (e.g., radiological assessments); example models include regression methods and support-vector machines (SVM).

As an artificial intelligence tool, machine learning may best be introduced to the clinic through structured use cases; in the case of breast cancer, these may include the application of artificial intelligence to identify suspicious microcalcifications (118) and, given the variability of visual density assessments (119), the quantification of breast fibroglandular tissue volume (25). The American college of radiology recommend using the BI-RADS categories for characterizing breast lesions. This method relies on the radiologist's experience and is limited by inter-observer variance.

Neural networks are machine learning models that consist of multiple interconnected layers. The study of neural networks is termed deep learning. Lately, deep learning has surpassed traditional image processing models in the segmentation and detection of novel imaging biomarkers (120). Convolutional neural networks are a type of neural network that has convolutional layers and hidden layers, and they have profound diagnostic performance. For example, a 3D deep convolution neural network can be used to identify and localize malignant breast lesions in DCE images, previously demonstrating 90.8% sensitivity and 69.3% specificity (121, 122). Another potential application is fibroglandular tissue and BPE assessment; while BI-RADS defines relevant categories, it does not establish percentage values for their quantification. A large proportion of fibroglandular tissue in the breast correlates with breast cancer risk (23, 26, 119, 123). Robust fibroglandular tissue quantification can be an efficient tool for clinicians to process large amount of breast MRI data and support more accurate breast cancer

risk assessments (124). Independent of fibroglandular tissue quantification, computer-aided BPE quantification in DCE images has shown potential to be an imaging biomarker of breast cancer (125). For breast image segmentation and tumor volume quantification, several algorithmic routines have been demonstrated, e.g., (123, 124, 126–128); however, deep computational neural networks (i.e., U-nets) have shown particular promise for improving robustness and accuracy of results (129–131). **Figure 2B** shows the segmented fibroglandular tissue overlaid on anatomical DCE breast images. Based on fully automated computerized approaches, BPE DCE-MRI recently has been reported applicable in screening potential risk factors of breast cancer to regionalize the parenchymal tissues and their vasculature (125).

Radiomics involves extracting quantitative features from medical images, such as tumor size, shape, and textures, and patient-level data, such as the genetic data, to determine the underlying relationship between these features and pathologies (121, 132–136). A radiomics study of BPE DCE-MRI was able to differentiate subtypes of triple negative breast cancer (137). Another study combining BPE and T_2-weighted breast MRI predicted NAC response with high accuracy (138). Texture parameters used as features in the support-vector machine learning approach show accurate prediction of benign and malignant breast lesions (133, 138–142). Texture parameters can consist of statistical and grey-level metrics in the sub-1cm region of interest in DCE images (139), the ADC map histogram combined with DCE-derived parametric maps (140, 141), and the parenchymal texture analysis (133). Finally, radiogenomics aims to identify imaging biomarkers and incorporates with phenotypic and genotypic metrics to support the execution of radiomics studies (142).

Machine learning has applications in breast lesion detection and classification, as well as predicting NAC response. Machine learning can bring together data from many studies and reduce the variability of radiologists' annotation methods on breast lesions. The current limitations of machine learning are the training requirement of large datasets and lack of standardized machine learning models to extract features from these datasets. Lastly, the decision-making process of machine learning can be considered a "black box"; it is difficult to intuitively explain how and why a certain answer is produced by machine learning models.

Ultra-High Field MR Scanners

7 Tesla

As indicated by the improved fat-water contrast visible in **Figure 1**, the positive predictive value and cancer detection rates of MRI increase at higher magnetic fields (143). However, the issue of transmit B_1 inhomogeneity is greater at ultra-high fields, and it becomes necessary to utilize a local transmit coil for breast MRI at 7T (144). Given the proximity to the breasts and the greater net magnetization inherent at higher static magnetic fields, a local RF coil may be used for both transmit and receive (28). However, owing to the asymmetric dielectric load presented by the torso, transmit B_1 inhomogeneity can still be pronounced throughout the breasts, leading to a linear signal drop-off toward the chest wall. In response, adiabatic pulse sequences have been developed to compensate for B_1 inhomogeneity and improve tip angle uniformity (145). Alternatively, transmit coil designs exploiting transmission line techniques, e.g., forced current excitation (90, 146), have been shown to produce excellent B_1 homogeneity throughout the breast to the chest wall [7.2% B_1 coefficient of variation reported in (147)] and facilitate the use of standardized pulse sequences. As with lower static fields, the received SNR is further improved by utilizing a 7T array coil insert (148–151).

Ultra-High Field Safety

The potential for RF power deposition to cause localized tissue heating is more apparent at higher fields. The amount of power dissipated in a given mass of tissue is quantified as specific absorption rate, and operational safety limits are stipulated by the International Electrotechnical Commission (152). The safety of local transmit coils must be validated, typically through thermometry measurements and electromagnetic simulation of the specific coil design. While higher specific absorption rate is expected for women with greater breast tissue density, their resulting levels for routine 7T pulse sequences are generally well within safety limits (153, 154). Furthermore, a preliminary simulation study indicates the presence of breast implants has no significant effects on specific absorption rate or tissue heating (155).

CONCLUSIONS AND FUTURE DIRECTIONS

The current and emerging MRI techniques discussed in this paper are summarized in **Table 1**. For a multifaceted disease such as cancer, multi-parametric approach through which both structural and functional information can be elucidated simultaneously is a necessity to overcome the limitations of current MR based clinical modalities. In comparison to the stand-alone modalities, mpMRI enables both visualization and quantification. Quantifying varied cancer traits, including but not limited to, tumor architecture, tumor microenvironment, vascularization and angiogenesis, tumor heterogeneity, cellularity, metabolite concentration, and receptor status in parallel with image reconstruction through the combination of modalities would inevitably improve the status quo in detecting and treating breast cancer (156). Furthermore, individual modalities that appear far-removed from standalone efficacy may be ideal adjuncts for an mpMRI approach; for example, Weiss et al. recently demonstrated a promising approach to predict personalized response to NAC using a combination of DCE and DWI; however, the accuracy of their mathematical model would be strengthened by personalized measurements of elastic properties of the breast, potentially through MRE (157). Ultimately, mpMRI incorporating one or more emerging methods has the potential to afford improved specificity and deliver excellent

TABLE 1 | Comparison of current and emerging MRI techniques.

	Imaging techniques		Clinical applications	Features and strengths	Limitations
Current MRI techniques	Structural imaging		T_1 and T_2 weighted bilateral fat suppression imaging	Superior sensitivity for breast tumors; preferable for dense breast imaging	Low tumoral contrast, as tumor is surrounded by breast fat and fibroglandular tissue
	Contrast Enhanced Perfusion MRI	Dynamic Contrast Enhanced (DCE) MRI	Routinely utilized for distinguishing malignant vs benign cancers	Microvasculature and hypersensitivity in malignant tumors	Affected by hormones (menstrual cycle)
		Background Parenchymal Enhancement (BPE) MRI	Breast cancer predicting odds for patients at risk (32)	Whole breast area enhancement; tissue specific differences in normal tissues	Recent studies fail to correlate positive biopsy rate with specificity or sensitivity (33)
Emerging MRI techniques	Diffusion Weighted MRI (Gaussian)	Diffusion Weighted Imaging (DWI)	Potential tissue cellularity-based approach	Improved lesion detection for voxel-wise calculation (47, 48); higher resolution achievable (e.g., 0.8 mm) (47); yields superior quality when used in combination with MRS or other multiparametric modalities (46)	Inconsistency in obtaining high-quality breast DWI but can be solved with protocol standardization and QA procedure (see (49) for more details)
		Diffusion Tensor Imaging (DTI)	Potentially differentiating breast cancer subtypes (54, 55)	Distinction of malignant vs benign lesions	Reproducible results with higher accuracy remain a challenge
	Diffusion Weighted MRI (Non-Gaussian)	Diffusion Kurtosis Imaging	Potential to differentiate heterogenous tumor microstructures (62)	Applicable for intracellular structures, e.g., membranes and organelles (61); improved unsuppressed fat signal (63)	Low SNR; longer scanning time and higher magnetic gradient strength for high b-value acquisition
		Intravoxel Incoherent Motion	Promising results in differentiating malignant vs benign lesions; neoadjuvant chemotherapy (NAC) prediction	Tissue diffusion and microcapillary perfusion based; contrast Agents are not required;	Low SNR; longer scanning time and higher magnetic gradient strength for high b-value acquisition
	Magnetic Resonance Spectroscopy (MRS)	Proton Spectroscopy	Potential biomarker for malignant breast cancer	Highest sensitivity and simplest data acquisition	Issues related to reproducibility across clinical sites (74)
		Multinuclear Spectroscopy	Potential in identifying 'at risk' population by monitoring metabolism-based results	Tumor malignancy transformation study	Low SNR
		Magnetization Transfer	Potential in monitoring response to NAC; differentiating malignant tumors vs benign lesions	Facilitates detection of low concentration chemicals	Low SNR, benefits from higher magnetic field strength (7T)
	Other techniques	Sodium MRI	Potentially differentiating malignant tumors based on sodium concentration (101)	No chemical or spectral shift observed; based on sodium/potassium ion channels in the body	Could be overlapped with other sodium/potassium ion channel related disorder
		Susceptibility-Weighted MRI	Potential microcalcifications in breast tissues (otherwise only visible using mammography)	Potential to determine ductal carcinoma *in situ* that are often missed	Possibility for MRI related artifacts in images
		MR Elastography	Applicable for differentiating malignant vs benign lesions	Characterization of biomechanical tissue properties (microenvironmental stiffness)	Requires breast in contact with soft sternal driver
		Electrical Properties Tomography	Differentiate malignant vs benign lesions; invasive ductal carcinoma vs ductal carcinoma *in situ* (112)	Utilizes undesirable distortions in transmit field	Poor spatial resolution
		Machine Learning	Lesion detection, lesion classification, and predicting response after NAC	Brings together data from a large number of studies, and reduces inter-reader variability caused by readers' different annotations in breast tumor masks	Lack of standardization: no standard method for segmentation and feature extraction. Requires large datasets for training. The decision-making process is a 'black box,' hard to understand

accuracy for the prediction, detection, and monitoring of breast cancer (158).

Both DWI and ^{1}H MRS are considered important approaches to pursue the analysis of tumor growth and treatment response *in vivo* (159). Advanced DWI methods that have the potential to distinguish tumors, given distinct signatures of cellularity and intravoxel heterogeneity, hold great potential in the noninvasive differentiation of tumor subtypes. Specifically, the fractional order calculus model (160) can derive micrometer-scale diffusion metrics that may reflect nuclear morphometry. To elicit sensitivity to shorter-scale diffusion, this method requires acquisitions with at least five b-values in the high range of b = 3000–4000 s/mm^2. While one retrospective study failed to show improved utility of fractional order calculus model parameters as compared to DWI ADC, the maximum b-value acquisitions included in the study (b = 1500 s/mm^2) were insufficient to properly evaluate the fractional order calculus model (161). Regarding ^{1}H MRS, current issues surrounding inter-site reproducibility of single-voxel MRS may be mitigated through automated voxel placement or full 3D magnetic resonance spectroscopic imaging (74), particularly if following standardized process for acquisition, post-processing, and analysis (162). Continued development of MT techniques, including amide proton transfer, also show promise for differentiating tumor subtypes and predicting treatment outcome. DWI, MRS, and amide proton transfer all will benefit from the growing footprint of 7T MR scanners and continued progress toward U.S. Food and Drug Administration approval of clinical breast cancer applications at 7T. Positron emission tomography (PET) as a stand-alone imaging technique is known to have a high diagnostic ability for metastasis through imaging of the breast and adjacent lymph nodes. The diagnosis and characterization of primary tumors using PET has been shown to be improved when used simultaneously in conjugation with MRI, owing to the strengths of the individual modalities (163), but more research on combined PET/MRI modality is required to provide enough supportive evidence of their higher sensitivities. Radiation associated with the tracer in PET could be another concern; however, Melsaether et al. have demonstrated 50% reduction in total radiation dose when switching from PET/computed tomography to PET/MRI in a population of breast cancer patients, implying a safer mode of imaging and diagnosis in comparison to the former (164).

Finally, the rapidly advancing field of machine learning will facilitate more impactful applications for breast cancer detection and management, likely improving specificity, positive predictive value, and differentiation of tumor subtypes through MRI. Moreover, simultaneous assessments of biomarkers and their genomics data through radiogenomics is likely to prove instrumental in the future as we advance toward precision health or personalized medicine and simultaneously decrease the MRI associated false positive rates.

AUTHOR CONTRIBUTIONS

AC, XL, and JR drafted the manuscript. XL generated new figures. All authors contributed to manuscript revision and approved the submitted version.

FUNDING

The authors acknowledge support from the Purdue University Center for Cancer Research (NIH grant P30CA023168) and the Showalter Trust. XL and JR are partially funded by NIH grants R03EB026231 and R01EB024408.

ACKNOWLEDGMENTS

The authors thank the Purdue Graduate School, the Interdisciplinary Biomedical Sciences (IBSC) program, and the Showalter Trust for bestowing graduate fellowship support to AC.

REFERENCES

1. American Cancer Society. *Cancer Facts & Figures*. Available online at: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2020/cancer-facts-and-figures-2020.pdf (accessed February 9, 2020).
2. Heller SL, Moy L. MRI breast screening revisited. *J Magn Reson Imaging.* (2019) 49:1212–21. doi: 10.1002/jmri.26547
3. Kuhl CK, Strobel K, Bieling H, Leutner C, Schild HH, Schrading S. Supplemental breast MR imaging screening of women with average risk of breast cancer. *Radiology.* (2017) 283:361–70. doi: 10.1148/radiol.2016161444
4. Shahid H, Wiedenhoefer JF, Dornbluth C, Otto P, Kist KA. An overview of breast MRI. *J Appl Radiol.* (2016) 45:7–13. Available online at: https://appliedradiology.com/articles/an-overview-of-breast-mri
5. Kuhl CK, Mielcareck P, Klaschik S, Leutner C, Wardelmann E, Gieseke J, et al. Dynamic breast MR imaging: are signal intensity time course data useful for differential diagnosis of enhancing lesions? *Radiology.* (1999) 211:101–10. doi: 10.1148/radiology.211.1.r99ap38101
6. Orel SG. Differentiating benign from malignant enhancing lesions identified at MR imaging of the breast: are time-signal intensity curves an accurate predictor? *Radiology.* (1999) 211:5–7. doi: 10.1148/radiology.211.1.r99ap395
7. Kuhl CK, Keulers A, Strobel K, Schneider H, Gaisa N, Schrading S. Not all false positive diagnoses are equal: on the prognostic implications of false-positive diagnoses made in breast MRI versus in mammography/digital tomosynthesis screening. *Breast Cancer Research.* (2018) 20:13. doi: 10.1186/s13058-018-0937-7
8. National Breast Cancer Foundation. *Male Breast Cancer*. Available online at: https://www.nationalbreastcancer.org/male-breast-cancer (accessed February 9, 2020).
9. American Cancer Society. *Breast Cancer in Men*. Available online at: https://www.cancer.org/cancer/breast-cancer-in-men.html (accessed February 9, 2020).
10. Shaw A, Smith B, Howlett D. Male breast carcinoma and the use of MRI. *Radiology Case Reports.* (2015) 6:455. doi: 10.2484/rcr.v6i3.455
11. Shin K, Martaindale S, Whitman GJ. Male breast magnetic resonance imaging: when is it helpful? our experience over the last decade. *Curr Probl Diagn Radiol.* (2019) 48:196–203. doi: 10.1067/j.cpradiol.2018.01.002
12. Liu N, Johnson KJ, Ma CX. Male breast cancer: an updated surveillance, epidemiology, and end results data analysis. *Clin Breast Cancer.* (2018) 18:997–1002. doi: 10.1016/j.clbc.2018.06.013

13. Wang F, Shu X, Meszoely I, Pal T, Mayer IA, Yu Z, et al. Overall mortality after diagnosis of breast cancer in men vs women. *JAMA Oncol.* (2019) 5:1589–96. doi: 10.1001/jamaoncol.2019.2803
14. Food and Drug Administration. *Male Breast Cancer: Developing Drugs for Treatment Draft - Guidance for Industry*. Rockville, MD: U.S. Department of health and human services; (2019). Available online at: https://www.fda.gov/media/130061/download (accessed February 9, 2020).
15. Silvestri V, Barrowdale D, Mulligan AM, Neuhausen SL, Fox S, Karlan BY, et al. Male breast cancer in *BRCA1* and *BRCA2* mutation carriers: pathology data from the Consortium of Investigators of Modifiers of *BRCA1/2*. *Breast Cancer Res.* (2016) 18:15. doi: 10.1186/s13058-016-0671-y
16. Huuse EM, Moestue SA, Lindholm EM, Bathen TF, Nalwoga H, Krüger K, et al. *In vivo* MRI and histopathological assessment of tumor microenvironment in luminal-like and basal-like breast cancer xenografts. *J Magn Reson Imaging.* (2012) 35:1098–107. doi: 10.1002/jmri.23507
17. Ahmed Sultan A, Hanry Al-backry M, Mohamed Alhefney E, Ezat Mosa A, Abdullah Farahat HE. Role of MR spectroscopy and diffusion-weighted imaging in diagnosis of orbital masses. *Egypt J Radiol Nucl Med.* (2018) 49:45–53. doi: 10.1016/j.ejrnm.2017.11.005
18. Belli P, Costantini M, Bufi E, Giardina GG, Rinaldi P, Franceschini G, et al. Diffusion magnetic resonance imaging in breast cancer characterisation: correlations between the apparent diffusion coefficient and major prognostic factors. *Radiol Med.* (2015) 120:268–76. doi: 10.1007/s11547-014-0442-8
19. Li G, Chen R, Hao L, Lin L. Three dimensional MREIT for breast cancer detection on open MRI scanners. In: *Proceedings of the 2012 IEEE International Conference on Information and Automation*. Shenyang; New York, NY: IEEE (2012). p. 446–50. doi: 10.1109/ICInfA.2012.6246847
20. Partridge SC, McDonald ES. Diffusion weighted magnetic resonance imaging of the breast: protocol optimization, interpretation, and clinical applications. *Magn Reson Imaging Clin N Am.* (2013) 21:601–24. doi: 10.1016/j.mric.2013.04.007
21. Penet M-F, Mikhaylova M, Li C, Krishnamachary B, Glunde K, Pathak AP, et al. Applications of molecular MRI and optical imaging in cancer. *Future Med Chem.* (2010) 2:975–88. doi: 10.4155/fmc.10.25
22. Chu W, Jin W, Liu D, Wang J, Geng C, Chen L, et al. Diffusion-weighted imaging in identifying breast cancer pathological response to neoadjuvant chemotherapy: a meta-analysis. *Oncotarget.* (2018) 9:7088–100. doi: 10.18632/oncotarget.23195
23. Checka CM, Chun JE, Schnabel FR, Lee J, Toth H. The relationship of mammographic density and age: implications for breast cancer screening. *Am J Roentgenol.* (2012) 198:292–509. doi: 10.2214/AJR.10.6049
24. Sardanelli F, Giusepprtti GM, Panizza P, Bazzocchi M, Fausto A, Simonetti G, et al. Sensitivity of MRI versus mammography for detecting foci of multifocal, multicentric breast cancer in fatty and dense breasts using the whole-Breast pathologic examination as a gold standard. *Am J Roentgenol.* (2004) 183:1149–57. doi: 10.2214/ajr.183.4.1831149
25. Morris E, Comstock C, Lee C. *ACR BI-RADS® Magnetic Resonance Imaging. ACR BI-RADS® Atlas, Breast Imaging Reporting and Data System*. Reston, VA: American College of Radiology. (2013) doi: 10.1016/j.mric.2013.04.006
26. 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
27. Medved M, Li H, Abe H, Sheth D, Newstead GM, Olopade OI, et al. Fast bilateral breast coverage with high spectral and spatial resolution (HiSS) MRI at 3T. *J Magn Reson Imaging.* (2017) 46:1341–8. doi: 10.1002/jmri.25658
28. Brown R, Storey P, Geppert C, McGorty K, Klautau Leite AP, Babb J, et al. Breast MRI at 7 tesla with a bilateral coil and robust fat suppression. *J Magn Reson Imaging.* (2014) 39:540–9. doi: 10.1002/jmri.24205
29. Delille J-P, Slanetz PJ, Yeh ED, Kopans DB, Garrido L. Physiologic changes in breast magnetic resonance imaging during the menstrual cycle: perfusion imaging, signal enhancement, and influence of the T_1 relaxation time of breast tissue. *Breast J.* (2005) 11:236–41. doi: 10.1111/j.1075-122X.2005.21499.x
30. Kuhl CK, Bieling HB, Gieseke J, Kreft BP, Sommer T, Lutterbey G, et al. Healthy premenopausal breast parenchyma in dynamic contrast-enhanced MR imaging of the breast: normal contrast medium enhancement and cyclical-phase dependency. *Radiology.* (1997) 203:137–44. doi: 10.1148/radiology.203.1.9122382
31. Newitt D, Hylton N. Data from: Multi-center breast DCE-MRI data and segmentations from patients in the I-SPY 1/ACRIN 6657 trials. *Cancer Imag Arch.* (2016) 198:W373–80. doi: 10.7937/K9/TCIA.2016.HdHpgJLK
32. King V, Brooks JD, Bernstein JL, Reiner AS, Pike MC, Morris EA. Background parenchymal enhancement at breast MR imaging and breast cancer risk. *Radiology.* (2011) 260:50–60. doi: 10.1148/radiol.11102156
33. DeMartini WB, Liu F, Peacock S, Eby PR, Gutierrez RL, Lehman CD. Background parenchymal enhancement on breast MRI: impact on diagnostic performance. *Am J Roentgenol.* (2012) 198:W373–W80. doi: 10.2214/AJR.10.6272
34. Kuhl CK, Kooijman H, Gieseke J, Schild HH. Effect of B_1 inhomogeneity on breast mr imaging at 3.0 T. *Radiology.* (2007) 244:929–30. doi: 10.1148/radiol.2443070266
35. Azlan CA, Di Giovanni P, Ahearn TS, Semple SIK, Gilbert FJ, Redpath TW. B_1 transmission-field inhomogeneity and enhancement ratio errors in dynamic contrast-enhanced MRI (DCE-MRI) of the breast at 3T. *J Magn Reson Imaging.* (2010) 31:234–9. doi: 10.1002/jmri.22018
36. Sung K, Daniel BL, Hargreaves BA. Transmit B_1^+ field inhomogeneity and T_1 estimation errors in breast DCE-MRI at 3 tesla. *J Magn Reson Imaging.* (2013) 38:454–9. doi: 10.1002/jmri.23996
37. Azlan CA, Ahearn TS, Di Giovanni P, Semple SIK, Gilbert FJ, Redpath TW. Quantification techniques to minimize the effects of native T_1 variation and B_1 inhomogeneity in dynamic contrast enhanced MRI of the breast at 3 T. *Magn Reson Med.* (2012) 67:531–40. doi: 10.1002/mrm.23021
38. Wright SM, Wald LL. Theory and application of array coils in MR spectroscopy. *NMR in Biomedicine.* (1997) 10:394–410. doi: 10.1002/(SICI)1099-1492(199712)10:8<394::AID-NBM494>3.0.CO;2-0
39. Konyer NB, Ramsay EA, Bronskill MJ, Plewes DB. Comparison of MR imaging breast coils. *Radiology.* (2002) 222:830–4. doi: 10.1148/radiol.2223001310
40. Marshall H, Devine PM, Shanmugaratnam N, Fobel R, Siegler P, Piron CA, et al. Evaluation of multicoil breast arrays for parallel imaging. *J Magn Reson Imaging.* (2010) 31:328–38. doi: 10.1002/jmri.22023
41. Nnewihe AN, Grafendorfer T, Daniel BL, Calderon P, Alley MT, Robb F, et al. Custom-fitted 16-channel bilateral breast coil for bidirectional parallel imaging. *Magn Reson Med.* (2011) 66:281–9. doi: 10.1002/mrm.22771
42. Hancu I, Fiveland E, Park K, Giaquinto RO, Rohling K, Wiesinger F. Flexible, 31-channel breast coil for enhanced parallel imaging performance at 3T. *Magn Reson Med.* (2016) 75:897–905. doi: 10.1002/mrm.25655
43. Li T, Yu T, Li L, Lu L, Zhuo Y, Lian J, et al. Use of diffusion kurtosis imaging and quantitative dynamic contrast-enhanced MRI for the differentiation of breast tumors. *J Magn Reson Imaging.* (2018) 48:1358–66. doi: 10.1002/jmri.26059
44. Zhou J, Chen E, Xu H, Ye Q, Li J, Ye S, et al. Feasibility and diagnostic performance of voxelwise computed diffusion-weighted imaging in breast cancer. *J Magn Reson Imaging.* (2018) 49:1610–6. doi: 10.1002/jmri.26533
45. Solomon E, Nissan N, Furman-Haran E, Seginer A, Shapiro-Feinberg M, Degani H, et al. Overcoming limitations in diffusion-weighted MRI of breast by spatio-temporal encoding. *Magn Reson Med.* (2015) 73:2163–73. doi: 10.1002/mrm.25344
46. Pinker K, Moy L, Sutton EJ, Mann RM, Weber M, Thakur SB, et al. Diffusion-Weighted imaging with apparent diffusion coefficient mapping for breast cancer detection as a stand-alone parameter: comparison with dynamic contrast-enhanced and multiparametric magnetic resonance imaging. *Invest Radiol.* (2018) 53:587–95. doi: 10.1097/RLI.0000000000000465
47. Barentsz MW, Taviani V, Chang JM, Ikeda DM, Miyake KK, Banerjee S, et al. Assessment of tumor morphology on diffusion-weighted (DWI) breast MRI: diagnostic value of reduced field of view DWI. *J Magn Reson Imaging.* (2015) 42:1656–65. doi: 10.1002/jmri.24929
48. Taviani V, Alley MT, Banerjee S, Nishimura DG, Daniel BL, Vasanawala SS, et al. High-resolution diffusion-weighted imaging of the breast with multiband 2D radiofrequency pulses and a generalized parallel imaging reconstruction. *Magn Reson Med.* (2016) 77:209–20. doi: 10.1002/mrm.26110
49. Newitt DC, Zhang Z, Gibbs JE, Partridge SC, Chenevert TL, Rosen MA, et al. Test-retest repeatability and reproducibility of ADC measures by breast

DWI: results from the ACRIN 6698 trial. *J Magn Reson Imaging.* (2019) 49:1617–28. doi: 10.1002/jmri.26539
50. Partridge SC, Nissan N, Rahbar H, Kitsch AE, Sigmund EE. Diffusion-weighted breast MRI: clinical applications and emerging techniques. *J Magn Reson Imaging.* (2017) 45:337–55. doi: 10.1002/jmri.25479
51. Eyal E, Shapiro-Feinberg M, Furman-Haran E, Grobgeld D, Golan T, Itzchak Y, et al. Parametric diffusion tensor imaging of the breast. *Invest Radiol.* (2012) 47:284–91. doi: 10.1097/RLI.0b013e3182438e5d
52. Furman-Haran E, Grobgeld D, Nissan N, Shapiro-Feinberg M, Degani H. Can diffusion tensor anisotropy indices assist in breast cancer detection? *J Magn Reson Imaging.* (2016) 44:1624–32. doi: 10.1002/jmri.25292
53. Ye Z, Zhao N, Lin J, Gary SE, Viox JD, Song C, et al. Quantification of benign and malignant breast tumor cellularity. In: *Proceedings of the ISMRM 27th Annual Meeting & Exhibition* 2019. Montréal, QC; Concord, CA: ISMRM (2019). p. 277. Available online at: https://index.mirasmart.com/ISMRM2019/PDFfiles/0277.html (accessed February 9, 2020).
54. Onaygil C, Kaya H, Ugurlu MU, Aribal E. Diagnostic performance of diffusion tensor imaging parameters in breast cancer and correlation with the prognostic factors. *J Magn Reson Imaging.* (2017) 45:660–72. doi: 10.1002/jmri.25481
55. Ozal ST, Inci E, Gemici AA, Turgut H, Cikot M, Karabulut M. Can 3.0 tesla diffusion tensor imaging parameters be prognostic indicators in breast cancer? *Clin Imaging.* (2018) 51:240–7. doi: 10.1016/j.clinimag.2018.03.022
56. Partridge SC, Murthy RS, Ziadloo A, White SW, Allison KH, Lehman CD. Diffusion tensor magnetic resonance imaging of the normal breast. *Magn Reson Imaging.* (2010) 28:320–8. doi: 10.1016/j.mri.2009.10.003
57. Tagliafico A, Rescinito G, Monetti F, Villa A, Chiesa F, Fisci E, et al. Diffusion tensor magnetic resonance imaging of the normal breast: reproducibility of DTI-derived fractional anisotropy and apparent diffusion coefficient at 3.0 T. *Radiol Med.* (2012) 117:992–1003. doi: 10.1007/s11547-012-0831-9
58. Wang Y, Zhang X-P, Li Y-L, Li X-T, Hu Y, Cui Y, et al. Optimization of the parameters for diffusion tensor magnetic resonance imaging data acquisition for breast fiber tractography at 1.5 T. *Clin Breast Cancer.* (2014) 14:61–7. doi: 10.1016/j.clbc.2013.09.002
59. Nissan N, Furman-Haran E, Feinberg-Shapiro M, Grobgeld D, Eyal E, Zehavi T, et al. Tracking the mammary architectural features and detecting breast cancer with magnetic resonance diffusion tensor imaging. *J Vis Exp.* (2014) 94:e52048. doi: 10.3791/52048
60. Reisert M, Weigel M, Eyal E, Grobgeld D, Degani H, Hennig J. Diffusion tensor-based reconstruction of the ductal tree. In: *Proceedings of the ISMRM 19th Annual Meeting & Exhibition 2011*. Montréal, QC; Concord, CA: ISMRM (2011). p. 1011. Available online at: https://cds.ismrm.org/protected/11MProceedings/files/1011.pdf (accessed February 9, 2020).
61. Jensen JH, Helpern JA. MRI quantification of non-Gaussian water diffusion by kurtosis analysis. *NMR Biomed.* (2010) 23:698–710. doi: 10.1002/nbm.1518
62. Tang L, Zhou XJ. Diffusion MRI of cancer: from low to high b-values. *J Magn Reson Imaging.* (2019) 49:23–40. doi: 10.1002/jmri.26293
63. Mlynarska-Bujny A, Bickelhaupt S, König F, Laun F, Lederer W, Daniel H, et al. Einflussvon nicht vollständig unterdrücktem Fettsignal auf die Diffusions-Kurtosis-Bildgebung in der MRI-Mammografie [Influence of incompletely suppressed fat signal on diffusion kurtosis imaging in MR mammography]. *Fortschr Röntgenstr.* (2019) 191:308. doi: 10.1055/s-0037-1682122
64. Bihan DL, Breton E, Lallemand D, Grenier P, Cabanis E, Laval-Jeantet M. MR imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders. *Radiology.* (1986) 161:401–407. doi: 10.1148/radiology.161.2.3763909
65. Iima M. Investigation of new cancer diagnosis using non-Gaussian diffusion MRI and IVIM. *Impact.* (2018) 2018:41–3. doi: 10.21820/23987073.2018.12.41
66. Iima M, Kataoka M. IVIM MRI of the Breast. In: Bihan DL, Iima M, Federau C, Sigmund EE, editors. *Intravoxel Incoherent Motion (IVIM) MRI: Principles and Applications*. New York, NY: Jenny Stanford (2018). p. 173–94. doi: 10.1201/9780429427275-8
67. Dijkstra H, Dorrius MD, Wielema M, Jaspers K, Pijnappel RM, Oudkerk M, et al. Semi-automated quantitative intravoxel incoherent motion analysis and its implementation in breast diffusion-weighted imaging. *J Magn Reson Imaging.* (2016) 43:1122–31. doi: 10.1002/jmri.25086
68. Liu C, Liang C, Liu Z, Zhang S, Huang B. Intravoxel incoherent motion (IVIM) in evaluation of breast lesions: comparison with conventional DWI. *Eur J Radiol.* (2013) 82:e782–e9. doi: 10.1016/j.ejrad.2013.08.006
69. Cho GY, Gennaro L, Sutton EJ, Zabor EC, Zhang Z, Giri D, et al. Intravoxel incoherent motion (IVIM) histogram biomarkers for prediction of neoadjuvant treatment response in breast cancer patients. *Eur J Radiol Open.* (2017) 4:101–7. doi: 10.1016/j.ejro.2017.07.002
70. Tkáč I, Öz G, Adriany G, Ugurbil K, Gruetter R. In vivo ^{1}H NMR spectroscopy of the human brain at high magnetic fields: metabolite quantification at 4T vs 7T. *Magn Reson Med.* (2009) 62:868–79. doi: 10.1002/mrm.22086
71. Bolan PJ, Nelson MT, Yee D, Garwood M. Imaging in breast cancer: magnetic resonance spectroscopy. *Breast Cancer Res.* (2005) 7:149. doi: 10.1186/bcr1202
72. Baltzer PAT, Dietzel M. Breast lesions: diagnosis by using proton MR spectroscopy at 1.5 and 3.0 T—systematic review and meta-analysis. *Radiology.* (2013) 267:735–746. doi: 10.1148/radiol.13121856
73. Rosi A, Grande S, Luciani AM, Palma A, Giovannini C, Guidoni L, et al. Role of glutathione in apoptosis induced by radiation as determined by ^{1}H MR spectra of cultured tumor cells. *Radiat Res.* (2007) 167:268–82 doi: 10.1667/RR0578.1
74. Bolan PJ, Kim E, Herman BA, Newstead GM, Rosen MA, Schnall MD et al. MR spectroscopy of breast cancer for assessing early treatment response: results from the ACRIN 6657 MRS trial. *J Magn Reson Imaging.* (2017) 46:290–302. doi: 10.1002/jmri.25560
75. Glunde K, Penet M-F, Jiang L, Jacobs MA, Bhujwalla ZM. Choline metabolism-based molecular diagnosis of cancer: an update. *Expert Rev Mol Diagn.* (2015) 15:735–47. doi: 10.1586/14737159.2015.1039515
76. Sah RG, Agarwal K, Sharma U, Parshad R, Seenu V, Jagannathan NR. Characterization of malignant breast tissue of breast cancer patients and the normal breast tissue of healthy lactating women volunteers using diffusion MRI and in vivo ^{1}H MR spectroscopy. *J Magn Reson Imaging.* (2015) 41:169–74. doi: 10.1002/jmri.24507
77. Coum A, Ouldamer L, Noury F, Barantin L, Saint-Hilaire A, Vilde A. In vivo MR spectroscopy of human breast tissue: quantification of fatty acid composition at a clinical field strength (3 T). *MAGMA.* (2016) 29:1–4. doi: 10.1007/s10334-015-0506-3
78. Dimitrov IE, Douglas D, Ren J, Smith NB, Webb AG, Sherry AD, et al. In vivo determination of human breast fat composition by ^{1}H magnetic resonance spectroscopy at 7 T. *Magn Reson Med.* (2012) 67:20–6. doi: 10.1002/mrm.22993
79. Monaco ME. Fatty acid metabolism in breast cancer subtypes. *Oncotarget.* (2017) 8:29487–500. doi: 10.18632/oncotarget.15494
80. de Graaf RA, Klomp DWJ, Luijten PR, Boer VO. Intramolecular zero-quantum-coherence 2D NMR spectroscopy of lipids in the human breast at 7 T. *Magn Reson Med.* (2014) 71:451–7. doi: 10.1002/mrm.24701
81. Jagannathan NR. Application of in vivo MR methods in the study of breast cancer metabolism. *NMR Biomed.* (2019) 32:e4032. doi: 10.1002/nbm.4032
82. Esmaeili M, Moestue SA, Hamans BC, Veltien A, Kristian A, Engebråten O, et al. In vivo ^{31}P magnetic resonance spectroscopic imaging (MRSI) for metabolic profiling of human breast cancer xenografts. *J Magn Reson Imaging.* (2015) 41:601–609. doi: 10.1002/jmri.24588
83. Schmitz AMT, Veldhuis WB, Menke-Pluijmers MBE, van der Kemp WJM, van der Velden TA, Viergever MA, et al. Preoperative indication for systemic therapy extended to patients with early-stage breast cancer using multiparametric 7-tesla breast MRI. *PLoS One.* (2017) 12:e0183855. doi: 10.1371/journal.pone.0183855
84. van der Kemp WJM, Wijnen JP, Luijten PR, Klomp DWJ. Saturation-transfer effects and longitudinal relaxation times of ^{31}P metabolites in fibroglandular breast tissue at 7T. *Magn Reson Med.* (2015) 76:402–407. doi: 10.1002/mrm.25871
85. van der Kemp WJM, van der Velden TA, Schmitz AM, Gilhuijs KG, Luijten PR, Klomp DWJ, et al. Shortening of apparent transverse relaxation time

of inorganic phosphate as a breast cancer biomarker. *NMR Biomed.* (2018) 32:e4011. doi: 10.1002/nbm.4011
86. Aboagye EO, Bhujwalla ZM. Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells. *Cancer Res.* (1999) 59:80–84.
87. Franks SE, Kuesel AC, Lutz NW, Hull WE. ^{31}P MRS of human tumor cells: effects of culture media and conditions on phospholipid metabolite concentrations. *Anticancer Res.* (1996) 16:1365–74.
88. Krikken E, van der Kemp WJM, van Diest PJ, van Dalen T, van Laarhoven HWM, Luijten PR, et al. Early detection of changes in phospholipid metabolism during neoadjuvant chemotherapy in breast cancer patients using phosphorus magnetic resonance spectroscopy at 7T. *NMR Biomed.* (2019) 32:e4086. doi: 10.1002/nbm.4086
89. Shaka A, Keeler J, Frenkiel T, Freeman R. An improved sequence for broadband decoupling: WALTZ-16. *J Magn Reson.* (1983) 52:335–8. doi: 10.1016/0022-2364(83)90207-X
90. McDougall MP, Cheshkov S, Rispoli J, Malloy C, Dimitrov I, Wright SM. Quadrature transmit coil for breast imaging at 7 tesla using forced current excitation for improved homogeneity. *J Magn Reson Imaging.* (2014) 40:1165–73. doi: 10.1002/jmri.24473
91. Glunde K, Jie C, Bhujwalla ZM. Molecular causes of the aberrant choline phospholipid metabolism in breast cancer. *Cancer Res.* (2004) 64:4270–6. doi: 10.1158/0008-5472.CAN-03-3829
92. Cheshkov S, Dimitrov IE, Rispoli J, Cui J, McDougall M, Wright S, et al. Protocol for investigating *in vivo* glucose metabolism in human breast cancer by ^{13}C MRS at 7T. In: *Proceedings of the 25th Annual Meeting of ISMRM.* Honolulu (2017). p. 2946.
93. Wolff SD, Balaban RS. Magnetization transfer contrast (MTC) and tissue water proton relaxation *in vivo*. *Magn Reson Med.* (1989) 10:135–44. doi: 10.1002/mrm.1910100113
94. Arlinghaus LR, Dortch RD, Whisenant JG, Kang H, Abramson RG, Yankeelov TE. Quantitative magnetization transfer imaging of the breast at 3.0 T: reproducibility in healthy volunteers. *Tomography.* (2016) 2:260–6. doi: 10.18383/j.tom.2016.00142
95. Dula AN, Arlinghaus LR, Dortch RD, Dewey BE, Whisenant JG, Ayers GD, et al. Amide proton transfer imaging of the breast at 3 T: establishing reproducibility and possible feasibility assessing chemotherapy response. *Magn Reson Med.* (2013) 70:216–24. doi: 10.1002/mrm.24450
96. Virostko J, Sorace AG, Wu C, Ekrut D, Jarrett AM, Upadhyaya RM, et al. Magnetization transfer MRI of breast cancer in the community setting: reproducibility and preliminary results in neoadjuvant therapy. *Tomography.* (2019) 5:44–52. doi: 10.18383/j.tom.2018.00019
97. Chan KWY, Jiang L, Cheng M, Wijnen JP, Liu G, Huang P, et al. CEST-MRI detects metabolite levels altered by breast cancer cell aggressiveness and chemotherapy response. *NMR Biomed.* (2016) 29:806–16. doi: 10.1002/nbm.3526
98. Krikken E, Khlebnikov V, Zaiss M, Jibodh RA, van Diest PJ, Luijten PR, et al. Amide chemical exchange saturation transfer at 7 T: a possible biomarker for detecting early response to neoadjuvant chemotherapy in breast cancer patients. *Breast Cancer Res.* (2018) 20:51. doi: 10.1186/s13058-018-0982-2
99. Donahue MJ, Donahue PCM, Rane S, Thompson CR, Strother MK, Scott AO, et al. Assessment of lymphatic impairment and interstitial protein accumulation in patients with breast cancer treatment-related lymphedema using CEST MRI. *Magn Reson Med.* (2016) 75:345–55. doi: 10.1002/mrm.25649
100. Klomp DWJ, Dula AN, Arlinghaus LR, Italiaander M, Dortch RD, Zu Z, et al. Amide proton transfer imaging of the human breast at 7T: development and reproducibility. *NMR Biomed.* (2013) 26:1271–7. doi: 10.1002/nbm.2947
101. Kaggie JD, Hadley JR, Badal J, Park DJ, Parker DL, Morrell G, et al. A 3 T sodium and proton composite array breast coil. *Magn Reson Med.* (2014) 71:2231–42. doi: 10.1002/mrm.24860
102. Ouwerkerk R, Jacobs MA, Macura KJ, Wolff AC, Stearns V, Mezban SD, et al. Elevated tissue sodium concentration in malignant breast lesions detected with non-invasive ^{23}Na MRI. *Breast Cancer Res Treat.* (2007) 106:151–60. doi: 10.1007/s10549-006-9485-4
103. Zaric O, Pinker K, Zbyn S, Strasser B, Robinson S, Minarikova L, et al. Quantitative sodium mr imaging at 7 T: initial results and comparison with diffusion-weighted imaging in patients with breast tumors. *Radiology.* (2016) 280:39–48. doi: 10.1148/radiol.2016151304
104. Gilles R, Zafrani B, Guinebretière JM, Meunier M, Lucidarme O, Tardivon AA, et al. Ductal carcinoma in situ: MR imaging-histopathologic correlation. *Radiology.* (1995) 196:415–9. doi: 10.1148/radiology.196.2.7617854
105. Cheshkov S, Gilbert G, Dimitrov I, By S, Rispoli J, McDougall M, et al. *In-vivo* breast microcalcification detection via susceptibility weighted imaging at 7T. In: Proceedings of the ISMRM 22nd Annual Meeting & Exhibition 2014. Milan; Concord, CA: ISMRM (2014). p. 3282. Available online at: https://cds.ismrm.org/protected/14MProceedings/files/3282.pdf (accessed February 9, 2020).
106. Sinkus R, Siegmann K, Xydeas T, Tanter M, Claussen C, Fink M. MR elastography of breast lesions: understanding the solid/liquid duality can improve the specificity of contrast-enhanced MR mammography. *Magn Reson Med.* (2007) 58:1135–44. doi: 10.1002/mrm.21404
107. Bohte AE, Nelissen JL, Runge JH, Holub O, Lambert SA, de Graaf L, et al. Breast magnetic resonance elastography: a review of clinical work and future perspectives. *NMR Biomed.* (2018) 31:e3932. doi: 10.1002/nbm.3932
108. Ma D, Gulani V, Seiberlich N, Liu K, Sunshine JL, Duerk JL, et al. Magnetic resonance fingerprinting. *Nature.* (2013) 495:187–92. doi: 10.1038/nature11971
109. Chen Y, Panda A, Pahwa S, Hamilton JI, Dastmalchian S, McGivney DF. Three-dimensional MR fingerprinting for quantitative breast imaging. *Radiology.* (2019) 290:33–40 doi: 10.1148/radiol.2018180836
110. Panda A, Chen Y, Ropella-Panagis K, Ghodasara S, Stopchinski M, Seyfried N., et al. Repeatability and reproducibility of 3D MR fingerprinting relaxometry measurements in normal breast tissue. *J Magn Reson Imaging.* (2019) 50:1133–43. doi: 10.1002/jmri.26717
111. Katscher U, Berg CAT. Electric properties tomography: biochemical, physical and technical background, evaluation and clinical applications. *NMR Biomed.* (2017) 30:e3729. doi: 10.1002/nbm.3729
112. Shin J, Kim MJ, Lee J, Nam Y, M-o K, Choi N, et al. Initial study on in vivo conductivity mapping of breast cancer using MRI. *J Magn Reson Imaging.* (2015) 42:371–8. doi: 10.1002/jmri.24803
113. Cavallo Marincola B, Telesca M, Zaccagna F, Reimer F, Anzidei M, Catalano C, et al. Can unenhanced MRI of the breast replace contrast-enhanced MRI in assessing response to neoadjuvant chemotherapy? *Acta Radiol.* (2019) 60:35–44. doi: 10.1177/0284185118773512
114. Alhamami M, Cheng W, Lyu Y, Allen C, Zhang X-A, Cheng H-LM. Manganese-porphyrin-enhanced MRI for the detection of cancer cells: a quantitative *in vitro* investigation with multiple clinical subtypes of breast cancer. *PLoS ONE.* (2018) 13:e0206720. doi: 10.1371/journal.pone.0206720
115. Ganesh T, Mokhtari RB, Alhamami M, Yeger H, Cheng H-LM. Manganese-enhanced MRI of minimally gadolinium-enhancing breast tumors. *J Magn Reson Imaging.* (2015) 41:806–813. doi: 10.1002/jmri.24608
116. Boehm-Sturm P, Haeckel A, Hauptmann R, Mueller S, Kuhl CK, Schellenberger EA. Low-molecular-weight iron chelates may be an alternative to gadolinium-based contrast agents for T_1-weighted contrast-enhanced MR imaging. *Radiology.* (2018) 286:537–46. doi: 10.1148/radiol.2017170116
117. Zhou Z, Qutaish M, Han Z, Schur RM, Liu Y, Wilson DL, et al. MRI detection of breast cancer micrometastases with a fibronectin-targeting contrast agent. *Nat Commun.* (2015) 6:7984. doi: 10.1038/ncomms8984
118. Allen B. How structured use cases can drive the adoption of artificial intelligence tools in clinical practice. *J Am Coll Radiol.* (2018) 15:1758–60. doi: 10.1016/j.jacr.2018.09.002
119. Sprague BL, Conant EF, Onega T, Garcia MP, Beaber EF, Herschorn SD, et al. Variation in mammographic breast density assessments among radiologists in clinical practice: a multicenter observational study. *Ann Intern Med.* (2016) 165:457–64. doi: 10.7326/M15-2934
120. Reig B, Heacock L, Geras KJ, Moy L. Machine learning in breast MRI. *J Magn Reson Imaging.* (2020) 55:57–68. doi: 10.1002/jmri.26852
121. Machireddy A, Thibault G, Tudorica A, Afzal A, Mishal M, Kemmer K, et al. Early prediction of breast cancer therapy response using multiresolution fractal analysis of DCE-MRI parametric maps. *Tomography.* (2019) 5:90–8. doi: 10.18383/j.tom.2018.00046

122. Zhou J, Luo L-Y, Dou Q, Chen H, Chen C, Li G-J, et al. Weakly supervised 3D deep learning for breast cancer classification and localization of the lesions in MR images. *J Magn Reson Imaging.* (2019) 50:1144–51. doi: 10.1002/jmri.26721
123. Ertas G, Doran SJ, Leach MO. A computerized volumetric segmentation method applicable to multi-centre MRI data to support computer-aided breast tissue analysis, density assessment and lesion localization. *Med Biol Eng Comput.* (2016) 55:57–68. doi: 10.1007/s11517-016-1484-y
124. Wu S, Weinstein SP, Conant EF, Kontos D. Automated fibroglandular tissue segmentation and volumetric density estimation in breast MRI using an atlas-aided fuzzy C-means method. *Med Phys.* (2013) 40:112302. doi: 10.1118/1.4829496
125. Wu S, Zuley ML, Berg WA, Kurland BF, Jankowitz RC, Sumkin JH, et al. DCE-MRI background parenchymal enhancement quantified from an early versus delayed post-contrast sequence: association with breast cancer presence. *Sci Rep.* (2017) 7:2115. doi: 10.1038/s41598-017-02341-8
126. Jafri NF, Newitt DC, Kornak J, Esserman LJ, Joe BN, Hylton NM. Optimized breast MRI functional tumor volume as a biomarker of recurrence-free survival following neoadjuvant chemotherapy. *J Magn Reson Imaging.* (2013) 40:476–82. doi: 10.1002/jmri.24351
127. Mustra M, Grgic M, Rangayyan RM. Review of recent advances in segmentation of the breast boundary and the pectoral muscle in mammograms. *Med Biol Eng Comput.* (2016) 54:1003–24. doi: 10.1007/s11517-015-1411-7
128. Klifa C, Carballido-Gamio J, Wilmes L, Laprie A, Lobo C, DeMicco E. Quantification of breast tissue index from MR data using fuzzy clustering. In: *Proceedings of the 26th Annual International Conference of the IEEE Engineering in Medicine Biology Society* 2004. San Francisco, CA; New york, NY: IEEE (2004). p.1667–70.
129. Fashandi H, Kuling G, Lu Y, Wu H, Martel AL. An investigation of the effect of fat suppression and dimensionality on the accuracy of breast MRI segmentation using U-nets. *Med Phys.* (2019) 46:1230–44. doi: 10.1002/mp.13375
130. Zhang Y, Chen J-H, Chang K-T, Park VY, Kim MJ, Chan S, et al. Automatic breast and fibroglandular tissue segmentation in breast MRI using deep learning by a fully-convolutional residual neural network U-net. *Acad Radiol.* (2019) 26:1526–35 doi: 10.1016/j.acra.2019.01.012
131. Dalmiş MU, Litjens G, Holland K, Setio A, Mann R, Karssemeijer N, et al. Using deep learning to segment breast and fibroglandular tissue in MRI volumes. *Med Phys.* (2017) 44:533–46. doi: 10.1002/mp.12079
132. Chitalia RD, Kontos D. Role of texture analysis in breast MRI as a cancer biomarker: a review. *J Magn Reson Imaging.* (2018) 49:927–38. doi: 10.1002/jmri.26556
133. Gastounioti A, Conant EF, Kontos D. Beyond breast density: a review on the advancing role of parenchymal texture analysis in breast cancer risk assessment. *Breast Cancer Res.* (2016) 18:91. doi: 10.1186/s13058-016-0755-8
134. Li H, Zhu Y, Burnside ES, Huang E, Drukker K, Hoadley KA, et al. Quantitative MRI radiomics in the prediction of molecular classifications of breast cancer subtypes in the TCGA/TCIA data set. *npj Breast Cancer.* (2016) 2:16012. doi: 10.1038/npjbcancer.2016.12
135. Bickelhaupt S, Jaeger PF, Laun FB, Lederer W, Daniel H, Kuder TA, et al. Radiomics based on adapted diffusion kurtosis imaging helps to clarify most mammographic findings suspicious for cancer. *Radiology.* (2018) 287:761–70. doi: 10.1148/radiol.2017170273
136. Liu Z, Li Z, Qu J, Zhang R, Zhou X, Li L, et al. Radiomics of multi-parametric MRI for pretreatment prediction of pathological complete response to neoadjuvant chemotherapy in breast cancer: a multicenter study. *Clin Cancer Res.* (2019) 50:1468–77. doi: 10.1158/1078-0432.CCR-18-3190
137. Wang J, Kato F, Oyama-Manabe N, Li R, Cui Y, Tha KK, et al. Identifying triple-negative breast cancer using background parenchymal enhancement heterogeneity on dynamic contrast-enhanced MRI: a pilot radiomics study. *PLoS ONE.* (2015) 10:e0143308. doi: 10.1371/journal.pone.0143308
138. Pinker-Domenig K, Tahmassebi A, Wengert G, Helbich TH, Bago-Horvath Z, Morris EA, et al. Abstract 579: magnetic resonance imaging of the breast and radiomics analysis for an improved early prediction of the response to neoadjuvant chemotherapy in breast cancer patients. *Cancer Res.* (2018) 78:579. doi: 10.1158/1538-7445.AM2018-579
139. Gibbs P, Onishi N, Sadinski M, Gallagher KM, Hughes M, Martinez DF. Characterization of sub-1 cm breast lesions using radiomics analysis. *J Magn Reson Imaging.* (2019) 50:1468–1477. doi: 10.1002/jmri.26732
140. Vidić I, Egnell L, Jerome NP, Teruel JR, Sjøbakk TE, Østlie A, et al. Support vector machine for breast cancer classification using diffusion-weighted MRI histogram features: preliminary study. *J Magn Reson Imaging.* (2018) 47:1205–16. doi: 10.1002/jmri.25873
141. Xie T, Zhao Q, Fu C, Bai Q, Zhou X, Li L, et al. Differentiation of triple-negative breast cancer from other subtypes through whole-tumor histogram analysis on multiparametric MR imaging. *Eur Radiol.* (2019) 29:2535–44. doi: 10.1007/s00330-018-5804-5
142. Pinker K, Chin J, Melsaether AN, Morris EA, Moy L. Precision medicine and radiogenomics in breast cancer: new approaches toward diagnosis and treatment. *Radiology.* (2018) 287:732–47. doi: 10.1148/radiol.2018172171
143. Lourenco AP, Donegan L, Khalil H, Mainiero MB. Improving outcomes of screening breast MRI with practice evolution: initial clinical experience with 3T compared to 1.5T. *J Magn Reson Imaging.* (2013) 39:535–9. doi: 10.1002/jmri.24198
144. Menezes GLG, Stehouwer BL, Klomp DWJ, van der Velden TA, van den Bosch MAAJ, Knuttel FM, et al. Dynamic contrast-enhanced breast MRI at 7T and 3T: an intra-individual comparison study. *SpringerPlus.* (2016) 5:13. doi: 10.1186/s40064-015-1654-7
145. van Kalleveen IML, Boer VO, Luijten PR, Klomp DWJ. Tilt optimized flip uniformity (TOFU) RF pulse for uniform image contrast at low specific absorption rate levels in combination with a surface breast coil at 7 tesla. *Magn Reson Med.* (2015) 74:482–8. doi: 10.1002/mrm.25415
146. Cui J, Bosshard JC, Rispoli JV, Dimitrov IE, Cheshkov S, McDougall MP, et al. A switched-mode breast coil for 7 T MRI using forced-current excitation. *IEEE Trans Biomed Eng.* (2015) 62:1777–83. doi: 10.1109/TBME.2015.2403850
147. Cheshkov S, Dimitrov I, Koning W, Rispoli J, McDougall M, Wright S, et al. Integration of 2-channel parallel transmission with forced current excitation for improved B_1 homogeneity in breast imaging at 7T. In: *Proceedings of the 21st Annual Meeting of ISMRM* 2013. Salt Lake City, UT; Concord, CA: iSMRM (2013). p. 4407. Available online at: https://cds.ismrm.org/protected/13MProceedings/files/4407.PDF (accessed February 9, 2020).
148. van de Bank B, Voogt I, Italiaander M, Stehouwer BL, Boer VO, Luijten PR, et al. Ultra high spatial and temporal resolution breast imaging at 7T. *NMR Biomed.* (2013) 26:367–75. doi: 10.1002/nbm.2868
149. By S, Rispoli JV, Cheshkov S, Dimitrov I, Cui J, Seiler S, et al. A 16-channel receive, forced current excitation dual-transmit coil for breast imaging at 7T. *PLoS ONE.* (2014) 9:e113969. doi: 10.1371/journal.pone.0113969
150. Kim J, Santini T, Bae KT, Krishnamurthy N, Zhao Y, Zhao T, et al. Development of a 7 T RF coil system for breast imaging. *NMR Biomed.* (2017) 30:e3664. doi: 10.1002/nbm.3664
151. Krikken E, Steensma BR, Voogt IJ, Luijten PR, Klomp DWJ, Raaijmakers AJE, et al. Homogeneous B_1^+ for bilateral breast imaging at 7 T using a five dipole transmit array merged with a high density receive loop array. *NMR Biomed.* (2019) 32:e4039. doi: 10.1002/nbm.4039
152. International Electrotechnical Commission. *60601: Medical Electrical Equipment-Part 2-33 Edition 3.1: Particular Requirements for the Safety of Magnetic Resonance Equipment for Medical Diagnosis*. Geneva: IEC (2013).
153. Fiedler TM, Ladd ME, Bitz AK. RF safety assessment of a bilateral four-channel transmit/receive 7 tesla breast coil: SAR versus tissue temperature limits. *Med Phys.* (2017) 44:143–57. doi: 10.1002/mp.12034
154. Li X, Rispoli JV. Toward 7T breast MRI clinical study: safety assessment using simulation of heterogeneous breast models in RF exposure. *Magn Reson Med.* (2019) 81:1307–21. doi: 10.1002/mrm.27395
155. Li X, Chen X, Steckner M, Rispoli J. An initial simulation study of breast implants for clinical breast MRI. In: *Proceedings of the ISMRM 27th Annual Meeting & Exhibition* 2019. Montréal, QC; Concord, CA: ISMRM (2019). p. 1443. Available online at: https://index.mirasmart.com/ISMRM2019/PDFfiles/1443.html (accessed February 9, 2020).
156. Marino MA, Helbich T, Baltzer P, Pinker-Domenig K. Multiparametric MRI of the breast: a review. *J Magn Reson Imaging.* (2018) 47:301–15. doi: 10.1002/jmri.25790

157. Weis JA, Miga MI, Arlinghaus LR, Li X, Abramson V, Chakravarthy AB, et al. Predicting the response of breast cancer to neoadjuvant therapy using a mechanically coupled reaction-diffusion model. *Cancer Res.* (2015) 75:4697–707. doi: 10.1158/0008-5472.CAN-14-2945
158. Leithner D, Wengert GJ, Helbich TH, Thakur S, Ochoa-Albiztegui RE, Morris EA, et al. Clinical role of breast MRI now and going forward. *Clin Radiol.* (2018) 73:700–14. doi: 10.1016/j.crad.2017.10.021
159. Canese R, Pisanu ME, Mezzanzanica D, Ricci A, Paris L, Bagnoli M, et al. Characterisation of *in vivo* ovarian cancer models by quantitative ^{1}H magnetic resonance spectroscopy and diffusion-weighted imaging. *NMR Biomed.* (2012) 25:632–42. doi: 10.1002/nbm.1779
160. Zhou XJ, Gao Q, Abdullah O, Magin RL. Studies of anomalous diffusion in the human brain using fractional order calculus. *Magn Reson Med.* (2010) 63:562–9. doi: 10.1002/mrm.22285
161. Bickelhaupt S, Steudle F, Paech D, Mlynarska A, Kuder TA, Lederer W, et al. On a fractional order calculus model in diffusion weighted breast imaging to differentiate between malignant and benign breast lesions detected on X-ray screening mammography. *PLoS ONE.* (2017) 12:e0176077. doi: 10.1371/journal.pone.0176077
162. Wilson M, Andronesi O, Barker PB, Bartha R, Bizzi A, Bolan PJ, et al. Methodological consensus on clinical proton MRS of the brain: review and recommendations. *Magn Reson Med.* (2019) 82:527–50. doi: 10.1002/mrm.27742
163. Taneja S, Jena A, Goel R, Sarin R, Kaul S. Simultaneous whole-body ^{18}F-FDG PET-MRI in primary staging of breast cancer: a pilot study. *Eur J Radiol.* (2014) 83:2231–9. doi: 10.1016/j.ejrad.2014.09.008
164. Melsaether A, Raad RA, Pujara AC, Ponzo FD, Pysarenko KM, Jhaveri K, et al. Comparison of whole-body ^{18}F FDG PET/MR imaging and whole-body ^{18}F FDG PET/CT in terms of lesion detection and radiation dose in patients with breast cancer. *Radiology.* (2016) 281:193–202. doi: 10.1148/radiol.2016151155

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 in Public Health

REVIEW
published: 29 October 2020
doi: 10.3389/fpubh.2020.509714

Metformin and Chemoprevention: Potential for Heart-Healthy Targeting of Biologically Aggressive Breast Cancer

*Veronica C. Jones, Eric C. Dietze, Tijana Jovanovic-Talisman, Jeannine S. McCune and Victoria L. Seewaldt**

City of Hope Comprehensive Cancer Center, Duarte, CA, United States

Currently, tamoxifen is the only drug approved for reduction of breast cancer risk in premenopausal women. The significant cardiovascular side effects of tamoxifen, coupled with lack of a survival benefit, potential for genotoxicity, and failure to provide a significant risk-reduction for estrogen receptor-negative breast cancer, all contribute to the low acceptance of tamoxifen chemoprevention in premenopausal women at high-risk for breast cancer. While other prevention options exist for postmenopausal women, there is a search for well-tolerated prevention agents that can simultaneously reduce risk of breast cancers, cardiovascular disease, and type-2 diabetes. Metformin is a well-tolerated oral biguanide hypoglycemic agent that is prescribed worldwide to over 120 million individuals with type-2 diabetes. Metformin is inexpensive, safe during pregnancy, and the combination of metformin, healthy lifestyle, and exercise has been shown to be effective in preventing diabetes. There is a growing awareness that prevention drugs and interventions should make the "whole woman healthy." To this end, current efforts have focused on finding low toxicity alternatives, particularly repurposed drugs for chemoprevention of breast cancer, including metformin. Metformin's mechanisms of actions are complex but clearly involve secondary lowering of circulating insulin. Signaling pathways activated by insulin also drive biologically aggressive breast cancer and predict poor survival in women with breast cancer. The mechanistic rationale for metformin chemoprevention is well-supported by the scientific literature. Metformin is cheap, safe during pregnancy, and has the potential to provide heart-healthy breast cancer prevention. On-going primary and secondary prevention trials will provide evidence whether metformin is effective in preventing breast cancer.

Keywords: breast cancer, prevention, metformin, chemoprevention, diabetes, heart disease

OPEN ACCESS

Edited by:
Dorothy Teegarden, Purdue University, United States

Reviewed by:
Davide Serrano, European Institute of Oncology (IEO), Italy
Natascia Marino, Indiana University, United States

****Correspondence:***
Victoria L. Seewaldt
vseewaldt@coh.org

Specialty section:
This article was submitted to Family Medicine and Primary Care, a section of the journal Frontiers in Public Health

Received: *20 November 2019*
Accepted: *24 September 2020*
Published: *29 October 2020*

Citation:
Jones VC, Dietze EC, Jovanovic-Talisman T, McCune JS and Seewaldt VL (2020) Metformin and Chemoprevention: Potential for Heart-Healthy Targeting of Biologically Aggressive Breast Cancer. Front. Public Health 8:509714. doi: 10.3389/fpubh.2020.509714

CURRENT BREAST CANCER PREVENTION STRATEGIES

Currently, tamoxifen is the only drug approved for reducing risk of breast cancer in premenopausal women. The approval of tamoxifen was based on the first National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention Trial (P1) (1, 2). The P1 trial demonstrated that high-risk women who took tamoxifen had a "50% decrease in the incidence of estrogen receptor-positive breast cancer" (1). Results from the P1 trial underlined the decision of the US Food and

Drug Administration (FDA) in October 1998 to approve tamoxifen as a chemoprevention agent for premenopausal high-risk women.

In 2013, the risk reduction benefit of tamoxifen was also shown in a meta-analysis of four randomized controlled trials (3): (1) Royal Marsden (4, 5), (2) International Breast Cancer Intervention Study (IBIS-1) (6, 7), (3) P1 (1, 2), and (4) Italian Randomized Tamoxifen Trial (8, 9). This analysis showed a 33% reduction ($p < 0.0001$) in all breast cancers (10, 11) in high-risk women who took tamoxifen chemoprevention vs. placebo controls (3). As in the P1 trial, the observed reduction was primarily due a decrease in the numbers ER-positive breast cancer (44% in invasive breast cancers ($p < 0.0001$) and DCIS ($p = 0.009$). Although tamoxifen-prevention was given for 5-years, follow-up evaluation of the high-risk subjects provide evidence that the long-term risk-reduction in subjects who took tamoxifen may persist up to 10 years (3).

The benefit of tamoxifen appears to be in risk-reduction of ER+ breast cancer; tamoxifen has failed to demonstrate in high-risk women (1) a significant risk reduction for ER- breast cancer and (2) a survival benefit. An extended analysis (median 16 years) of IBIS-I study participants, continues to shows in the tamoxifen vs. placebo arms "no difference in the number of breast cancer deaths ($p = 0.8$)" (12).

Despite initial recommendations by the FDA and American Society for Clinical Oncology, very few women take tamoxifen (11); it is estimated that only 5–12% of women offered tamoxifen chemoprevention elect to take tamoxifen (11).

Tamoxifen has been shown to increase risk for cardiovascular events, including venous thrombosis, pulmonary embolism, and stroke, and increases risk for endometrial cancer (12–14). Other side effects of tamoxifen include hot flashes, dyspareunia, depression, cataracts, weight gain, and bone loss in premenopausal women (12–15). Consistent with the increased risk of endometrial cancer in humans, a 2013 study in rats showed that 13-week tamoxifen treatment increased DNA point mutations in the liver (16). Lastly, a concern was raised that tamoxifen may be less active in the 5–10% of individuals who carried homozygous variant of the *CYP2D2* gene; this gene variant has low activity to convert tamoxifen to its more active metabolite, 4-hydroxytamoxifen. Lacking in the analysis was a consideration of the concentration of 4-hydroxytamoxifen required to saturate ER; consequently, prospective clinical studies did not demonstrate a reduction in tamoxifen efficacy in individuals with the *CYP2D2* variant (17).

While tamoxifen is the only agent approved for breast cancer prevention in premenopausal women, other agents have been approved for postmenopausal women. In the NSABP Study of Tamoxifen and Raloxifene (STAR) trial (raloxifene 60 mg vs. tamoxifen 20 mg), raloxifene was shown to reduce the incidence of breast cancer in postmenopausal women (18). Raloxifene does not increase the risk of endometrial cancer, however, the incidence of ischemic heart disease and stroke was equivalent to the risk associated with tamoxifen (18). IBIS-II tested anastrozole (1.0 mg) vs. placebo in postmenopausal women; the study found a significant decrease in breast cancer in women who took anastrozole; there was no increased incidence of fractures or cardiovascular disease (19). In the Mammary Prevention.3 trial (MAP.3) exemestane (25 mg) vs. placebo in postmenopausal women was associated with a decreased incidence of both ductal carcinoma *in situ* and invasive breast cancer; with a median follow-up of 3 years, side effects and impact on quality of life were minimal (20).

NEED FOR HEART-HEALTHY BREAST CANCER CHEMOPREVENTION

Women are not just at risk for breast cancer but also face the risk of developing heart disease, obesity, and type-2 diabetes. Furthermore, with the risk of currently available chemoprevention agents potentiating cardiovascular disease, there is a need to identify agents that can effectively target both conditions: breast cancer and cardiovascular disease. To this end, current efforts have focused on finding alternative prevention strategies that have the potential to reduce not just breast cancer but also reduce the risk for cardiometabolic diseases. Potential strategies have included exercise, aspirin, and metformin.

Metformin

Metformin (1,1-dimethylbiguanide hydrochloride) is a well-tolerated oral agent that is prescribed for first-line treatment of type-2 diabetes (21, 22) and is approved for treatment of polycystic ovary and gestational diabetes (23). Metformin is well-tolerated by the majority of patients; common metformin side effects include lack of appetite, epigastric pain, nausea, and diarrhea (24). The most significant potential side effect is lactic acidosis; consequently, metformin is not prescribed in individuals with kidney and/or liver disease (23, 25). The mechanism of action of metformin remains a topic of current investigations. It is accepted that metformin inhibits hepatic gluconeogenesis and decreases intestinal absorption of glucose, secondarily decreasing circulating insulin (21, 26). Metformin is also thought to indirectly increase insulin sensitivity by increasing peripheral glucose utilization (21).

Until recently, most clinical care has focused on treatment of type-2 diabetes rather than its prevention. However, several well-controlled studies have shown that it is possible to prevent type-2 diabetes through a combination of diet, exercise, and metformin. The Diabetes Prevention Program/Diabetes Prevention Program Outcomes Study (DPP/DPPOS) is the largest and longest clinical trial of metformin for the prevention of type-2 diabetes (27, 28). Study participants in the DPP/DPPOS cohort have over 15 years prospective assessment of the impact of metformin and lifestyle modification on type-2 diabetes, cardiovascular events, safety, and fiscal outcomes (27). Metformin and intensive lifestyle modification resulted in a 50% type-2 diabetes risk-reduction in women with a history of type-2 diabetes (29). Based on findings from the DPP/DPPOS study, in 2014, the American Diabetes Association (ADA) published formal recommendations for prevention of type-2 diabetes (30). Recommendations included: (1) individuals with impaired glucose tolerance or a HgbA1c 5.7–6.4 should be referred to a life-style modification (7% weight loss target) and moderate physical activity (e.g., walking) for 150 min/week (30). These recommendations may also prove beneficial in modifying breast cancer risk; as outlined below,

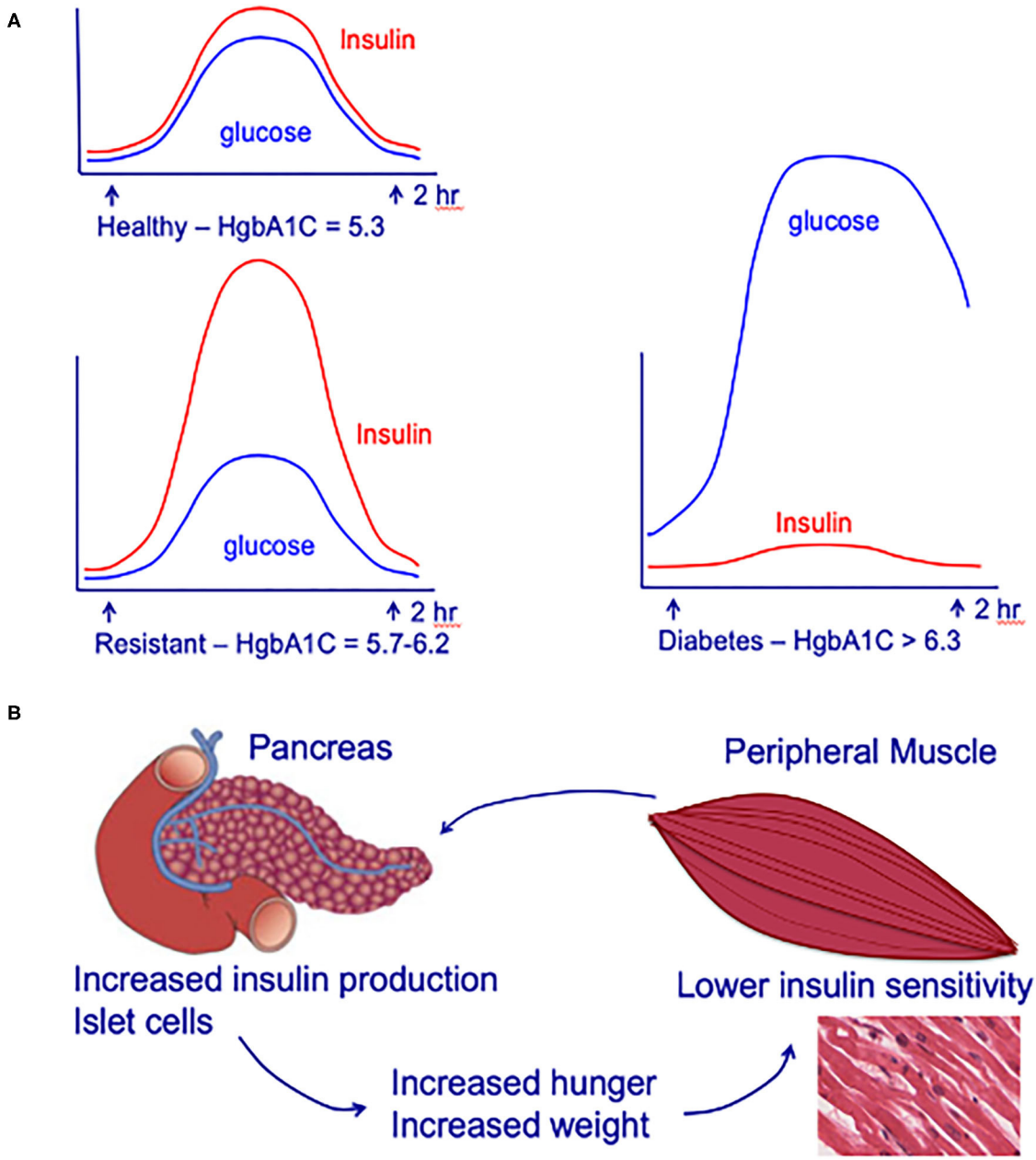


FIGURE 1 | (A) Circulating insulin and glucose levels in healthy individuals (Healthy), insulin-resistant individuals (Resistant), and individuals with type-2 diabetes (Diabetes) at baseline and at 2 h after eating. **(B)** Impact of insulin-resistance on pancreatic islet cells, peripheral muscle, and individual. Insulin resistance in peripheral muscle tissue results in increased insulin demands from the pancreas. Increased circulating insulin drives hunger and increases weight, leading to a positive feedback loop that increases the chance of an individual developing type-2 diabetes. Adapted from (42).

metformin is undergoing testing for primary and secondary breast cancer prevention.

Metformin and Breast Cancer: Epidemiology Studies

Population-based studies provide evidence that cancer incidence and mortality decreased in individuals with cancer who took metformin (31–33). In a retrospective study of women with breast cancer who received neoadjuvant chemotherapy individuals who took metformin had a higher rate of pathologic complete remission vs. those did not [24 vs. 8%, $p = 0.007$; (34)]. In a 2014 meta-analysis, individuals who took metformin had a lower incidence of breast cancer (SRR = 0.94; 95% CI, 0.90–0.99) (35). These epidemiologic studies represent a starting point for

recent prospective clinical trials testing the impact of metformin on primary and secondary breast cancer prevention.

Epidemiology studies investigating the impact of metformin on breast cancer incidence are limited by several factors. These factors include: (1) racial and ethnic differences in body mass index (BMI), (2) inability of BMI to precisely identify individuals who are metabolically unhealthy, and (3) the heterogeneity of breast cancer as a disease. A BMI $\geq$30 kg/m^2 is the most frequently used measure of adiposity (36). BMI is an inexact measure of risk, particularly when comparing individuals of different race and ethnicity. Muscle tissue weighs significantly more per unit volume than adipose tissue; consequently fit, muscular individuals can be mistakenly identified as overweight (BMI 25–30 kg/m^2) or obese.

BMI is not a precise measure of metabolic health. Over the past 20 years, the observation has been made that some individuals with a BMI $>$ 30 kg/m^2 are metabolically healthy, "metabolically healthy obese" (37). In contrast to individuals who are obese but metabolically healthy, there are also individuals with a normal BMI (BMI $<$25 kg/m^2) who have abnormal metabolic profiles and are at increased risk for cardiovascular disease and type-2 diabetes. Current definition of metabolically unhealthy individuals with a normal BMI includes (1) BMI $<$25 kg/m^2, (2) insulin-resistance, hypertriglyceridemia, (3) abdominal fat distribution, and (4) elevated blood pressure (37).

TYPE-2 DIABETES, METFORMIN, AND BREAST CANCER SUBTYPES

Type-2 diabetes is well-established to increase a woman's risk of developing breast cancer. The association between Type-2 diabetes and breast cancer subtypes, however, remains a work in progress, particularly since the majority of studies are underpowered. A case-control study of 916 postmenopausal women with breast cancer cases and 1,094 population-based controls conducted by Garcia-Esquinas et al. found that type-2 diabetes was associated with a 2.25-fold increased risk for triple negative breast cancer (TNBC) (38); this study was limited by a low number of TNBC and the study of only postmenopausal women. The Carolina Breast Cancer Study included 225 women with TNBC; no statistical association was found between type-2 diabetes and TNBC; unfortunately, this study did not test for the association between insulin-resistance and TNBC (39). A case-case study by Lara-Medina et al. of Latinas with breast cancer (469 women with TNBC) found no statistical association between type-2 diabetes and TNBC (40).

The most complete and well-designed epidemiologic study was a retrospective multi-center population-based case-case study of 4,557 women with breast cancer ages 20–69 years old performed by Chen et al.; 1,446 women had TNBC (41). The investigators identified that women with type-2 diabetes had a 38% (95% CI: 1.01–1.89) increased odds of having TNBC (vs. women without type-2 diabetes) (41).

Interestingly, Chen et al. also found that current and extended-time metformin use (13–24 months metformin) within 2 years of diagnosis, increased the odds of a woman having TNBC (OR = 1.54; 95% CI: 1.07–2.22 and OR = 1.80; 95% CI:1.13–2.85, respectively) (41). These latter results are puzzling, given the ability of insulin to activate signaling pathways that drive the aggressive biology of TNBC and the known ability of metformin to lower circulating insulin.

Epidemiologic studies are powerful tools for generating associations but do not test mechanisms. First off, as pointed out by Chen et al., it may be that the women who had the most poorly controlled diabetes (41), were the individuals who had the longest use of metformin; HgbA1c values for these individuals were not reported. While the number of women

TABLE 1 | Select list of clinically relevant known metformin pharmacokinetic and pharmacodynamic genes.

Gene	Protein	Effect	References
SLC22A1	OCT1	Low-function alleles linked to less reduction in HgbA1c	(46–54)
SLC22A2	OCT2	Change in metformin PK; no known clinical impact	(53)
SLC22A3	OCT3	Changes in metformin PK; no known clinical impact	(54)
SLC47A1	MATE1	Alleles linked to increased reduction in HgbA1c	(47, 50, 55)
SCLa7A2	MATE2	Low-function alleles linked to less reduction in HgbA1c	(55, 56)
SRR	Serine racemase	Metabolic changes	(57)
ATM	ATM	Low- and high-function alleles linked to change in HgbA1c	(58–60)
LBK/STK11	Upstream regulator of AMPK	Decreased ovulation in women with polycystic ovarian syndrome.	(47, 61)
PKRAA1, PKRAA2, PKRAB2	AMPK sub-units	Incidence type-2 diabetes	(47)
ABCC8-KNKJ11	Subunit beta cell potassium channel	Incidence type-2 diabetes	(47)

using metformin were carefully determined, it is not clear that the investigators incorporated insulin-use (insulin-dependent type-2 diabetes) in their risk models. Furthermore, these risk models do not account for individuals with insulin-resistance (**Figure 1**). Ultimately, the studies by Chen et al. are extremely important because they highlight how complex the associations between metformin-use, insulin-use, and TNBC are likely to be and underscore the importance of window-of-opportunity trials and ongoing prospective metformin prevention trials (such as MA-32, described below).

METFORMIN TRANSPORT AND MECHANISM OF ACTION

After oral administration, the oral bioavailability is 55 ± 16% (mean ± standard deviation); metformin is predominantly absorbed in the small intestine (43). Metformin is excreted unchanged in the urine and has a half-life between 4 and 8 h (44). Metformin's absorption and renal clearance is primarily mediated by OCT2/MATE1/MATE2-K (organic cation transporter 2/multidrug and toxin extrusion 1/ multidrug and toxin extrusion 2-K) (45). There are frequent polymorphisms in OCT2, MATE1, and MATE2-K that impact clearance metformin [**Table 1**; (46, 62)]. Up to 9% of non-Hispanic Whites exhibit an "OCT1 null phenotype" (46). To date, there have been variable findings in pharmacogenomic studies in humans. However, there is evidence that cancer cell lines with high MATE2 expression may be resistant metformin's growth inhibitor effects (63).

Despite metformin being one of our oldest medications, the precise molecular mechanism(s) underlying metformin's insulin-lowering effects, as well as its potential anti-neoplastic potential, are not completely understood. It is well-accepted that metformin inhibits hepatic gluconeogenesis and secondarily lowers circulating insulin. However, the precise mechanism(s) of metformin-action remains a work in progress. Two major pathways are thought to account for the main actions of metformin and metformin's proposed anti-cancer effects (**Figure 2**); both pathways converge on mammalian target of rapamycin (mTOR): (1) AMPK (adenosine monophosphate-activated protein kinase) independent, driven by metformin's

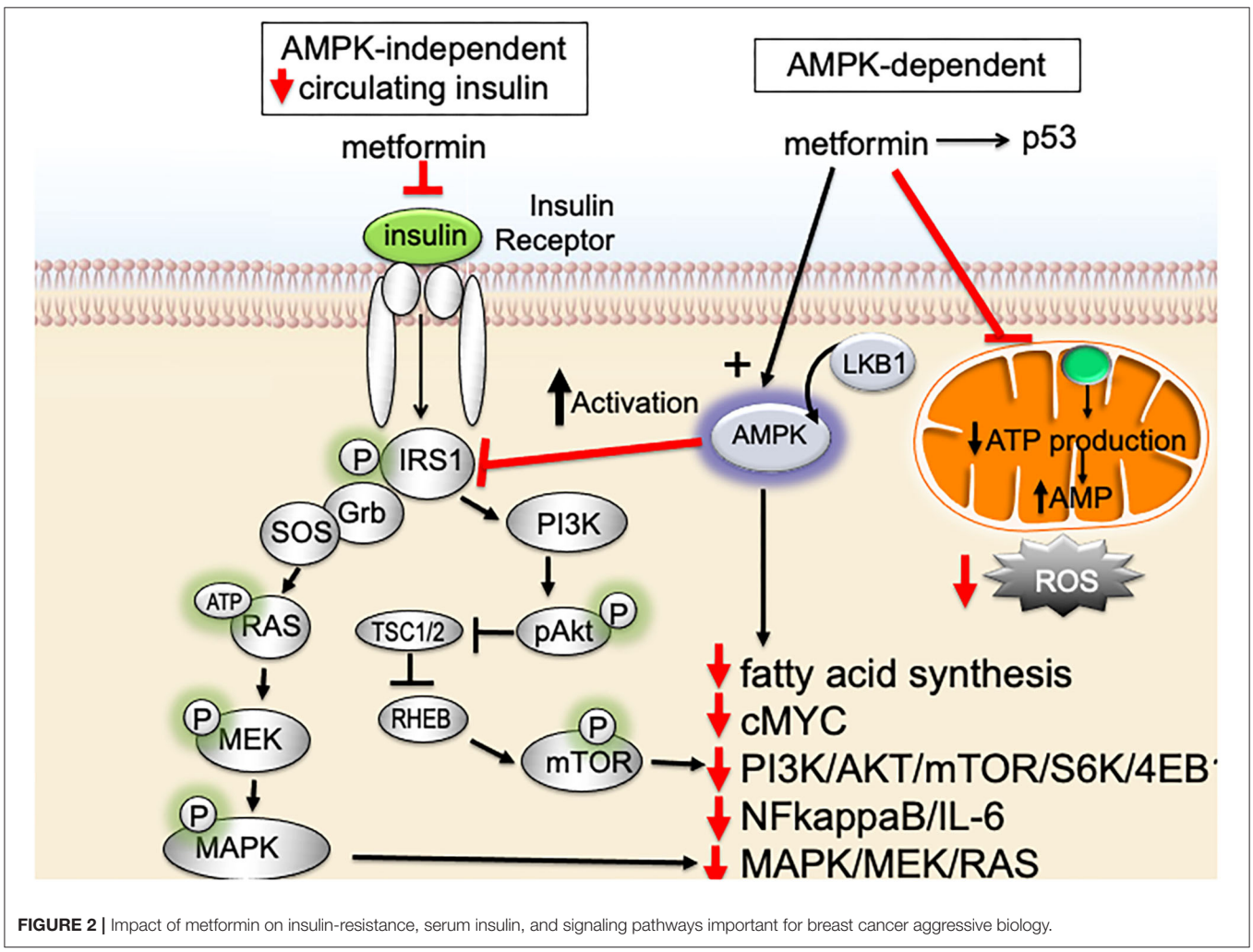


FIGURE 2 | Impact of metformin on insulin-resistance, serum insulin, and signaling pathways important for breast cancer aggressive biology.

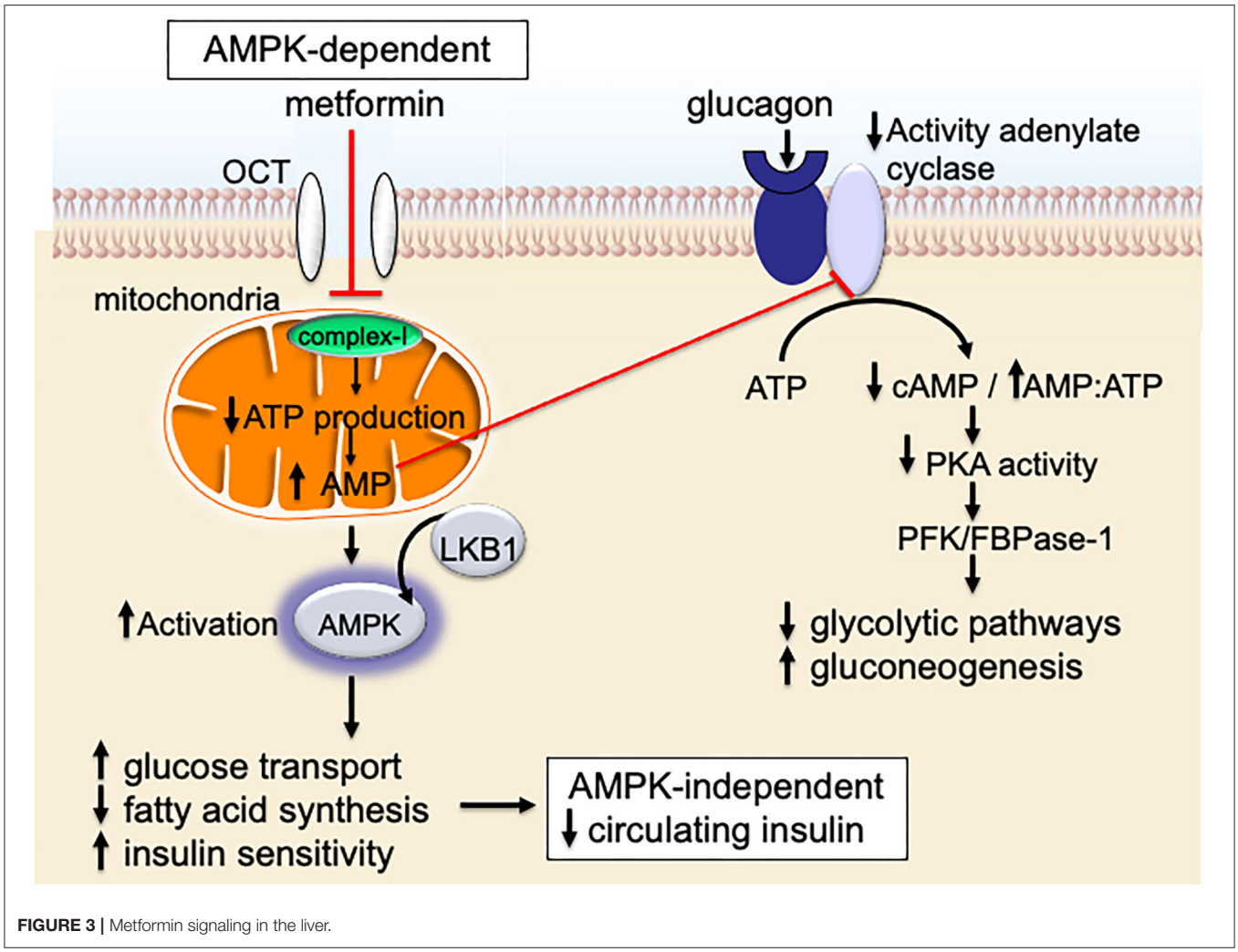


FIGURE 3 | Metformin signaling in the liver.

ability to secondarily lower serum insulin and (2) AMPK-dependent, regulated by metformin-suppression inhibition of mitochondrial complex-I (complex-I).

Metformin signals via an AMPK-independent pathway; in this pathway metformin secondarily lowers circulating insulin levels and inhibits insulin/insulin-like growth factor-1 (IGF-1)-signaling. Under nutrient-rich circumstances, IGF-1 binds to the IGF-1 receptor (IGF-1R) leading to activation of (1) PI3K (phosphatidylinositol-3-kinase)/AKT/mTOR-network signaling and (2) RAS/RAF/mitogen activated protein kinase (MAPK) [**Figure 2**; (64)]. Activation of PI3K/MAPK-pathways increase cell proliferation and activates signaling pathways associated with aggressive cancer biology in humans. By lowering circulating insulin, metformin inhibits IGF-1/IGF-1R signaling and inhibits PI3K- and MAPK-signaling pathways (**Figure 2**).

Metformin also signals through an AMPK-dependent pathway; in this pathway, metformin first inhibits the mitochondrial electron transport protein complex-I (65, 66). Inhibition of complex-I, in turn, blocks production of mitochondrial adenosine-5′-triphosphate (ATP), increases the AMP/ATP ratio, results in a reduction of AMP, and lowers hepatic energy state [**Figures 2**, **3**; (65–69)]. This hepatic energy state restriction leads to AMP binding to AMPK and, thereby, increasing AMPK's affinity for serine-threonine liver kinase B1 (LKB1) (70, 71). AMPK-LKB1-activaiton inhibits AKT/mTOR-network signaling leading to downstream inhibition of S6-Kinase (S6K) and 4E binding protein-1 (4EB-1). Metformin's inhibition of mTOR suppress additional downstream cancer-promoting pathways including (1) Nuclear Factor kappa-light-chain-enhancer of activated B cells NFkB/interleukin-6 (IL6), (2) MAPK/Ras, and (3) cMyc [**Figure 2**; (64, 72, 73)]. NFkB, IL6, MAPK, Ras, and cMyc together play a role in tissue inflammation, metabolism, and immune cell signaling.

Increasing attention has been paid to identifying molecular mechanisms that promote chemotherapy-resistance. Kevin Struhl's group first showed in 2009 that 0.1 mM metformin *in vitro* blocked transformation and killed cancer-like stem cells (74). The combination of metformin and doxorubicin in a mouse xenograft model (metformin 100 μg/ml) exhibited synergy. These results provided a potentially novel mechanism of action for metformin and an experimental rationale for using the

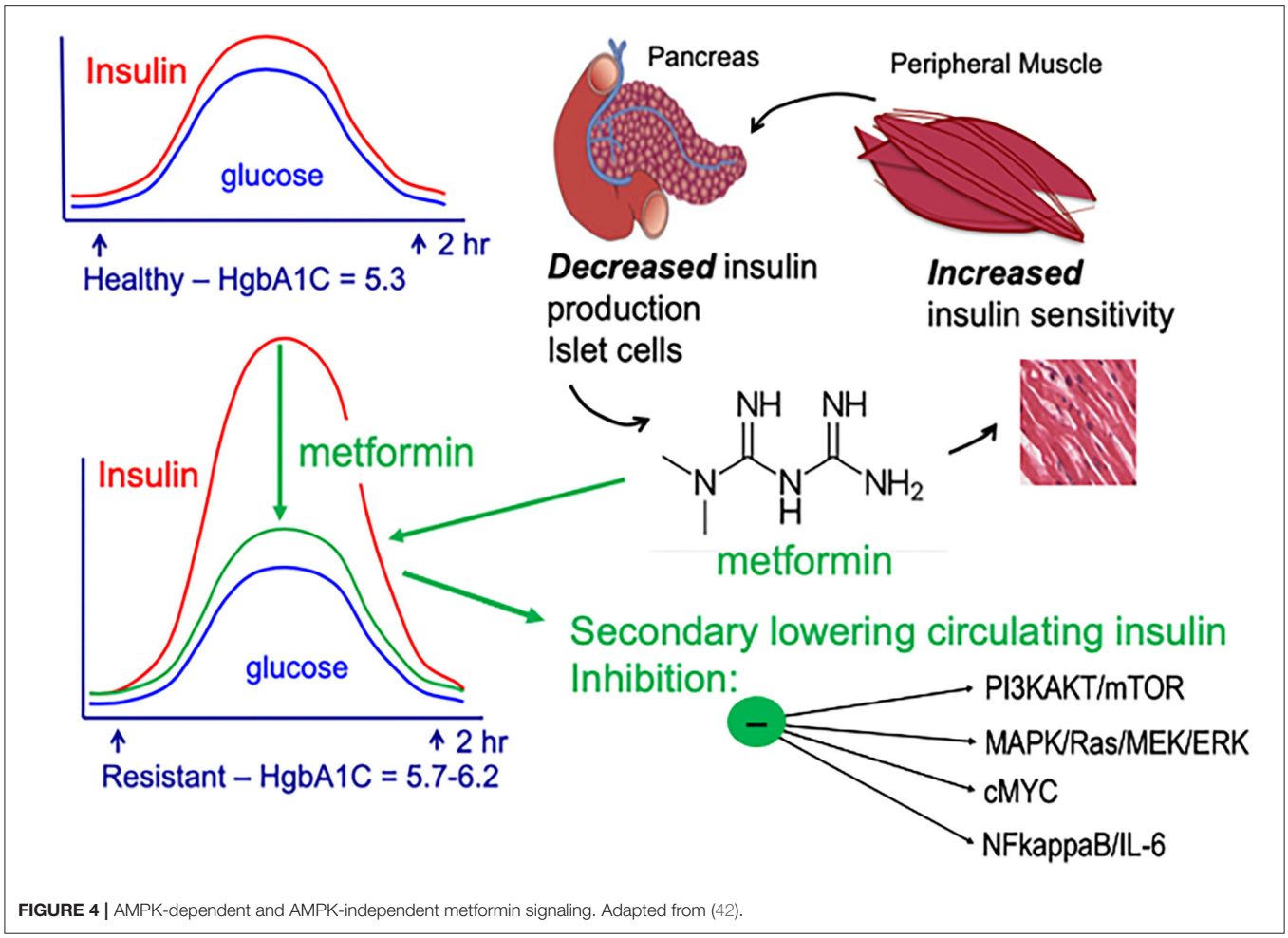


FIGURE 4 | AMPK-dependent and AMPK-independent metformin signaling. Adapted from (42).

combination of metformin and chemotherapy. The metformin doses in this study, however, were supratherapeutic and this very interesting mechanism of metformin-action remains an area of active investigation.

There is also evidence that metformin acts on the tumor microenvironment. Metformin increases intracellular oxygen; this increase is thought to reduce tumor hypoxia (75). Metformin's decrease in hypoxia has been shown to inhibit hypoxia-inducible factor 1 (HIF1) and vascular endothelial growth factor A (VEGFA) driven angiogenesis; there is also evidence for a direct anti-tumor effect on endothelial cells (76, 77). Metformin's increase in tumor oxygenation and or activation of AMPK is thought to shift cancer associated macrophages from a M2 to an M1 phenotype (78). Metformin has been shown to reduce programmed death-ligand 1 (PD-L1) expression on cancer cells, increase lymphocyte anti-tumor cytotoxicity, and downregulate myeloid derived tumor cell activity (79–82). Taken together, these findings highlight a potential role for metformin to be used in concert with immune-therapy.

Current Consensus

While the study of metformin's molecular mechanisms of actions remain an area of active research, there is a growing consensus of the key signaling targets of metformin. The following consensus statement for metformin's key mechanisms of actions is updated from Pernicova and Korbonits (83):

- Metformin alters cellular energy metabolism and promotes metabolic reprogramming.
- Metformin acts to lower glucose and increase insulin-sensitivity: (1) primarily by inhibiting hepatic gluconeogenesis and glucagon-signaling and (2) to a lesser degree, in the skeletal muscle by increasing glucose uptake.
- Metformin lowers circulating glucose by inhibiting hepatic gluconeogenesis and opposing glucagon-action.
- Mitochondria complex-1 is a key target of metformin-signaling.
- Antihyperglycemic effect of metformin remains an area of active investigation, more work is needed.
- Metformin impacts lipid metabolism primarily via activation of 5′-AMP-AMPK.

- Anti-cancer effects of metformin are hypothesized to be: (1) indirect—decrease in circulating insulin and (2) direct—energetic stress. However, additional studies are needed.
- Metformin induces energetic stress in cancer cells.
- AMPK-mediation inhibition of mTOR is important for much of metformin's anticancer activity.
- Impact of metformin on cancer stem-like cells needs validation *in vivo* and in human clinical trials.
- Metformin may have direct and indirect anti-tumor effects on the tumor microenvironment.

RATIONALE FOR METFORMIN'S ABILITY TO PREVENT BIOLOGICALLY AGGRESSIVE BREAST CANCERS

In breast cancer, particularly TNBC and basal-type breast cancer, activation of PI3K/AKT/mTOR-signaling pathway is associated with poor prognosis (84, 85). Activation of the PI3K/AKT/mTOR results in cell cycle progression, apoptosis-resistance, and invasion (86, 87). PI3K/AKT/mTOR is a regulator of glucose metabolism and aerobic glycolysis (Warburg effect) (88–90). The Warburg effect is directly linked to aggressive cancer biology due to its impact on glycolysis/glucose-uptake; increased glycolysis/glucose-uptake promotes increased growth, mitochondrial dysfunction, and apoptosis-resistance. Metformin targets the PI3K/AKT/mTOR pathway and promotes metabolic reprogramming. These actions support the use of metformin for prevention of biologically aggressive breast cancers (**Figures 2**–**4**).

Prevention options for premenopausal women who carry a deleterious germline BRCA mutation are limited. There is strong scientific rationale for testing metformin in chemoprevention of breast cancer in *BRCA* mutation carriers: (1) metformin activates AMPK and (2) signaling networks regulated by both AMPK and BRCA1, include PTEN, p53, and acetyl coenzyme A carboxylase alpha (ACCA) (83, 91, 92). AMPK regulates the phosphorylation/dephosphorylation cycles of ACCA (93, 94). Given that AMPK and BRCA1 both inactivate ACCA, it is hypothesized that metformin might compensate for BRCA1-loss. Further rational for metformin prevention in BRCA1 mutation carriers has been provided by Cuyas et al. (95). Introduction of BRCA1 mutation*185delAG* in MCF10A cells resulted in metabolic reprograming including (1) mitochondrial activation, (2) increased glucose- and glutamine-dependent activation of the tricarboxylic acid cycle (TCA), and (3) increased production of acetyl-CoA and malonyl-CoA (95). Metformin was shown *in vitro* to inhibit (1) mitochondrial biosynthetic capacity, (2) the TCA cycle, and (3) generation of lipogeneic precursors. The authors hypothesize that the ability of metformin to block ("starve") mitochondrial-generated biosynthesis, might provide further rationale for using metformin for cancer prevention in women with germline *BRCA1*-mutation (95). As described below, to date, the epidemiologic and clinical trials using metformin have yielded conflicting results. The ability of metformin to prevent biologically aggressive breast cancers, particularly TNBC, requires the completion of the on-going prospective trials, such as MA-32.

Clinical Studies

Dr. Pamela Goodwin has been a pioneer in the use of metformin for lowering insulin and breast cancer chemoprevention; she has developed some of the first trials testing metformin. In a trial of 32 women (4 dropout) with early stage breast cancer and fasting insulin of $\geq$45 pmol/L and glucose <7.0 mmol/L, administration of metformin 1500 mg per day for 6 months was associated with a 22.4% decrease in serum insulin [$p = 0.024$; (34)]. This study provided the rational for subsequent randomized clinical trials using metformin vs. placebo.

Window-of-opportunity trials provide important insight into metformin's mechanisms of action but have had conflicting results. In a Scottish trial, Hadad et al. tested the impact of metformin 500 mg ramp up and then 1,000 mg twice a day on Ki-67 and gene expression on 8 pilot women and a further 47 women with primary breast cancer; 7/32 women receiving metformin withdrew due to gastrointestinal upset (96). In women receiving metformin, Ki-67 fell significantly following metformin in both the pilot study ($p = 0.041$) and in the metformin arm ($p = 0.027$) but was unchanged in women who did not take metformin (96). Gene expression studies showed a decrease in mRNA expression in genes regulating AMPK; further analysis demonstrated that tumor necrosis factor receptor signaling, and mTOR- and AMPK-signaling were impacted by metformin (96).

The results by Hadad et al. contrast with a second window of opportunity trial. In a double-blind pre-surgical trial Bonanni et al. (2008-004912-10) randomized 200 non-diabetic women to metformin 850 mg/day vs. placebo for 4 weeks prior to surgery (97). Unlike findings by Hadad et al., Bonanni et al. observed no statistical difference in Ki-67 between arms (97). However, there was a differential impact on Ki-67 based on insulin-resistance (measured by homeostatic model assessment—HOMA). In women with HOMA >2.8 there was a 10.5% decrease in mean Ki-67 vs. an 11% increase in women with HOMA <2.8 (p-interaction = 0.045); women with Luminal B breast cancer had the greatest benefit [$p = 0.005$; (97)]. Further, biomarker analysis showed that this trial represented a significant accomplishment, given the difficulty of coordinating window-of-opportunity trials; importantly, this trial provided a key piece of evidence that non-diabetic metabolically unhealthy women may benefit from metformin chemoprevention (97). A third window-of-opportunity trial reported by Kalinsky et al. in women with early stage breast cancer and a BMI $\geq$30 reported that in women taking 1,500 mg metformin there were no significant differences in Ki-67 for either DCIS or invasive breast cancer (98). There has been significant discussion about the differences observed in these: trials; one potential difference is that women in the Scottish trial had larger breast cancers and therefore, had larger tumors for analysis [see Kalinsky and Hershman for a more in-depth analysis (99)]. Still, given the short duration of window-of-opportunity trials, longer duration trials with a cancer endpoint are required. See **Table 2A** for additional clinical

TABLE 2 | Review of metformin in breast cancer treatment or prevention.

ClinicalTrials.gov (reference if available)	Study	Study design	Inclusion	Endpoint and results (if available)
(A) Adjuvant, window-of-opportunity, and secondary prevention trials				
Breast phase II (34)	Insulin-lowering effects of metformin in women with early stage breast cancer	Metformin 500 mg tid × 6 months	IBC completed therapy with fasting insulin of ≥45 pmol/L and glucose <7.0 mmol/L	Serum insulin ***Results:*** Metformin was associated with a 22.4% decrease in serum insulin ($p = 0.024$)
NCT00897884 (100)	Clinical and biologic effects of metformin in early stage breast cancer	Window-of-opportunity. Single group. Metformin 500 mg tid × 3 weeks	Early stage disease. Women 18–70 years; T1-4; presurgical	Comparison pre- and post-operative biopsy; Ki67 ***Results:*** HOMA significantly reduced; Ki67 decreased 36.5–33% $p = 0.016$ TUNEL increased from 0.56 to 1.05 $p = 0.004$
NCT00909506	Efficacy and safety of adjuvant metformin for operable breast cancer patients	Window-of-opportunity. Metformin 500 mg × 1–2 weeks; then 500 mg bid weeks 3–24	Operable breast cancer BMI>23; no medications except tamoxifen	Weight loss
NCT00930579 (98)	Effects of metformin on AMP/mTOR pathway	Window-of-opportunity. Metformin 1,500 mg qd for >12 weeks before surgery	Operable breast cancer; BMI >30 overweigh and obese women with newly diagnosed breast cancer	***Results:*** No significant differences in Ki67 for DCIS or invasive breast cancer
NCT00933309 (101)	Impact of obesity and obesity treatments on breast cancer	Exemestane with metformin 1,000 mg per day and Rosiglitazone	Postmenopausal obese, ER+ metastatic breast cancer	Dose-limiting toxicity ***Results:*** Metformin was well-tolerated
NCT01042379	I-SPY 2 TRIAL: neoadjuvant and personalized adaptive novel agents to treat breast cancer	Window-of-opportunity. Randomized novel drugs in combination w/ standard chemotherapy	Presurgical breast cancer—neoadjuvant chemotherapy	Pathologic complete remission rate
NCT01101438 (MA-32) (102)	A phase III randomized trial of metformin vs. placebo in early stage breast cancer	Randomization to 1 of 2 treatment arms	Patients stratified by ER/PR status, BMI, HER2 status, and prior chemotherapy	Disease free survival Metabolic parameters: ***Results*** at 6 months: Weight −3.0%, glucose −3.8%, insulin −11.1%
NCT01310231 (103)	A trial of standard chemotherapy with metformin (vs. placebo) in women with metastatic breast cancer	Standard chemotherapy Metformin 850 bid vs. placebo	Metastatic breast cancer 1–4th line chemotherapy	***Results***: No significant impact on RR, PRS, or OS
NCT01650506	Study of Erlotinib and metformin in triple-negative breast cancer	Phase I to establish maximum tolerated dose	Open label single arm. Diagnosis of triple-negative breast cancer	Maximum tolerated dose
NCT01980823	Pre-surgical trial of the combination of metformin and atorvastatin in newly diagnosed operable breast cancer	Window-of-opportunity. Metformin 500 mg a.m. and 1,000 mg p.m. w/atorvastatin 80 mg or at least 2 weeks prior to surgery	Histologically confirmed DCIS or IBC who undergo CNB followed by surgery	Ki-67
NCT02145559 (104)	Pharmacodynamic study of sirolimus and metformin in patients w/advanced solid tumors	Pharmaco-dynamics study	Phase 1	Investigation of combination therapy in targeting mTOR pathway ***Results***: No dose limiting toxicities. No significant differences in fasting glucose, insulin, p70S6K
NCT02278965	Metformin and omega-3 fatty acids in women with a history of early stage breast cancer	Metformin 850 mg bid and Omega-3 1,120 mg bid × 12 months	Stage 1–3; no evidence of disease at entry	Safety and feasibility

(Continued)

TABLE 2 | Continued

ClinicalTrials.gov (reference if available)	Study	Study design	Inclusion	Endpoint and results (if available)
(A) Adjuvant, window-of-opportunity, and secondary prevention trials				
NCT02874430	Metformin hydrochloride and doxycycline in treating patients with localized breast or uterine cancer	Metformin days 1–3; then 2x per day on day 4. Treatment repeats every 7 days	Breast or Uterine cancer; localized; no neoadjuvant chemotherapy	Increased caveolin in cancer associated fibroblasts
NCT03238495	Randomized trial of neo-adjuvant chemotherapy with or without metformin for HER2 positive operable breast cancer (HERMET)	Randomized taxotere, Carboplatin, Herceptin + Pertuzumab With or without metformin	cT1c-cT4a-d HER2+ breast cancer	Pathologic complete response
Instituto Europeo di Oncologica 2006-006236-22 (105)	Use of metformin to reduce serum level of testosterone and improve the metabolic picture for women treated with breast cancer	Metformin 1,000 vs. 1,500 mg/d × 3 months	Postmenopausal with history of IBC and 6 months post-surgery, on TAM for at least 6 months and plan to continue, or at least 6 months post-chemo	1,500 mg/d decreased testosterone by 23% ($p < 0.01$)
Instituto Europeo di Oncologica 2007-000306-70 (105)	Effect of metformin on biomarker activity in primary breast cancer.	Window-of-opportunity trial. Metformin 500 mg/d × 1 week; then metformin 1,000 mg/d × 1 week vs. placebo	Menopausal; Stage 1–2 IBC, >1 cm, no history of diabetes High risk of recurrence due to elevated testosterone	3.4% decrease in Ki-67 ($p = 0.02$)
Instituto Europeo di Oncologica 2008-004912-10 (97, 106, 107)	A randomized double-blind pre-surgical phase II study on activity of metformin on breast cancer cell proliferation	Window-of opportunity trial. Metformin 850 mg/d × 3 days; then metformin 850 mg bid day 4–28 vs. placebo; 4 weeks prior to surgery	Presurgical-Stage IIII IBC patient not suitable for neoadjuvant therapy	No overall change in Ki-67 10.5% decrease in Ki-67 if HOMA >2.8 (p for interaction = 0.045)
ClinicalTrials.gov (reference if available)	**Study title**	**Study design**	**Inclusion**	**Primary endpoint**
(B) Primary prevention and presurgical trials				
ACTRN 12610000219088	Phase I trial metformin followed by reduction mammoplasty	500 mg/d × 1 week; then 1,000 mg/d × 4 weeks; then reduction mammoplasty	Women age 40–60	AMPK signaling and aromatase expression in reduction mastectomy
NCT01302379 (108)	Reach for Health study: Obesity-related mechanisms and mortality in breast cancer survivors	Metformin Placebo Lifestyle interventions 2 × 2 design	Breast cancer survivor; no active disease Overweight or obese	Study powered for metformin vs. placebo and weight loss vs. control. Metformin associated with decrease in serum insulin, estradiol, testosterone
NCT01793948	Metformin hydrochloride vs. placebo in overweight and obese patients at elevated risk for breast cancer	850 mg qd × 30 days; then bid × 11 months vs. placebo	Postmenopausal and high risk for breast cancer with BMI ≥25	Changes in mammary epithelial phosphorylated proteins
NCT01905046	Metformin hydrochloride vs. placebo in preventing breast cancer in obese premenopausal women with atypical hyperplasia or *in situ* breast cancer	850 mg qd × 4 weeks; then 850 mg bid vs. placebo × 24 months	Premenopausal, BMI >25, prior AH, LCIS or DCIS, >1.66% Gail or known BRCA carrier, and cytological atypia	1^O Endpoint: Regression of atypia at 12 and 24 months 2^O Endpoint: Changes in phosphorylated proteins
NCT02028221	Phase II study of metformin for reduction of obesity-associated breast cancer risk	850 mg × 1 month; then 850 mg bid × 11 months vs. placebo	Premenopausal women age 30–45 with BMI of 25 or greater and metabolic syndrome	Change in breast density from baseline at 6 and 12 months
NCT02431676	Survivorship promotion in reducing IGF-1 trial	Metformin Coach directed behavioral weight loss Self-control weight loss	Breast cancer Prostate cancer Lung cancer	Serum IGF-1 IGF-1/IGFBP3 ratio

(Continued)

TABLE 2 | Continued

ClinicalTrials.gov (reference if available)	Study title	Study design	Inclusion	Primary endpoint
(B) Primary prevention and presurgical trials				
NCT04300790	Study to evaluate the effect of Metformin in prevention of hyperglycemia in HR+/HER2- PI3KCA-mutant advanced breast cancer patients [METALLICA]	Metformin Alpelisib Fulvestrant	Prevention hyperglycemia in cancer patients	Number of patients with grade 3–4 hyperglycemia

IBC, invasive breast cancer; DCIS, ductal carcinoma in situ; qd, one a day; bid, twice a day; tid, three times a day; Tam, Tamoxifen; BMI, body mass index; HOMA, Homeostasis Model Assessment; CNB, core needle biopsy; RR, recurrence rate; PFS, progression free survival; OS, overall survival.
AH, atypical hyperplasia; LCIS, lobular carcinoma in situ; DCIS, ductal carcinoma in situ; qd, one a day; bid, twice a day; tid, three times a day; Tam, Tamoxifen; BMI, body mass index; RPPM, reverse phase proteomic microarray profiling.

and window-of-opportunity metformin trials in women with breast cancer.

Currently many ongoing prospective clinical studies are testing the metformin for primary and secondary prevention of breast cancer (**Tables 2A,B**). Together, these clinical studies represent an important investment by the National Institute of Health, United States (NIH), European Cancer trials groups, and the National Cancer Institute, Canada (NCIC) (**Table 2**). The largest adjuvant (secondary prevention) trial is NCIC MA-32, comparing metformin 850 mg p.o. twice a day vs. placebo (NCT01101438) in women with breast cancer; the endpoint of this trial is breast cancer recurrence. After 2,382 women were enrolled, in 2012, the eligibility criteria were amended to mandate TNBC status for patients with T1cN0 disease and at least one adverse tumor characteristic for patients with T2N0 tumors. Interim analysis of the first 500 women taking metformin entered in MA-32, showed at 6 months there was a significant decrease in weight (−3.0%), serum glucose (−3.8%), and serum insulin (−11.1%) (102); further results from this trial are pending. ACTRN12610000219088 is currently testing the impact of metformin (1,000 mg) on LKB1 and AMPK signaling; NCT0430079 tests the impact of metformin in preventing grade 3–4 in (1) men and (2) post-menopausal women receiving treatment for ER/PR+, HER2-not amplified advanced breast cancer, with a PI3K-mutation [METALLICA trial]. Primary prevention studies include (1) NCT01793948: randomized testing the impact of metformin on postmenopausal women with high breast density, (2) NCT01905046: metformin vs. placebo in high-risk premenopausal women (including BRCA mutation carriers) with cytologic atypia, and (3) NCT01905046: randomized testing of whether metformin alters breast density, serum IGF-1/IGFBP-e ratios, IGF-2, and leptin/adiponectin ratios, body weight/body composition (109). See **Table 2B** for additional trials. Given the wealth of primary and secondary metformin chemoprevention trials, it is anticipated that over the next 5 years, these trials will provide important insights into whether metformin is a viable chemoprevention agent for breast cancer.

METFORMIN AND HEART-HEALTHY PREVENTION OF BIOLOGICALLY AGGRESSIVE BREAST CANCERS

Metformin is cheap, safe during pregnancy, and has shown to prevent type-2 diabetes. There is a need for prevention drugs that target both ER+ and ER- breast cancer as well as providing prevention for cardiometabolic disease. Metformin clearly lowers insulin-signaling; signaling pathways activated by insulin are known to drive biologically aggressive breast cancer and predict poor survival in women with breast cancer. Despite the fact that metformin targets many key breast cancer pathways, there is much to be learned about whether metformin can prevent breast cancer and/or breast cancer recurrence. Window-of-opportunity trials provide important clues to metformin's impact on normal and malignant breast tissue, but results have not been entirely consistent. Currently, it is unclear which breast cancer subtypes may benefit the most from metformin. It is likely that MA-32 will provide answers to many of these questions. There is also much to be learned about metformin, insulin resistance, and BMI; specifically, whether metformin's impact is only in women who are metabolically unhealthy and/or have high BMI, or whether metformin can benefit all women. Biomarker studies that define key signaling pathways impacted by metformin will be critical to design and inform future clinical trials. Over the next 5 years on-going primary and secondary prevention trials will show (or not show) the ability of metformin to prevent breast cancer. Hopefully, these studies will not just provide a yes/no answer also provide the biomarkers to determine which women will maximally benefit from metformin. In the words of several of my patients "Please do not quote statistics at me; these statistics are about other women. If I take a prevention agent, I want to know if the prevention agent is working in my breasts."

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 National Institutes of Health/National Cancer Institute (NIH/NCI) grants R01CA170851, P20CA24619, R01CA192914, and U01CA189283 (all to VS) and P30CA033572-Biomarkers Core. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1. Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and bowel project P-1 study. *J Natl Cancer Inst.* (1998) 90:1371–88. doi: 10.1093/jnci/90.18.1371
2. Fisher B, Costantino JP, Wickerham DL, Cecchini RS, Cronin WM, Robidoux A, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and bowel project P-1 study. *J Natl Cancer Inst.* (2005) 97:1652–62. doi: 10.1093/jnci/dji372
3. Cuzick J, Sestak I, Bonanni B, Costantino JP, Cummings S, DeCensi A, et al. Selective oestrogen receptor modulators in prevention of breast cancer: an updated meta-analysis of individual participant data. *Lancet.* (2013) 381:1827–34. doi: 10.1016/S0140-6736(13)60140-3
4. Powles T, Eeles R, Ashley S, Easton D, Chang J, Dowsett M, et al. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomised chemoprevention trial. *Lancet.* (1998) 352:98–101. doi: 10.1016/S0140-6736(98)85012-5
5. Powles TJ, Ashley S, Tidy A, Smith IE, Dowsett M. Twenty-year follow-up of the Royal Marsden randomized, double-blinded tamoxifen breast cancer prevention trial. *J Natl Cancer Inst.* (2007) 99:283–90. doi: 10.1093/jnci/djk050
6. Cuzick J, Forbes J, Edwards R, Baum M, Cawthorn S, Coates A, et al. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomised prevention trial. *Lancet.* (2002) 360:817–24. doi: 10.1016/S0140-6736(02)09962-2
7. Cuzick J, Forbes JF, Sestak I, Cawthorn S, Hamed H, Holli K, et al. Long-term results of tamoxifen prophylaxis for breast cancer—96-month follow-up of the randomized IBIS-I trial. *J Natl Cancer Inst.* (2007) 99:272–82. doi: 10.1093/jnci/djk049
8. Veronesi U, Maisonneuve P, Costa A, Sacchini V, Maltoni C, Robertson C, et al. Prevention of breast cancer with tamoxifen: preliminary findings from the Italian randomised trial among hysterectomised women. Italian Tamoxifen Prevention study. *Lancet.* (1998) 352:93–7. doi: 10.1016/S0140-6736(98)04394-3
9. Veronesi U, Maisonneuve P, Rotmensz N, Bonanni B, Boyle P, Viale G, et al. Tamoxifen for the prevention of breast cancer: late results of the Italian Randomized Tamoxifen prevention trial among women with hysterectomy. *J Natl Cancer Inst.* (2007) 99:727–37. doi: 10.1093/jnci/djk154
10. Taylor R, Taguchi K. Tamoxifen for breast cancer chemoprevention: low uptake by high-risk women after evaluation of a breast lump. *Ann Fam Med.* (2005) 3:242–7. doi: 10.1370/afm.284
11. Donnelly LS, Evans DG, Wiseman J, Fox J, Greenhalgh R, Affen J, et al. Uptake of tamoxifen in consecutive premenopausal women under surveillance in a high-risk breast cancer clinic. *Br J Cancer.* (2014) 110:1681–7. doi: 10.1038/bjc.2014.109
12. Cuzick J, Sestak I, Cawthorn S, Hamed H, Holli K, Howell A, et al. Tamoxifen for prevention of breast cancer: extended long-term follow-up of the IBIS-I breast cancer prevention trial. *Lancet Oncol.* (2015) 16:67–75. doi: 10.1016/S1470-2045(14)71171-4
13. Francis PA, Regan MM, Fleming GF. Adjuvant ovarian suppression in premenopausal breast cancer. *N Engl J Med.* (2015) 372:1673. doi: 10.1056/NEJMc1502618
14. Prasad V, Diener-West M. Primary chemoprevention of breast cancer: are the adverse effects too burdensome? *CMAJ.* (2015) 187:E276–8. doi: 10.1503/cmaj.141627
15. Sestak I, Harvie M, Howell A, Forbes JF, Dowsett M, Cuzick J. Weight change associated with anastrozole and tamoxifen treatment in postmenopausal women with or at high risk of developing breast cancer. *Breast Cancer Res Treat.* (2012) 134:727–34. doi: 10.1007/s10549-012-2085-6
16. Kawamura Y, Hayashi H, Kurata Y, Hiratsuka K, Masumura K, Nohmi T. Evaluation of the genotoxicity of tamoxifen in the liver and kidney of F344 gpt delta transgenic rat in 3-week and 13-week repeated dose studies. *Toxicology.* (2013) 312:56–62. doi: 10.1016/j.tox.2013.07.014
17. Regan MM, Leyland-Jones B, Bouzyk M, Pagani O, Tang W, Kammler R, et al. CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the breast international group 1-98 trial. *J Natl Cancer Inst.* (2012) 104:441–51. doi: 10.1093/jnci/djs125
18. Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, et al. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP study of tamoxifen and raloxifene (STAR) P-2 trial. *JAMA.* (2006) 295:2727–41. doi: 10.1001/jama.295.23.joc60074
19. Cuzick J, Sestak I, Forbes JF, Dowsett M, Cawthorn S, Mansel RE, et al. Use of anastrozole for breast cancer prevention (IBIS-II): long-term results of a randomised controlled trial. *Lancet.* (2020) 395:117–22. doi: 10.1016/S0140-6736(19)32955-1
20. Goss PE, Ingle JN, Ales-Martinez JE, Cheung AM, Chlebowski RT, Wactawski-Wende J, et al. Exemestane for breast-cancer prevention in postmenopausal women. *N Engl J Med.* (2011) 364:2381–91. doi: 10.1056/NEJMoa1103507
21. Maruthur NM, Tseng E, Hutfless S, Wilson LM, Suarez-Cuervo C, Berger Z, et al. Diabetes medications as monotherapy or metformin-based combination therapy for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med.* (2016) 164:740–51. doi: 10.7326/M15-2650
22. Wilding SDAJ. *Clinical Obesity in Adults and Children*. 2nd ed. Malden, MA: Blackwell (2008).
23. Lautatzis ME, Goulis DG, Vrontakis M. Efficacy and safety of metformin during pregnancy in women with gestational diabetes mellitus or polycystic ovary syndrome: a systematic review. *Metabolism.* (2013) 62:1522–34. doi: 10.1016/j.metabol.2013.06.006
24. Triggle CR, Ding H. Metformin is not just an antihyperglycaemic drug but also has protective effects on the vascular endothelium. *Acta Physiol.* (2017) 219:138–51. doi: 10.1111/apha.12644
25. Lipska KJ, Bailey CJ, Inzucchi SE. Use of metformin in the setting of mild-to-moderate renal insufficiency. *Diabetes Care.* (2011) 34:1431–7. doi: 10.2337/dc10-2361
26. Horakova O, Kroupova P, Bardova K, Buresova J, Janovska P, Kopecky J, et al. Metformin acutely lowers blood glucose levels by inhibition of intestinal glucose transport. *Sci Rep.* (2019) 9:6156. doi: 10.1038/s41598-019-42531-0
27. Aroda VR, Knowler WC, Crandall JP, Perreault L, Edelstein SL, Jeffries SL, et al. Metformin for diabetes prevention: insights gained from the diabetes prevention program/diabetes prevention program outcomes study. *Diabetologia.* (2017) 60:1601–11. doi: 10.1007/s00125-017-4361-9
28. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* (2002) 346:393–403. doi: 10.1056/NEJMoa012512
29. Ratner RE, Christophi CA, Metzger BE, Dabelea D, Bennett PH, Pi-Sunyer X, et al. Prevention of diabetes in women with a history of gestational diabetes: effects of metformin and lifestyle interventions. *J Clin Endocrinol Metab.* (2008) 93:4774–9. doi: 10.1210/jc.2008-0772
30. American Diabetes A. 3. Prevention or delay of type 2 Diabetes: standards of medical care in Diabetes-2020. *Diabetes Care.* (2020) 43(Suppl. 1):S32–6. doi: 10.2337/dc20-S003
31. Zhou YY, Zhu GQ, Liu T, Zheng JN, Cheng Z, Zou TT, et al. Systematic review with network meta-analysis: antidiabetic medication and risk of hepatocellular carcinoma. *Sci Rep.* (2016) 6:33743. doi: 10.1038/srep33743

32. Yu H, Zhong X, Gao P, Shi J, Wu Z, Guo Z, et al. The potential effect of metformin on cancer: an umbrella review. *Front Endocrinol.* (2019) 10:617. doi: 10.3389/fendo.2019.00617
33. Rennert G, Rennert HS, Gronich N, Pinchev M, Gruber SB. Use of metformin and risk of breast and colorectal cancer. *Diabetes Res Clin Pract.* (2020) 165:108232. doi: 10.1016/j.diabres.2020.108232
34. Goodwin PJ, Pritchard KI, Ennis M, Clemons M, Graham M, Fantus IG. Insulin-lowering effects of metformin in women with early breast cancer. *Clin Breast Cancer.* (2008) 8:501–5. doi: 10.3816/CBC.2008.n.060
35. Gandini S, Puntoni M, Heckman-Stoddard BM, Dunn BK, Ford L, DeCensi A, et al. Metformin and cancer risk and mortality: a systematic review and meta-analysis taking into account biases and confounders. *Cancer Prev Res.* (2014) 7:867–85. doi: 10.1158/1940-6207.CAPR-13-0424
36. Heymsfield SB, Peterson CM, Thomas DM, Heo M, Schuna JM Jr. Why are there race/ethnic differences in adult body mass index-adiposity relationships? A quantitative critical review. *Obes Rev.* (2016) 17:262–75. doi: 10.1111/obr.12358
37. Brandao I, Martins MJ, Monteiro R. Metabolically healthy obesity-heterogeneity in definitions and unconventional factors. *Metabolites.* (2020) 10:48. doi: 10.3390/metabo10020048
38. Garcia-Esquinas E, Guino E, Castano-Vinyals G, Perez-Gomez B, Llorca J, Altzibar JM, et al. Association of diabetes and diabetes treatment with incidence of breast cancer. *Acta Diabetol.* (2016) 53:99–107. doi: 10.1007/s00592-015-0756-6
39. Millikan RC, Newman B, Tse CK, Moorman PG, Conway K, Dressler LG, et al. Epidemiology of basal-like breast cancer. *Breast Cancer Res Treat.* (2008) 109:123–39. doi: 10.1007/s10549-007-9632-6
40. Lara-Medina F, Perez-Sanchez V, Saavedra-Perez D, Blake-Cerda M, Arce C, Motola-Kuba D, et al. Triple-negative breast cancer in Hispanic patients: high prevalence, poor prognosis, and association with menopausal status, body mass index, and parity. *Cancer.* (2011) 117:3658–69. doi: 10.1002/cncr.25961
41. Chen H, Cook LS, Tang MC, Hill DA, Wiggins CL, Li CI. Relationship between diabetes and diabetes medications and risk of different molecular subtypes of breast cancer. *Cancer Epidemiol Biomarkers Prev.* (2019) 28:1802–8. doi: 10.1158/1055-9965.EPI-19-0291
42. Yee LD, Mortimer JE, Natarajan R, Dietze EC, Seewaldt VL. Metabolic health, insulin, and breast cancer: why oncologists should care about insulin. *Front Endocinol.* (2020) 20:58. doi: 10.3389/fendo.2020.00058
43. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, et al. Clinical pharmacokinetics of metformin. *Clin Pharmacokinet.* (2011) 50:81–98. doi: 10.2165/11534750-000000000-00000
44. Pawlyk AC, Giacomini KM, McKeon C, Shuldiner AR, Florez JC. Metformin pharmacogenomics: current status and future directions. *Diabetes.* (2014) 63:2590–9. doi: 10.2337/db13-1367
45. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenet Genomics.* (2012) 22:820–7. doi: 10.1097/FPC.0b013e3283559b22
46. Sundelin E, Gormsen LC, Jensen JB, Vendelbo MH, Jakobsen S, Munk OL, et al. Genetic polymorphisms in organic cation transporter 1 attenuates hepatic metformin exposure in humans. *Clin Pharmacol Ther.* (2017) 102:841–8. doi: 10.1002/cpt.701
47. Jablonski KA, McAteer JB, de Bakker PI, Franks PW, Pollin TI, Hanson RL, et al. Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle intervention in the diabetes prevention program. *Diabetes.* (2010) 59:2672–81. doi: 10.2337/db10-0543
48. Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, Castro RA, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest.* (2007) 117:1422–31. doi: 10.1172/JCI30558
49. Shu Y, Brown C, Castro RA, Shi RJ, Lin ET, Owen RP, et al. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. *Clin Pharmacol Ther.* (2008) 83:273–80. doi: 10.1038/sj.clpt.6100275
50. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus. *Pharmacogenomics J.* (2009) 9:242–7. doi: 10.1038/tpj.2009.15
51. Christensen MM, Brasch-Andersen C, Green H, Nielsen F, Damkier P, Beck-Nielsen H, et al. The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c. *Pharmacogenet Genomics.* (2011) 21:837–50. doi: 10.1097/FPC.0b013e32834c0010
52. Gambineri A, Tomassoni F, Gasparini DI, Di Rocco A, Mantovani V, Pagotto U, et al. Organic cation transporter 1 polymorphisms predict the metabolic response to metformin in women with the polycystic ovary syndrome. *J Clin Endocrinol Metab.* (2010) 95:E204–8. doi: 10.1210/jc.2010-0145
53. Shikata E, Yamamoto R, Takane H, Shigemasa C, Ikeda T, Otsubo K, et al. Human organic cation transporter (OCT1 and OCT2) gene polymorphisms and therapeutic effects of metformin. *J Hum Genet.* (2007) 52:117–22. doi: 10.1007/s10038-006-0087-0
54. Tzvetkov MV, Vormfelde SV, Balen D, Meineke I, Schmidt T, Sehrt D, et al. The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. *Clin Pharmacol Ther.* (2009) 86:299–306. doi: 10.1038/clpt.2009.92
55. Stocker SL, Morrissey KM, Yee SW, Castro RA, Xu L, Dahlin A, et al. The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin. *Clin Pharmacol Ther.* (2013) 93:186–94. doi: 10.1038/clpt.2012.210
56. Choi JH, Yee SW, Ramirez AH, Morrissey KM, Jang GH, Joski PJ, et al. A common 5′-UTR variant in MATE2-K is associated with poor response to metformin. *Clin Pharmacol Ther.* (2011) 90:674–84. doi: 10.1038/clpt.2011.165
57. Dong M, Gong ZC, Dai XP, Lei GH, Lu HB, Fan L, et al. Serine racemase rs391300 G/A polymorphism influences the therapeutic efficacy of metformin in Chinese patients with diabetes mellitus type 2. *Clin Exp Pharmacol Physiol.* (2011) 38:824–9. doi: 10.1111/j.1440-1681.2011.05610.x
58. van Leeuwen N, Nijpels G, Becker ML, Deshmukh H, Zhou K, Stricker BH, et al. A gene variant near ATM is significantly associated with metformin treatment response in type 2 diabetes: a replication and meta-analysis of five cohorts. *Diabetologia.* (2012) 55:1971–7. doi: 10.1007/s00125-012-2537-x
59. GoDarts, Group UDPS, Wellcome Trust Case Control C, Zhou K, Bellenguez C, Spencer CC, et al. Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. *Nat Genet.* (2011) 43:117–20. doi: 10.1038/ng.735
60. Tkac I. Replication of the association of gene variant near ATM and response to metformin. *Pharmacogenomics.* (2012) 13:1331–2. doi: 10.2217/pgs.12.115
61. Legro RS. Impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women: do we need a new drug? *J Clin Endocrinol Metab.* (2008) 93:4218–20. doi: 10.1210/jc.2008-1994
62. Mannino GC, Andreozzi F, Sesti G. Pharmacogenetics of type 2 diabetes mellitus, the route toward tailored medicine. *Diabetes Metab Res Rev.* (2019) 35:e3109. doi: 10.1002/dmrr.3109
63. Chowdhury S, Yung E, Pintilie M, Muaddi H, Chaib S, Yeung M, et al. MATE2 expression is associated with cancer cell response to metformin. *PLoS ONE.* (2016) 11:e0165214. doi: 10.1371/journal.pone.0165214
64. Heckman-Stoddard BM, Gandini S, Puntoni M, Dunn BK, DeCensi A, Szabo E. Repurposing old drugs to chemoprevention: the case of metformin. *Semin Oncol.* (2016) 43:123–33. doi: 10.1053/j.seminoncol.2015.09.009
65. Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature.* (2014) 510:542–6. doi: 10.1038/nature13270
66. Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA, et al. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science.* (2005) 310:1642–6. doi: 10.1126/science.1120781
67. Cool B, Zinker B, Chiou W, Kifle L, Cao N, Perham M, et al. Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. *Cell Metab.* (2006) 3:403–16. doi: 10.1016/j.cmet.2006.05.005
68. Savage DB, Choi CS, Samuel VT, Liu ZX, Zhang D, Wang A, et al. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense

oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *J Clin Invest.* (2006) 116:817–24. doi: 10.1172/JCI27300
69. Fullerton MD, Galic S, Marcinko K, Sikkema S, Pulinilkunnil T, Chen ZP, et al. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nat Med.* (2013) 19:1649–54. doi: 10.1038/nm.3372
70. Hardie DG. Neither LKB1 nor AMPK are the direct targets of metformin. *Gastroenterology.* (2006) 131:973. doi: 10.1053/j.gastro.2006.07.032
71. Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: from mechanisms of action to therapies. *Cell Metab.* (2014) 20:953–66. doi: 10.1016/j.cmet.2014.09.018
72. Mishra AK, Dingli D. Metformin inhibits IL-6 signaling by decreasing IL-6R expression on multiple myeloma cells. *Leukemia.* (2019) 33:2695–709. doi: 10.1038/s41375-019-0470-4
73. Sekino N, Kano M, Matsumoto Y, Sakata H, Akutsu Y, Hanari N, et al. Antitumor effects of metformin are a result of inhibiting nuclear factor kappa B nuclear translocation in esophageal squamous cell carcinoma. *Cancer Sci.* (2018) 109:1066–74. doi: 10.1111/cas.13523
74. Hirsch HA, Iliopoulos D, Tsichlis PN, Struhl K. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res.* (2009) 69:7507–11. doi: 10.1158/0008-5472.CAN-09-2994
75. Kurelac I, Umesh Ganesh N, Iorio M, Porcelli AM, Gasparre G. The multifaceted effects of metformin on tumor microenvironment. *Semin Cell Dev Biol.* (2020) 98:90–7. doi: 10.1016/j.semcdb.2019.05.010
76. Ye J, Chen K, Qi L, Li R, Tang H, Zhou C, et al. Metformin suppresses hypoxiainduced migration via the HIF1alpha/VEGF pathway in gallbladder cancer *in vitro* and *in vivo*. *Oncol Rep.* (2018) 40:3501–10. doi: 10.3892/or.2018.6751
77. Wang J, Li G, Wang Y, Tang S, Sun X, Feng X, et al. Suppression of tumor angiogenesis by metformin treatment via a mechanism linked to targeting of HER2/HIF-1alpha/VEGF secretion axis. *Oncotarget.* (2015) 6:44579–92. doi: 10.18632/oncotarget.6373
78. Liu Q, Tong D, Liu G, Gao J, Wang LA, Xu J, et al. Metformin inhibits prostate cancer progression by targeting tumor-associated inflammatory infiltration. *Clin Cancer Res.* (2018) 24:5622–34. doi: 10.1158/1078-0432.CCR-18-0420
79. Incio J, Tam J, Rahbari NN, Suboj P, McManus DT, Chin SM, et al. PlGF/VEGFR-1 signaling promotes macrophage polarization and accelerated tumor progression in obesity. *Clin Cancer Res.* (2016) 22:2993–3004. doi: 10.1158/1078-0432.CCR-15-1839
80. Scharping NE, Menk AV, Whetstone RD, Zeng X, Delgoffe GM. Efficacy of PD-1 blockade is potentiated by metformin-induced reduction of tumor hypoxia. *Cancer Immunol Res.* (2017) 5:9–16. doi: 10.1158/2326-6066.CIR-16-0103
81. Li L, Wang L, Li J, Fan Z, Yang L, Zhang Z, et al. Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian cancer. *Cancer Res.* (2018) 78:1779–91. doi: 10.1158/0008-5472.CAN-17-2460
82. Kim SH, Li M, Trousil S, Zhang Y, Pasca di Magliano M, Swanson KD, et al. phenformin inhibits myeloid-derived suppressor cells and enhances the anti-tumor activity of PD-1 blockade in melanoma. *J Invest Dermatol.* (2017) 137:1740–8. doi: 10.1016/j.jid.2017.03.033
83. Pernicova I, Korbonits M. Metformin–mode of action and clinical implications for diabetes and cancer. *Nat Rev Endocrinol.* (2014) 10:143–56. doi: 10.1038/nrendo.2013.256
84. Yang SX, Polley E, Lipkowitz S. New insights on PI3K/AKT pathway alterations and clinical outcomes in breast cancer. *Cancer Treat Rev.* (2016) 45:87–96. doi: 10.1016/j.ctrv.2016.03.004
85. Sobhani N, Roviello G, Corona SP, Scaltriti M, Ianza A, Bortul M, et al. The prognostic value of PI3K mutational status in breast cancer: a meta-analysis. *J Cell Biochem.* (2018) 119:4287–92. doi: 10.1002/jcb.26687
86. Xue G, Hemmings BA. PKB/Akt-dependent regulation of cell motility. *J Natl Cancer Inst.* (2013) 105:393–404. doi: 10.1093/jnci/djs648
87. Hoxhaj G, Manning BD. The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. *Nat Rev Cancer.* (2020) 20:74–88. doi: 10.1038/s41568-019-0216-7
88. Robey RB, Hay N. Is Akt the "Warburg kinase"?-Akt-energy metabolism interactions and oncogenesis. *Semin Cancer Biol.* (2009) 19:25–31. doi: 10.1016/j.semcancer.2008.11.010
89. Wu Z, Wu J, Zhao Q, Fu S, Jin J. Emerging roles of aerobic glycolysis in breast cancer. *Clin Transl Oncol.* (2020) 22:631–46. doi: 10.1007/s12094-019-02187-8
90. Lien EC, Lyssiotis CA, Cantley LC. Metabolic reprogramming by the PI3K-Akt-mTOR pathway in Cancer. *Recent Results Cancer Res.* (2016) 207:39–72. doi: 10.1007/978-3-319-42118-6_3
91. Fabian CJ, Kimler BF. Chemoprevention for high-risk women: tamoxifen and beyond. *Breast J.* (2001) 7:311–20. doi: 10.1046/j.1524-4741.2001.21570.x
92. Kumar NB, Vadaparampil ST, Mahajan N, Lilienfeld HS, Lee JH, Laronga C, et al. Metformin- a promising agent for chemoprevention in BRCA1 carriers. *Hereditary Genet.* (2012) 1:104. doi: 10.4172/2161-1041.1000104
93. Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. *Endocrinology.* (2003) 144:5179–83. doi: 10.1210/en.2003-0982
94. Dasgupta B, Chhipa RR. Evolving lessons on the complex role of AMPK in normal physiology and cancer. *Trends Pharmacol Sci.* (2016) 37:192–206. doi: 10.1016/j.tips.2015.11.007
95. Cuyas E, Fernandez-Arroyo S, Alarcon T, Lupu R, Joven J, Menendez JA. Germline BRCA1 mutation reprograms breast epithelial cell metabolism towards mitochondrial-dependent biosynthesis: evidence for metformin-based "starvation" strategies in BRCA1 carriers. *Oncotarget.* (2016) 7:52974–92. doi: 10.18632/oncotarget.9732
96. Hadad S, Iwamoto T, Jordan L, Purdie C, Bray S, Baker L, et al. Evidence for biological effects of metformin in operable breast cancer: a pre-operative, window-of-opportunity, randomized trial. *Breast Cancer Res Treat.* (2011) 128:783–94. doi: 10.1007/s10549-011-1612-1
97. Bonanni B, Puntoni M, Cazzaniga M, Pruneri G, Serrano D, Guerrieri-Gonzaga A, et al. Dual effect of metformin on breast cancer proliferation in a randomized presurgical trial. *J Clin Oncol.* (2012) 30:2593–600. doi: 10.1200/JCO.2011.39.3769
98. Kalinsky K, Crew KD, Refice S, Xiao T, Wang A, Feldman SM, et al. Presurgical trial of metformin in overweight and obese patients with newly diagnosed breast cancer. *Cancer Invest.* (2014) 32:150–7. doi: 10.3109/07357907.2014.889706
99. Kalinsky K, Hershman DL. Cracking open window of opportunity trials. *J Clin Oncol.* (2012) 30:2573–5. doi: 10.1200/JCO.2012.42.3293
100. Niraula S, Dowling RJ, Ennis M, Chang MC, Done SJ, Hood N, et al. Metformin in early breast cancer: a prospective window of opportunity neoadjuvant study. *Breast Cancer Res Treat.* (2012) 135:821–30. doi: 10.1007/s10549-012-2223-1
101. Esteva FJ, Moulder SL, Gonzalez-Angulo AM, Ensor J, Murray JL, Green MC, et al. Phase I trial of exemestane in combination with metformin and rosiglitazone in nondiabetic obese postmenopausal women with hormone receptor-positive metastatic breast cancer. *Cancer Chemother Pharmacol.* (2013) 71:63–72. doi: 10.1007/s00280-012-1977-9
102. Goodwin PJ, Parulekar WR, Gelmon KA, Shepherd LE, Ligibel JA, Hershman DL, et al. Effect of metformin vs placebo on and metabolic factors in NCIC CTG MA.32. *J Natl Cancer Inst.* (2015) 107:djv006. doi: 10.1093/jnci/djv006
103. Pimentel I, Lohmann AE, Ennis M, Dowling RJO, Cescon D, Elser C, et al. A phase II randomized clinical trial of the effect of metformin versus placebo on progression-free survival in women with metastatic breast cancer receiving standard chemotherapy. *Breast.* (2019) 48:17–23. doi: 10.1016/j.breast.2019.08.003
104. Sehdev A, Zha Y, Karrison TG, Janisch LA, Cohen EEW, Maitland ML, et al. A pharmacodynamic study of sirolimus and metformin in patients with advanced solid tumors. *J Clin Oncol.* (2017) 35(Suppl. 15):TPS11628. doi: 10.1200/JCO.2017.35.15_suppl.TPS11628
105. Campagnoli C, Pasanisi P, Abba C, Ambroggio S, Biglia N, Brucato T, et al. Effect of different doses of metformin on serum testosterone and insulin in non-diabetic women with breast cancer: a randomized study. *Clin Breast Cancer.* (2012) 12:175–82. doi: 10.1016/j.clbc.2012.03.004
106. Macis D, Gandini S, Guerrieri-Gonzaga A, Johansson H, Magni P, Ruscica M, et al. Prognostic effect of circulating adiponectin in a randomized 2 x

2 trial of low-dose tamoxifen and fenretinide in premenopausal women at risk for breast cancer. *J Clin Oncol.* (2012) 30:151–7. doi: 10.1200/JCO.2011.35.2237

107. Cazzaniga M, DeCensi A, Pruneri G, Puntoni M, Bottiglieri L, Varricchio C, et al. The effect of metformin on apoptosis in a breast cancer presurgical trial. *Br J Cancer.* (2013) 109:2792–7. doi: 10.1038/bjc.2013.657
108. Patterson RE, Marinac CR, Sears DD, Kerr J, Hartman SJ, Cadmus-Bertram L, et al. The effects of metformin and weight loss on biomarkers associated with breast cancer outcomes. *J Natl Cancer Inst.* (2018) 110:1239–47. doi: 10.1093/jnci/djy040
109. Martinez JA, Chalasani P, Thomson CA, Roe D, Altbach M, Galons JP, et al. Phase II study of metformin for reduction of obesity-associated breast cancer risk: a randomized controlled trial protocol. *BMC Cancer.* (2016) 16:500. doi: 10.1186/s12885-016-2551-3

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.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership