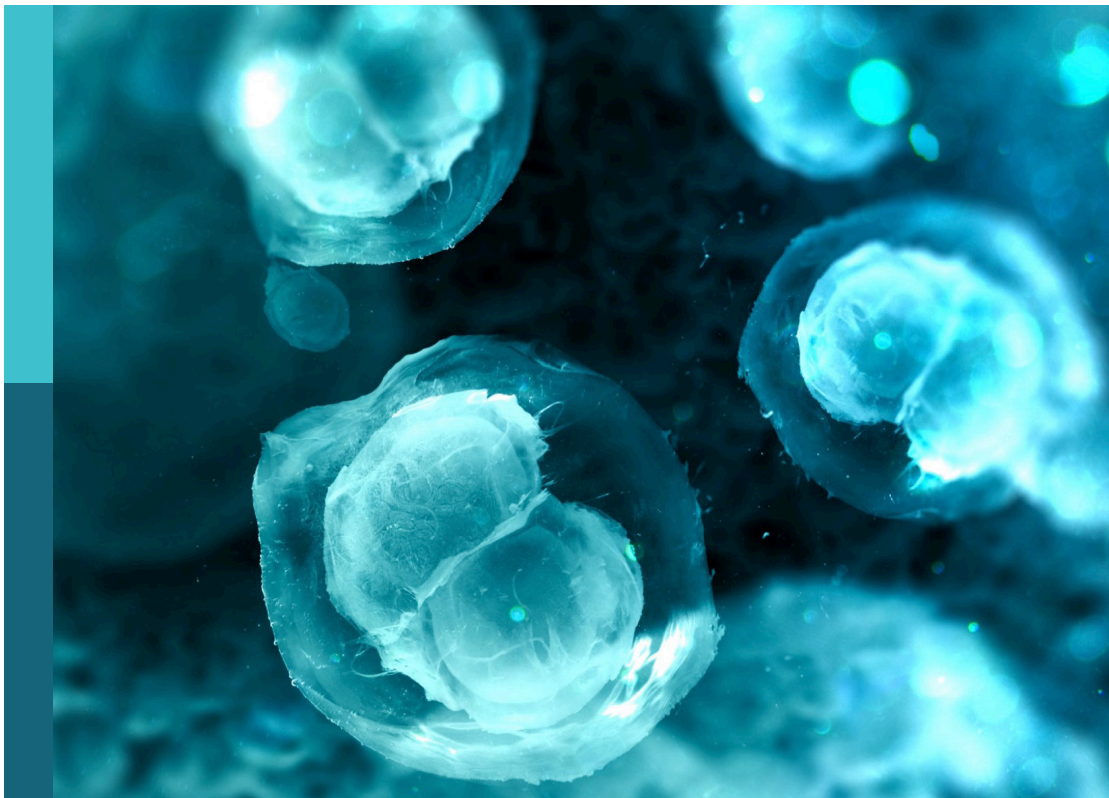
frontiers | Research Topics

Cancer pathogenesis: molecular and cellular mechanisms of tumor evolution, therapy-resistance and immune evasion

Edited by
Sambhavi Animesh, Ajinkya Revandkar and Lucio Miele

Published in
Frontiers in Cell and Developmental Biology



ISSN 1664-8714
ISBN 978-2-8325-5933-8
DOI 10.3389/978-2-8325-5933-8

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

Cancer pathogenesis: molecular and cellular mechanisms of tumor evolution, therapy-resistance and immune evasion

Topic editors

Sambhavi Animesh — Massachusetts General Hospital, Harvard Medical School, United States

Ajinkya Revandkar — Massachusetts General Hospital Cancer Center, United States

Lucio Miele — Louisiana State University, United States

Citation

Animesh, S., Revandkar, A., Miele, L., eds. (2025). *Cancer pathogenesis: molecular and cellular mechanisms of tumor evolution, therapy-resistance and immune evasion*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5933-8

Table of contents

frontiers | Frontiers in Cell and Developmental Biology

TYPE Review
PUBLISHED 27 March 2024
DOI 10.3389/fcell.2024.1353860

OPEN ACCESS

EDITED BY
Ajinkya Revandkar,
Massachusetts General Hospital Cancer Center, United States

REVIEWED BY
Pasqualino De Antonellis,
University of Toronto, Canada
Veronica Ferrucci,
University of Naples Federico II, Italy

*CORRESPONDENCE
Tapan Behl,
tapanbehl31@gmail.com
Bairong Shen,
bairong.shen@scu.edu.cn
Rajeev K. Singla,
rajeevsingla26@gmail.com

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

RECEIVED 11 December 2023
ACCEPTED 13 March 2024
PUBLISHED 27 March 2024

CITATION
Zheng M, Kumar A, Sharma V, Behl T, Sehgal A, Wal P, Shinde NV, Kawaduji BS, Kapoor A, Anwer MK, Gulati M, Shen B, Singla RK and Bungau SG (2024), Revolutionizing pediatric neuroblastoma treatment: unraveling new molecular targets for precision interventions. *Front. Cell Dev. Biol.* 12:1353860. doi: 10.3389/fcell.2024.1353860

Revolutionizing pediatric neuroblastoma treatment: unraveling new molecular targets for precision interventions

Min Zheng[1†], Ankush Kumar[2†], Vishakha Sharma[2], Tapan Behl[3*], Aayush Sehgal[4], Pranay Wal[5], Nirmala Vikram Shinde[6], Bhosale Sachin Kawaduji[6], Anupriya Kapoor[7], Md. Khalid Anwer[8], Monica Gulati[9,10], Bairong Shen[1*], Rajeev K. Singla[1,9*] and Simona Gabriela Bungau[11,12]

[1]Joint Laboratory of Artificial Intelligence for Critical Care Medicine, Department of Critical Care Medicine and Institutes for Systems Genetics, Frontiers Science Center for Disease-related Molecular Network, West China Hospital, Sichuan University, Chengdu, China, [2] Amity School of Pharmaceutical Sciences, Amity University, Mohali, Punjab, India, [3]Amity School of Pharmaceutical Sciences, Amity University, Mohali, Punjab, India, [4]GHG Khalsa College of Pharmacy, Ludhiana, Punjab, India, [5]Pranveer Singh Institute of Technology, Pharmacy, Kanpur, Uttar Pradesh, India, [6]SMBT College of Pharmacy, Ghoti Kh, Maharashtra, India, [7]School of Pharmaceutical Sciences, Chhatrapati Shahu Ji Maharaj University, Kanpur, Uttar Pradesh, India, [8]Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Alkharj, Saudi Arabia, [9]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, [10]Australian Research Consortium in Complementary and Integrative Medicine, Faculty of Health, University of Technology Sydney, Ultimo, NSW, Australia, [11]Department of Pharmacy, Faculty of Medicine and Pharmacy, University of Oradea, Oradea, Romania, [12]Doctoral School of Biomedical Sciences, University of Oradea, Oradea, Romania

Neuroblastoma (NB) is the most frequent solid tumor in pediatric cases, contributing to around 15% of childhood cancer-related deaths. The wide-ranging genetic, morphological, and clinical diversity within NB complicates the success of current treatment methods. Acquiring an in-depth understanding of genetic alterations implicated in the development of NB is essential for creating safer and more efficient therapies for this severe condition. Several molecular signatures are being studied as potential targets for developing new treatments for NB patients. In this article, we have examined the molecular factors and genetic irregularities, including those within insulin gene enhancer binding protein 1 (ISL1), dihydropyrimidinase-like 3 (DPYSL3), receptor tyrosine kinase-like orphan receptor 1 (ROR1) and murine double minute 2-tumor protein 53 (MDM2-P53) that play an essential role in the development of NB. A thorough summary of the molecular targeted treatments currently being studied in pre-clinical and clinical trials has been described. Recent studies of immunotherapeutic agents used in NB are also studied in this article.

Abbreviations: AKT, Ak strain transforming; CAR T, Chimeric antigen receptor T; CRD, Cysteine-rich domain; CRMP, Collapsin response mediator protein; DPYSL3, Dihydropyrimidinase-related protein 3; DNA, Deoxyribonucleic Acid; EMT, Epithelial-mesenchymal transitions; FDA, Food and Drug Administration; FZD, Frizzled receptors; HRNBL, High-risk neuroblastoma; ISL1, ISL LIM Homeobox 1; KRD, Kringle domain; MAPK, Mitogen-activated protein kinase; MDM2, Murine double minute 2; MIF, Migration inhibitory factor; MYCN, myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog; NB, Neuroblastoma; NCC, Neural crest cells; PI3K, phosphoinositide 3-kinase; ROR1, Receptor tyrosine kinase-like orphan receptor 1; RTK, Receptor Tyrosine Kinase; mAbs, Monoclonal antibodies, PD-L1, Programmed Cell Death Ligand; ORR, Objective response rate; TP53, Tumor protein p53.

Moreover, we explore potential future directions to discover new targets and treatments to enhance existing therapies and ultimately improve treatment outcomes and survival rates for NB patients.

KEYWORDS

neuroblastoma, molecular targets, immunotherapy, precision interventions, preclinical studies

1 Introduction

NB is a cancer that predominantly impacts young children and emerges in nerve tissues. It frequently begins in the adrenal glands above the kidneys but can also develop in nerve tissue along the spine, chest, abdomen, or pelvis (Neuroblastoma, 1931; Qiu and Matthay, 2022; Jacobson et al., 2023). It is one of the most common cancers in infants and is typically found in children under 5 years old (Pudela et al., 2020; Davidoff, 2021; Jha et al., 2023). In the United States, around 700 to 800 children are diagnosed with NB each year, constituting about 6% of childhood cancers. The relative survival rate over 5 years for children under 15 diagnosed with NB is about 82% (Horton, 2022; Mehrvar et al., 2023). NB is classified as embryonic due to its connection to neural crest cells (NCCs) during fetal development (Ben Amar et al., 2022). NCCs are unique cells vital in early embryogenesis, migrating extensively and contributing to various tissues, including the peripheral nervous system, adrenal glands, heart, and face (Pilon, 2021). They essentially serve as building blocks for these critical anatomical features. Substantial advancements have occurred in our understanding of the molecular mechanisms underlying the development and progression of NB (Gomez et al., 2022; Ponzoni et al., 2022). These research endeavors have identified new focal points for potential treatments (Steen et al., 2023). Genome-wide studies, including genome sequencing, have unveiled fundamental genetic changes driving NB growth (Bell, 2010; Kholodenko et al., 2018a; Tonini and Capasso, 2020). In this article, we have summarized novel targets for NB, such as ISL1 (Zhang et al., 2018; Zhang et al., 2019; Li et al., 2021), DPYSL3 (Cheung et al., 2008; Chicco et al., 2023), and ROR1 (Janovská and Bryja, 2017; Quezada and Lopez-Bergami, 2023), along with immunotherapy which is an emerging and promising treatment approach for this disease. Several identified targets are currently being tested as potential treatments for NB patients. This review offers the latest molecular insights regarding the development and progression of NB with a specific focus on genetic alterations/molecular pathways along with clinical management.

Additionally, we aim to offer perspectives on the potential advantages of combination therapy, which involves using inhibitors targeting multiple pathways. One of the prominent molecular features of the NB is MYCN amplification, which is linked to aggressive tumor growth and poor prognosis, along with an increase of chromosome 17q. This oncogene is amplified in 18%–38% of total genes (Aygun and Altungoz, 2019).

Furthermore, it is expected to detect deletion involving chromosomes 1p and 11q and instances of hyperploidy in NB cases (Attiyeh et al., 2005; Bartolucci et al., 2022). Amplification of the MYCN oncogene leads to overexpression of the MYCN protein, which promotes cell growth and proliferation. Anaplastic lymphoma kinase is another oncogene. TrkA (NTRK1) is another factor linked to a favorable prognosis. It is activated by attacking the NGF ligand and promotes cell differentiation, which causes spontaneous regression of NB (Brodeur et al., 2009).

The ongoing incorporation of cutting-edge treatments for individuals with NB into clinical trials and established clinical practices has led to gradual enhancements in patient survival rates (Filippi et al., 2020). Nevertheless, survivors of high-risk neuroblastoma (HRNB) still encounter long-term side effects stemming from their treatment (Veal et al., 2021). Furthermore, there is currently no curative treatment available for most of the approximately 50% of patients who face a relapse after being diagnosed with HRNB. As diagnostic and molecular profiling technologies progress rapidly, researchers are also witnessing the identification of potential targets for treatment (Advani et al., 2022). Molecular targeted therapy for genomic abnormalities and disrupted pathways offers a promising and innovative approach to NB treatment (Jones et al., 2019; Voicu et al., 2023). This approach holds the potential to enhance treatment effectiveness while minimizing adverse effects. In this context, we have explored the clinical scenario regarding NB therapies within the precision medicine framework. Substantial progress has been achieved in understanding the molecular causes of NB, revealing potential therapeutic targets. Genomic studies have identified genetic changes and disrupted pathways in NB growth. These findings may lead to more effective, less toxic treatments.

Authors also delve into their examination in ongoing clinical trials. Furthermore, future directions have been added for enhancing or creating more effective targeted therapies to improve the survival rates of NB patients while minimizing treatment-related side effects.

2 Molecular landscape of pediatric neuroblastoma

NB represents the predominant solid tumors outside the cranial cavity during childhood, particularly prevalent within the first year of life (Park et al., 2010; Irwin and Park, 2015). Its distinctive nature is that infants frequently manifest either localized or metastatic forms of the disease, which may undergo spontaneous regression intervention (Tolbert and Matthay, 2018). In contrast, older children can experience disease progression leading to morbidity or mortality despite prolonged and intensive therapeutic interventions (Ahmed et al., 2017). The "International Neuroblastoma Risk Group Staging System (INRGSS)" is a classification system employed in the medical field to stage and categorize NB (Monclair et al., 2009; Sokol and Desai, 2019; Irwin et al., 2021). NB exhibits significant variability, making it crucial to have a systematic approach to determine the disease's extent and guide treatment choices. There are various known molecular aspects

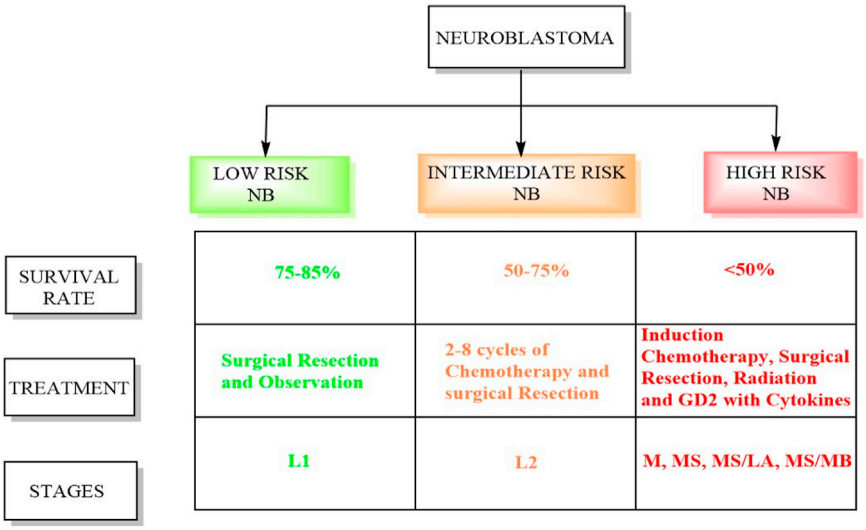


FIGURE 1
Difference between high-risk and low-risk NB.

in NB, such as MYCN amplification, ALK mutations, chromosomal abnormalities (Carén et al., 2008), changes in the pattern of DNA methylation, and tumor microenvironment (Tonini and Capasso, 2020; Raieli et al., 2021). Interactions of the tumor microenvironment with stromal cells and blood vessels also play a crucial role in NB progression. Neutrophins are other growth factors involved in developing and maintaining the nervous system, and their dysregulation further promotes tumor growth and survival.

Over 50 years ago, Conrad Waddington introduced the foundational principles of the 'epigenetic landscape' to elucidate the fundamental mechanisms governing normal cell differentiation (Baedke, 2013). These studies revealed that transcription factors play pivotal roles in shaping and directing cellular identity and navigating developmental pathways on Waddington's Hill. Interactions with diverse DNA regulatory elements within a specific epigenetic framework, coupled with chromatin, contribute to the stability of cellular lineages and fates, akin to the depth of grooves on the developmental hill. In a recent analysis, Flavahan and colleagues proposed the notion of aberrant epigenetic resistance and plasticity, attributing these phenomena to dysregulated chromatin regulator activity, remodeling, and DNA methylation as crucial contributors to tumorigenesis (Flavahan et al., 2017; Durinck and Speleman, 2018). Management approaches for asymptomatic individuals classified as low risk, with a projected survival rate exceeding 98%, typically entail either observation or surgical excision alone.

Conversely, intermediate-risk patients, characterized by a survival rate exceeding 90%, necessitate moderate doses of chemotherapy tailored to their response alongside surgical resection (Pinto et al., 2015). Patients classified as high risk undergo successive cycles of combination chemotherapy preceding surgical intervention, followed by consolidation therapy involving myeloablative autologous hematopoietic stem cell transplantation and localized radiation therapy (Tolbert and Matthay, 2018). Subsequently, the patients receive immunotherapy and differentiation therapy during the maintenance phase. The INRGSS incorporates tumor characteristics, metastasis, patient age, and specific biological markers (Newman et al., 2019; Campbell et al., 2023). Figure 1 displays the schematic summary between high-risk and low-risk neuroblastoma. Below is a detailed overview of the stages and risk groups in the INRGSS.

- Stage L1: This stage denotes NB confined to its origin point and has not spread to distant sites (Luksch et al., 2016). Tumors in this stage usually exhibit less aggressive biological features, indicating a more favorable prognosis. Patients in stage L1 are considered low-risk and typically have better treatment outcomes (Newman et al., 2019).
- Like stage L1, stage L2 NB is localized but characterized by unfavorable biological traits, suggesting a more aggressive disease. Patients in this stage are classified as intermediate risk, and their treatment may involve more intensive therapies than those in stage L1.
- Stage M indicates that NB has spread or metastasized to distant areas in the body, such as bones, bone marrow, lymph nodes, or other organs. The presence of metastases elevates the risk associated with the disease. Patients in stage M are classified as high-risk and typically require aggressive treatment strategies, including chemotherapy.
- Stage MS represents a subgroup of stage M and includes patients with metastatic NB who exhibit unique clinical features impacting treatment decisions. These circumstances might include patient age, tumor biology, or other relevant factors. Despite these unique features, patients in stage MS remain classified as high risk. The MS/LA subgroup comprises patients with metastatic NB who have a restricted number of metastatic sites. The presence of limited metastases can influence the treatment approach. In stage MS/MB, myc avian myelocytomatosis viral oncogene neuroblastoma (MYCN) amplification is a genetic alteration that increases the tumor's aggressiveness. This subgroup encompasses patients with metastatic NB who exhibit MYCN amplification.

2.1 Comparison between different molecular features in high-risk neuroblastoma (HRNB)

MYCN amplification, ALK mutations, chromosomal abnormalities, changes in the pattern of DNA methylation, and tumor microenvironment are the primary molecular features in HRNBs. MYCN is found in approximately 25% of all NB people with a poor prognosis. It regulates cell proliferation, and its over-expression disrupts cell cycles by inhibiting apoptosis (Rickman et al., 2018). MYCN positively influences the expression of essential cellular regulators, such as E2F and ID2 inhibitors, which regulate cell cycle progression (Woo et al., 2008). In a cohort study by Pugh and colleagues, the authors study the somatic mutations in HRNB on 240 affected people. 9.2% of the total cases comprised of ALK mutation, PTPN11 is expressed in 2.9%, ATRX (2.5%), and MYCN was observed to be 1.7%. Significantly elevated levels of germline variants were observed within the genes ALK, CHEK2, PINK1, and BARD1 (Pugh et al., 2013). Another study by Molenaar et al. showed that chromothripsis and neuritogenesis were two major gene alterations continuously occurring in HRNB. They found 7% cases of ALK mutations, 3% of TIAM1, and 18% of chromothripsis (Molenaar et al., 2012).

3 Therapies tailored to specific genetic and molecular alterations in neuroblastoma

3.1 Current targeted therapies and novel potential targets in NB

3.1.1 Targeting ISL1

ISL1, a transcription factor containing a LIM homeodomain first discovered as a protein that interacts with an enhancer region of the insulin gene, influences its expression (Karlsson et al., 1990). Recent research has highlighted the significant role of ISL1 in cancer progression, primarily attributed to its irregular expression (Agaimy et al., 2013). ISL1 is also associated with triple-negative breast cancer (Zhang et al., 2018), melanoma (Zhang et al., 2018), and gastric cancer (Guo et al., 2019) and has also been found to be a regulator of CD1 and c-Myc genes. ISL1 plays a role in controlling separate temporal gene expression patterns essential for the proliferation and differentiation of sympathetic neurons, either directly or indirectly. ALK, LMO1, and PROX1 are some of the genes modulated by the ISL1 gene, which activates the oncogenic pathways of NB (Cheung et al., 2008; Zhang et al., 2018). ISL1 and GATA3 synergistically activate the signaling pathways by tumor growth and differentiation, which can be a therapeutic target in NB (Zhang et al., 2019). It has prognostic value in gastric and bladder cancers and can serve as a biomarker in NB (Kitchen et al., 2015). ISL1 gene is aurora kinase A (AURKA), a widely distributed protein kinase with crucial functions in cell division (Fulcher and Sapkota, 2020). It is acknowledged to be a potent oncogene and a possible target for cancer (Fadaka et al., 2020; Du et al., 2021). A study also revealed that the ISL1 transcription factor enhances cell proliferation through phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway by upregulation of the AURKA enzyme, which is responsible for the survival of NB cells (Figure 2). This study revealed that ISL1 might offer a promising avenue for future therapeutic intervention. They found that PI3K inhibitor LY294002 induces apoptosis in cells. There was a dose-dependent increase in the epithelial marker E-cadherin, along with a corresponding decrease in the levels of mesenchymal markers in Western blot analysis. Notably, metastatic NB cells, SK-N-BE with MYCN amplification and SK-N-SH without MYCN amplification, already express ISL1.

Interestingly, when authors boost ISL1 expression using an overexpression vector, it amplifies the cells' ability to proliferate and migrate. These findings confirm that ISL1 functions as an oncogene in NB. Finally, they assert that ISL1 triggers epithelial-to-mesenchymal transition (EMT) in NB through PI3K/AKT pathway activation (Li et al., 2021).

3.1.2 DPYSL3 as a potential target

DPYSL3, alternatively labeled as collapsing response mediator protein 4 (CRMP4), represents a human gene responsible for producing a protein termed dihydropyrimidinase-like 3 (Ponnusamy et al., 2014). It is a member of the CRMP family and is involved in various cellular functions, encompassing aspects like neuronal development, guiding axons, and governing microtubule dynamics (Kanda et al., 2014; Ponnusamy et al., 2014). Specifically, DPYSL3 is predominantly found in the nervous system, promoting neurites, guiding axons, and shaping the structural framework of the nervous system (Kawahara et al., 2013; Manivannan et al., 2013). It engages in interactions with various proteins and molecules necessary for these processes. It helps enhance neurite growth, guides axons, and shapes the nervous system's structural framework (Desprez et al., 2023).

DPYSL3 gene is closely found in the cytosol of NB cells, where it co-localizes with f-actin. The co-localization from a rib-like structure inside the lamellipodia (Figure 3) (Chicco et al., 2023). The primary relation between this gene and F-actin states is an increased level of DPYSL3, which helps migrate B35 NB cells. Conversely, reducing DPYSL3 enhanced cell migration and disrupted lamellipodia (Rosslenbroich et al., 2005; Adam et al., 2020). Studies employing genetic methods have revealed an intriguing relationship between DPYSL3 levels and MYCN expression. It indicates that MYCN inhibits DPYSL3 in NB cells, possibly through an enhancer of zeste homolog 2 (EZH2). GSK3b may also play a role in mediating this negative regulation (Alabed et al., 2010). Furthermore, it is established that AKT phosphorylates and subsequently deactivates GSK-3b in NB cells. Inactivation of GSK-3b is linked to elevated MYCN protein expression (Chicco et al., 2023). Thus, an increase in MYCN level results in the suppression of DPYSL3. Tan and colleagues conducted a study investigating the correlation between DPYSL3 and MYCN in retinoic acid induced cell proliferation and differentiation, further increasing mRNA expression. They found that 72 kDA isoform was unchanged and 62 kDA isoform level increased (Tan et al., 2013). A previous study demonstrated that the 65 kDA form is less phosphorylated and undetected. This change depends on phosphatase regulated by retinoids (Gaetano et al., 1997). DPYSL3 is upregulated by GSK-3B, and their association has already been reported (Alabed et al., 2010; Ong Tone et al., 2010). Consequently, elevated levels of MYCN, either through

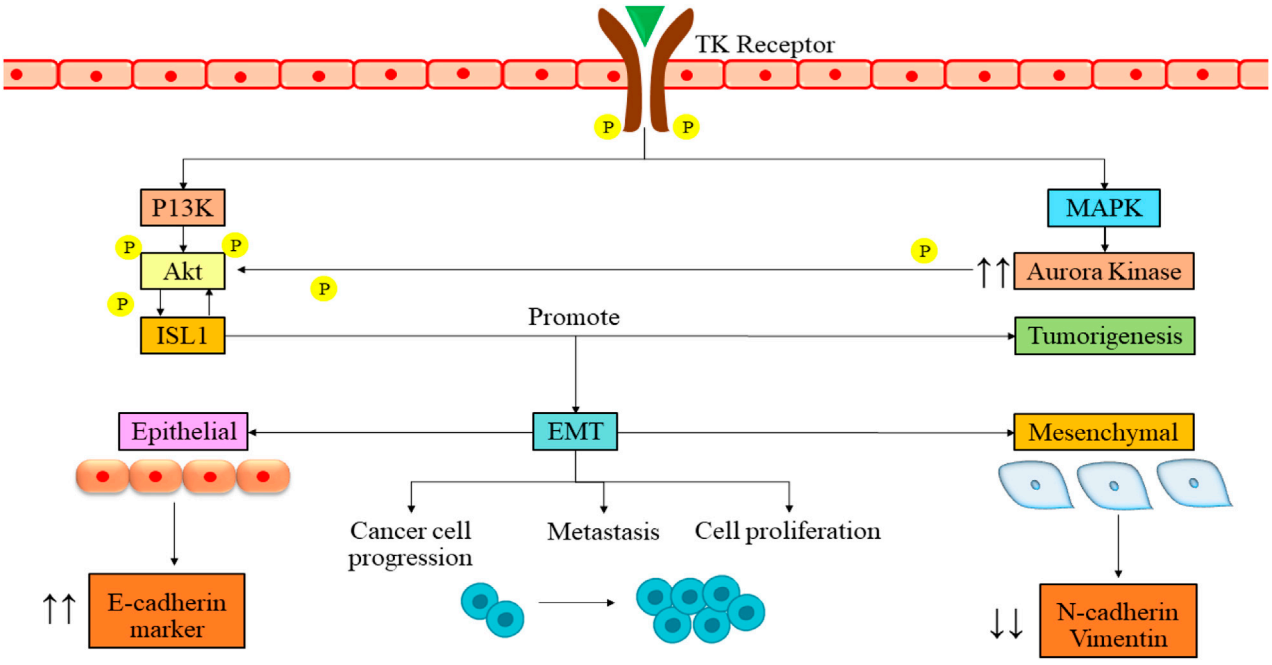


FIGURE 2
Signaling pathway of ISL1 in NB mediation. The binding of growth factor to the tyrosine kinase receptor leads to the cross-phosphorylation of the receptor. The phosphorylation then leads to activation of PI3K and MAPK pathways. These pathways further lead to the phosphorylation and activation of ISL1. The activation then leads to various cellular processes such as tumorigenesis, cancer cell progression, metastasis, and cell proliferation. PI3K/Akt, phosphatidylinositol 3-kinase/serine/threonine kinase family; EMT, epithelial to mesenchymal transition; ISL1, Insulin gene enhancer binding protein; MAPK, Mitigen-activated protein kinase; TK, Tyrosine kinase; P, Phosphorylation.

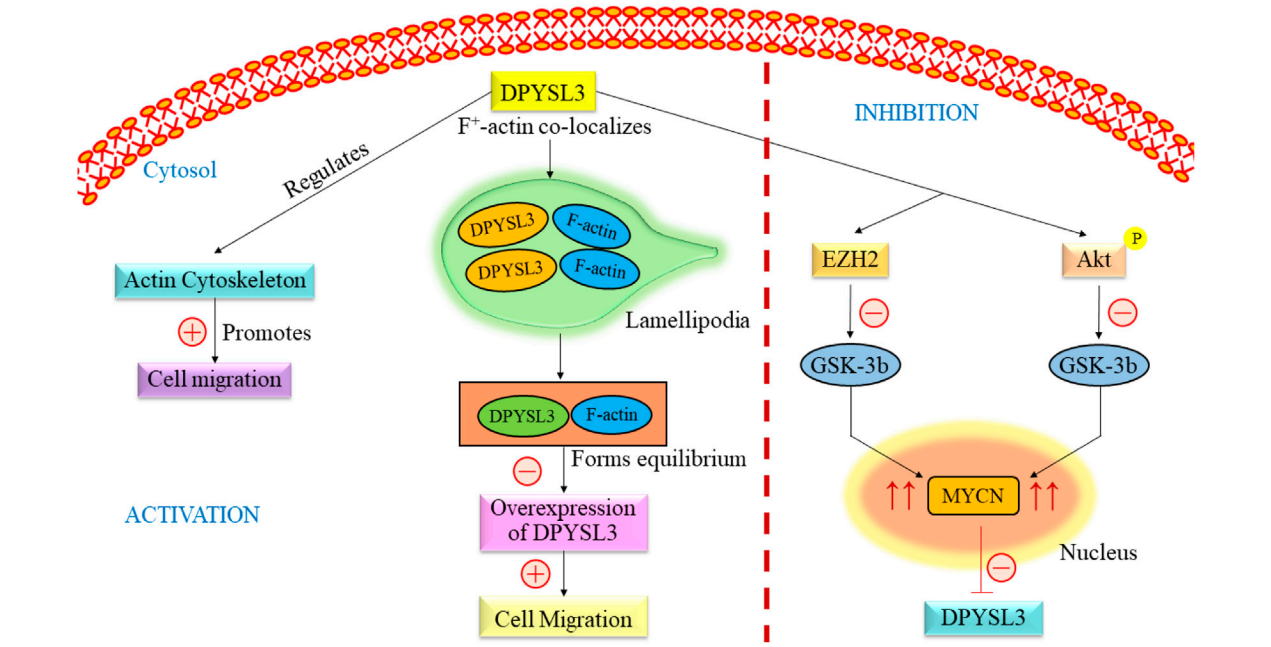


FIGURE 3
Overview of the signaling pathway of DPYSL3. DPYSL3 is primarily located in the nervous system and is crucial in enhancing neurite growth, guiding axons, and shaping the nervous system's structural framework. This gene further regulates the actin cytoskeleton, which promotes the cell migration. F-actin co-localizes in lamellipodia and maintains the equilibrium, causing overexpression of DYPSL3 following the promotion of cell migration. The level of DPYSL3 changes with a change in MYCN expression, which GSK-3β regulates. DYPSL3, Dihydropyrimidinase-like 3; EZH2, Enhancer of zeste homolog 2; Akt: Akt serine/threonine kinase family; MYCN, myc avian myelocytomatosis viral oncogene neuroblastoma.

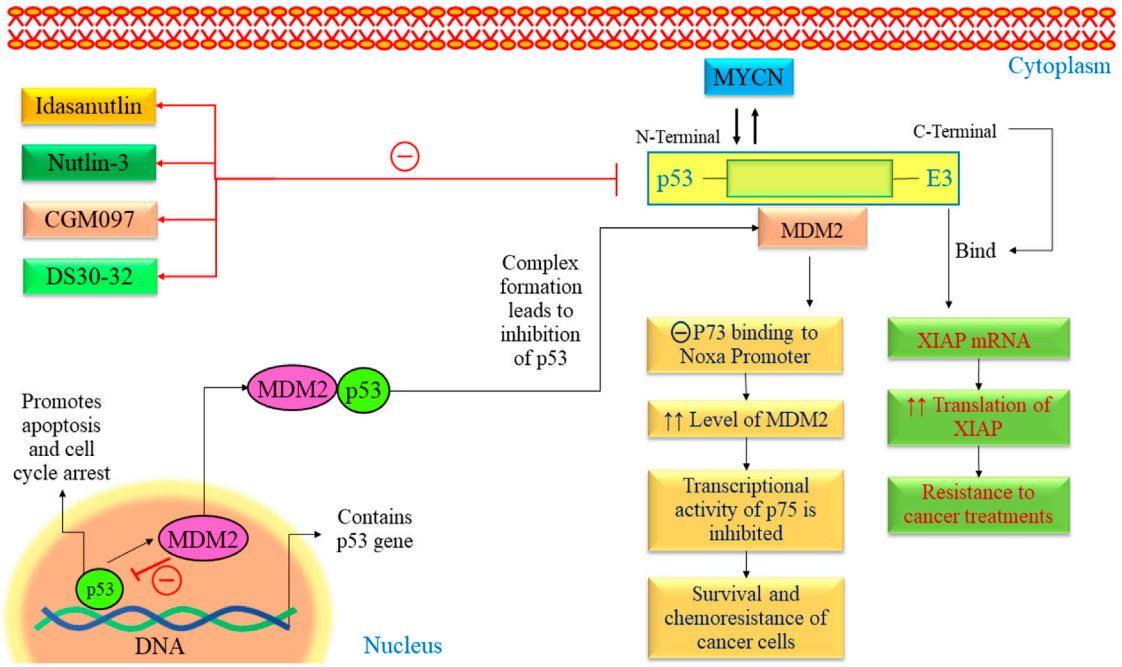


FIGURE 4
The p53-MDM2 pathway in NB. This pathway is mainly regulated by various proteins such as ARF, BMI-1, BHL, and FAK. All these pathways lead to alteration in cell cycle arrest, proliferation, angiogenesis, and promotion of apoptosis. FAK, Focal Adhesion Kinase; MYCN, myc avian myelocytomatosis viral oncogene neuroblastoma; MDM2, Murine double minute 2; VEGF, Vascular endothelial growth factor; TSLC1, Tumor suppressor in Lung Cancer; p53, Tumor protein p53.

amplification or due to Akt-mediated GSK-3b inactivation, would suppress DPYSL3 in NB cells (Le Grand et al., 2020; Gao et al., 2024). This mechanistic insight underscores the crucial prognostic significance of DPYSL3 expression (Tan et al., 2013).

3.1.3 MDM2-p53 as potential target

The exact cause of NB remains largely unknown. Recent advances in genetic research, including whole genome sequencing, have revealed mutations, amplifications, and gene rearrangements linked to NB development (Nicolai et al., 2015; Aygun, 2018). Researchers closely examine oncogenes and tumor suppressor genes to understand their crucial role in NB development. The p53 protein protects cells from genome instability and cancer (Balint and Vousden, 2001). P53 mutation is quite rare in NB (Kattner et al., 2019; Tonini and Capasso, 2020). The p53 tumor suppressor responds to deoxyribonucleic acid (DNA) damage by inducing apoptosis or causing cell cycle arrest (Carvajal and Manfredi, 2013). Over half of human cancers have TP53 mutations, often affecting its DNA binding domain and reducing its transcriptional activity (Olivier et al., 2010; Parrales and Iwakuma, 2015). It implies that p53 is experiencing adverse effects through alternative pathways (Figure 4). MDM2 is upregulated in retinoid-induced NB, and irradiation causes DNA damage. In NB, increased p53 activity suggested a potential for inducing apoptosis in these tumors by boosting p53 (Kim and Shohet, 2009). Several investigations have indicated the presence of consistent MDM2 levels in NB cell lines, even in the absence of MDM2 amplification (Cattelani et al., 2008; Cattelani et al., 2013). Elevated MDM2 expression is a recurring observation in NBs, occasionally attributed to single nucleotide polymorphisms (SNPs) in the MDM2 promoter (Cattelani et al., 2013). Moreover, some studies indicate that MYCN contributes to the reduced p53 activity in neuroblastoma by transcriptionally activating MDM2 expression. All these findings suggested MDM2 as a potential therapeutic target in NB.

The MDM2 oncogene is overexpressed in various human cancers, including NB (Hamzehloie et al., 2012). Elevated levels of MDM2 in tumors are associated with a less favorable outlook for individuals with cancer. MDM2 is found to be a negative regulator of p53 and exhibits various oncogenic processes (Nag et al., 2014). It has been found that the N-terminal of MDM2 binds with p53, and C-terminal works as E3 ubiquitin ligase. Many studies have demonstrated that blocking the interaction between p53 and MDM2 using MDM2 antagonists can trigger apoptotic signaling induced by p53 in NB (Van Maerken et al., 2006; Xue et al., 2007). Using medication to inhibit these genes appears to hold potential as a practical treatment approach for NB.

3.1.4 ROR1 as a potential target

The ROR gene family encompasses two members, namely, ROR1 and ROR2, that exhibit a notable degree of evolutionary conservation among diverse organisms, from metazoans to humans (Zhang et al., 2012a; Yu et al., 2016; Dave, 2020; Mukhtar et al., 2020; Behl et al., 2021). These two proteins displayed a substantial amino acid homology of 58% and were effectively replicated in 1992 from the human NB cell line known as SH-SY5Y. ROR1 and ROR2 serve

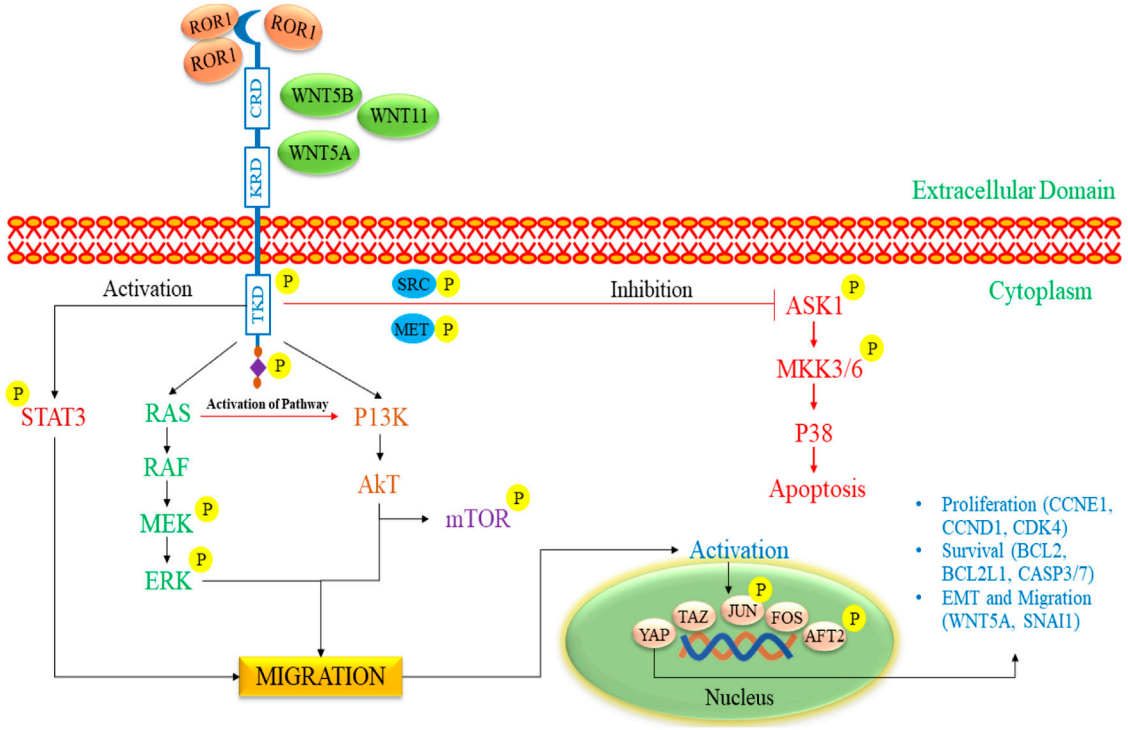


FIGURE 5
The signaling pathway of ROR1/2 in pediatric NB progression. The initiation of ROR1 signaling commences with the binding of a non-canonical WNT ligand, which triggers the formation of a complex involving either ROR1 and ROR2 or ROR1 and a FZD receptor. It triggers the phosphorylation of ROR1 by various kinases, inhibiting anti-apoptotic pathways and activating pathways such as MAPK/ERK, PI3K/AKT, and NF-κB. These pathways prompt cytoskeletal changes, enhance tumor cell migration, and promote gene expression linked to cell proliferation, survival, EMT, and therapy resistance. ROR1, Receptor tyrosine kinase-like orphan receptor 1; WNT, Wingless/Integrated; FZD, Frizzled receptors; RAS, Rat sarcoma; RAF, Rapidly Accelerated Fibrosarcoma; MEK, mitogen-activated extracellular signal-regulated kinase; ERK, extracellular signal-regulated kinases; mTOR: mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; Akt, Ak strain transforming; EMT, Epithelial-mesenchymal transitions; ASK1, Apoptosis signal-regulating kinase 1; MKK3/6, MAP kinase 3/6; STAT3, Signal transducer and activator of transcription 3.

as single-pass transmembrane receptors characterized by distinct structural attributes in their extracellular regions, including an immunoglobulin (Ig)-like domain, a cysteine-rich domain (CRD) and a kringle domain (KRD) (Masiakowski and Carroll, 1992; Masiakowski and Yancopoulos, 1998). The CRD present in ROR1 and ROR2 shares similarities with the CRD of the frizzled receptors (FZD), and it serves a vital function in facilitating the binding of WNT ligands. Notably, RORs are the only members of the RTK (Receptor Tyrosine Kinase) family that possess a KRD, and this domain has been demonstrated to be vital for the formation of hetero-oligomers between ROR1 and ROR2 (Henry et al., 2015). An exceptionally high percentage of tumors positive for ROR2 were discovered in specific cancer types. These included breast cancer, where 87% of the tumors exhibited ROR2 expression, glioblastoma with over 90% ROR2-positive tumors, and n NB, where 80% of the tumors showed ROR2 presence (Kim et al., 2015). ROR1 prefers expression over ROR2 in B-cell chronic lymphocytic leukemia (B-CLL) cells. Moreover, it maintains constitutive expression on B-CLL cells, even in the face of B-cell activation induced by CD40L and IL-4. ROR1 is associated with high expression in gastric, mRNA, B-CLL, and non-small carcinoma cell lines (Green et al., 2008). In CLL cells, the expression of ROR1 may be regulated by IL-6 through the Stat3 pathway (Li et al., 2010). In one study, the administration of retinoic acid has been shown to enhance the number of cells from their stem-cell stage to a mature neuronal state, acquiring typical neuronal traits such as the inhibition of proliferation. It states that the modulation of ROR1 by retinoic acid could induce differentiation and reduce cancer growth (Mishra et al., 2018).

The activation of ROR1 signaling commences with the attachment of a non-canonical WNT ligand, which triggers the formation of a complex comprising ROR1 (as shown in Figure 5) (Zhang et al., 2012b). This intricate structure can involve interactions between ROR1 and ROR2 or ROR1 and a FZD receptor. The signaling cascade encompasses the phosphorylation of ROR1 by multiple kinases exerting dual effects (Castro and Lopez-Bergami, 2022). On the one hand, this phosphorylation suppresses anti-apoptotic pathways, while on the other hand, it activates downstream pathways such as MAPK/ERK, PI3K/AKT, and NF-κB. Activating these downstream pathways has various consequences (Menck et al., 2021; Quezada and Lopez-Bergami, 2023). This process may result in cytoskeletal rearrangements correlated with elevated tumor cell migration. Furthermore, it can induce a transcriptional response that leads to the upregulation of genes facilitating cell proliferation, cell survival, EMT, or resistance to therapy (Zhou et al., 2020; Zhang et al., 2021).

TABLE 1 Clinical trials comprising drug therapies and vaccines with immunotherapeutic agents. NB, Neuroblastoma; HRNB: High-Risk Neuroblastoma; CART, Chimeric Antigen Receptor T cells; PSMA, Prostate-specific membrane antigen; EGFR: Epidermal Growth factor receptor; MTD: maximum tolerated dose; DNA, Deoxyribonucleic acid; IL2, Interleukin 2; NK cells, Natural Killer cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; RXRg, retinoid X receptor gamma.

Sr No.	Identifier	Phase	Status	Agent/Drug	Sample size	Start date	Tested sample	Conditions	Comments
1	NCT05754684	2	Recruiting	Natural killer cell, Dinutuximab beta, Interleukin-2	29	01-01-2022	Blood	Relapsed or refractory NB	Quadruple immunotherapy with NK cells, anti-GD2, IL-2 and GM-CSF and RXRg
2	NCT02573896	1	Active, not recruiting	Dinutuximab. NK cells, Lenalidomide	13	14-01-2019	Blood	Relapsed Refractory NB with expanded NK Cells	This trial determines the MTD of NK combined with Dinutuximab
3	NCT05272371	1	Recruiting	Dinutuximab beta in combination with chemotherapy	20	01-12-2021	Tissue	HRNB	To assess the safety and efficacy of patients treated with dinutuximab beta in combination with chemotherapy
4	NCT04239040	1	Recruiting	GVAX vaccine, Nivolumab, Ipilimumab	26	29-01-2020	Tissue	Relapsed, refractory HRNB, pediatric solid tumor	To check the safety and tolerated dose
5	NCT03635632	1	Recruiting	C7-RGD2, CART cells	94	23-04-2019	Tumor, blood sample	Relapsed and refractory NB	Treat patients with C7R-GD2.CART Cells
6	NCT06057948	2	Recruiting	OPT-821 (QS-21) and β-glucan	94	21-09-2023	Blood	HRNB and metastatic	Research on a vaccine combined with beta-glucan for individuals with NB
7	NCT04936529	2	Recruiting	GM-CSF, OPT-821, β-glucan	264	02-08-2021	Blood NB		The study seeks to assess a combination treatment using a bivalent vaccine, β-glucan, and GM-CSF for high-risk NB patients
8	NCT04049864	1	Recruiting	DNA vaccine, *Salmonella* oral vaccine, Lenalidomide	12	09-01-2019	Tumor sample	Relapsed NB	To test the safety and immune response of a DNA vaccine in relapsed NB patients with post-chemotherapy and stem cell transplantation
9	NCT04239040	1	Recruiting	GVAX vaccine, Nivolumab, Ipilimumab	26	29-01-2023	Tissue collection	Relapsed or refractory NB	This clinical research trial aims to investigate the development and utilization of GVAX when used in conjunction with nivolumab and ipilimumab, as a potential therapeutic approach for NB.
10	NCT05650749	1	Recruiting	GPC2 CAR T cells	30	3-05-2023	Blood	Refractory NB, relapsed NB, HRNB	To determine the MTD of GPC2 CAR T cells
11	NCT05990751	1	Not yet recruiting	GD2 CAR T cells	12	01-01-2024	NA	Relapsed or refractory NB	Targeting Chimeric antigen receptor
12	NCT03721068	1	Recruiting	iC9.GD2.CAR.IL-15 T-cells, Cyclophosphamide, Fludarabine	18	19-02-2019	Tissue	NB, osteosarcoma	To identify the maximum tolerated dose
13	NCT04637503	1,2	Recruiting	GD2, PSMA and CD276 CAR-T cells	100	18-11-2020	Blood	NB	To evaluate the safety and efficacy of 4SCAR-T cell therapy

(Continued on following page)

TABLE 1 (*Continued*) Clinical trials comprising drug therapies and vaccines with immunotherapeutic agents. NB, Neuroblastoma; HRNB: High-Risk Neuroblastoma; CART, Chimeric Antigen Receptor T cells; PSMA, Prostate-specific membrane antigen; EGFR: Epidermal Growth factor receptor; MTD: maximum tolerated dose; DNA, Deoxyribonucleic acid; IL2, Interleukin 2; NK cells, Natural Killer cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; RXRg, retinoid X receptor gamma.

Sr No.	Identifier	Phase	Status	Agent/Drug	Sample size	Start date	Tested sample	Conditions	Comments
14	NCT03618381	1	Recruiting	4-1BBζ EGFR806-EGFRt	44	22-02-2019	Blood	NB	EGFR806 CAR T Cell Immunotherapy
15	NCT05562024	1	Recruiting	TAA06 Injection	24	30-12-2022	Tissue	B7-H3-positive Relapsed/ Refractory NB	to check the tolerability, safety, and cytokinetic characteristics

3.2 Novel therapies (clinical trials) in implementing immunotherapy for pediatric neuroblastoma

NB is a complex cancer type with various subtypes and genetic variations, making it challenging to tailor immunotherapies to each case (Kholodenko et al., 2018b). Children have developing immune systems, which might not respond as effectively to immunotherapies and could lead to more severe side effects (Mackall et al., 2014; Varadé et al., 2021). Limited clinical data for pediatric patients with NB means there is a lack of knowledge regarding the safety and effectiveness of these treatments. Determining the appropriate dosage for children based on age and weight can be complicated, and striking the right balance between potential benefits and risks is crucial. Access to clinical trials for pediatric patients may also be limited, restricting their options for innovative treatments (Fletcher et al., 2018). NB's tumor microenvironment can suppress immune responses, hindering the efficacy of immunotherapies.

Additionally, NB can employ mechanisms to evade the immune system, making it harder to target the cancer cells effectively (Vanichapol et al., 2018; Veschi et al., 2019). Addressing relapse and resistance, developing combination therapies, and considering the psychological and emotional impact on pediatric patients and their families are also part of the challenge. The cost of immunotherapies and the ethical considerations surrounding their use in pediatric cases further complicate the situation (Unguru et al., 2013; Cabral et al., 2023). Despite these difficulties, ongoing research, collaboration, and a holistic approach offer hope for improved immunotherapy outcomes in the fight against pediatric NB. Table 1 comprises clinical trials of drug therapies and immunotherapeutic agents of the last 5 years.

In recent years, clinical trials have been conducted to assess innovative strategies for NB treatment (Esposito et al., 2017; Tolbert and Matthay, 2018). These approaches frequently incorporate the granulocyte-macrophage colony-stimulating factor (GM-CSF), which enhances immune activation and antibody-dependent cellular cytotoxicity (ADCC). Numerous current clinical trials explore combination therapies encompassing drugs and newly engineered immunotherapies. Table 1 depicts the ongoing trials on different immunotherapeutic targets in NB using novel combination therapies. NCT05754684 is a phase 2 study to check the safety and efficacy of quadruple immunotherapy with NK cells, IL-2, GM-CSF, and retinoid X receptor gamma (RXRg) with inclusion criteria of creatinine clearance of ≥40 mL/min/1.73 m^2. The condition for this trial is relapsed and refractory NB. This trial (NCT02573896) is being carried out to check the maximum tolerated dose (MTD) when NK cells combine with Dinutuximab. Dinutuximab is a chimeric antibody against GD2, mainly expressed in NB cells. This trial is performed by using Lenalidomide in combination. Trial number (NCT05272371) describes the study evaluation of Dinutuximab beta in combination with chemotherapy. Another clinical trial (NCT04239040) investigates the development and use of GVAX, a GM-CSF-secreting, autologous NB cell vaccine, combined with nivolumab and ipilimumab as a potential NB treatment. In a clinical trial (NCT03635632), scientists will extract blood samples from the patient. They will then enhance the GD2. C7R T cells by introducing a new gene using a specialized virus called a retroviral vector. The GD2. CAR gene enables the T cells to identify and destroy cancer cells, particularly those that are GD2-positive. Another gene called C7R will also be introduced to these cells to extend their survival. Subsequently, the modified T cells will undergo testing to ensure their ability to target and eliminate GD2-positive cancer cells. A trial NCT06057948, started on 21-09-2023, is carried out to test the treatment of β-glucan with bivalent vaccine. In one study (NCT03721068), the safety and efficacy of IL-15, iCaspase9 is being carried out on patients with relapsed and refractory NB.

4 Immunotherapeutic approaches to combat neuroblastoma

The positive outcomes observed in immunotherapy involving anti-GD2 monoclonal antibodies prompt an inquiry into whether NB possesses characteristics that make it susceptible to immune responses (Bhoopathi et al., 2021; Morandi et al., 2021). When discussing the immunogenicity of NB, it is essential to be precise in terminology (Webb et al., 2020; Paraboschi et al., 2021). If we consider "immunogenicity" in the context of cancer, the question arises as to whether NB can activate immune responses.

Despite the absence of evidence for an adaptive immune response, a substantial body of evidence indicates that NB, like many other human cancers, possesses inherent mechanisms designed to elude immune recognition (Cervantes-Villagrana et al., 2020). These mechanisms include decreased MHC class-I expression, suppressive myeloid cells, and the production of inhibitory factors like arginase-2 and TGF-β (Cornel et al., 2020; Dhatchinamoorthy et al., 2021). It enhances an intriguing possibility that the relatively low immune activity within NB may result from a lack of inherent danger signals and the tumor's active immune evasion mechanisms. This observation offers hope for the potential effectiveness of immunotherapeutic strategies. By developing

TABLE 2 Various immunotherapy approaches to combat neuroblastoma. NB, Neuroblastoma; ADCC, antibody-dependent cell-mediated cytotoxicity; CDC: Complement dependent cytotoxicity; PD1, Programmed Cell Death; PD-L1, Programmed Cell Death Ligand 1; CART Cells: Chimeric Antigen Receptor T cells; CTLA4, Cytotoxic T-lymphocyte associated protein 4; IL1/2, Interleukin 1/2; DNA: Deoxyribonucleic acid.

Neuroblastoma immunotherapy approaches	Description	Examples
Monoclonal antibodies	Administration of antibodies that target specific NB cell surface antigens, often leading to ADCC and CDC	Dinutuximab (Unituxin), Naxitumab
Immune checkpoint inhibitors	Blockade of immune checkpoint molecules like PD-1, PD-L1, or CTLA-4 to enhance the activity of immune cells (e.g., T cells) against NB cells	Nivolumab (Opdivo), Pembrolizumab (Keytruda)
CAR T Cell therapy	Genetic modification of patient's T cells to express CARs targeting NB antigens, leading to targeted cell killing	GD2-specific CAR T cells
Cytokine therapy	Administration of cytokines (e.g., IL-2, IL-12, IFN-γ) to boost the immune response against NB by enhancing T cell and NK cell activity	Interleukin-2 (Proleukin)
Vaccines	Utilization of NB-specific vaccines (e.g., peptide, dendritic cell, or DNA vaccines) to stimulate the immune system to recognize and attack tumor cells	NBL-004 (Vaccine targeting GD2)
Oncolytic viruses	Use gene-modified viruses that selectively infect and kill NB cells while inducing an immune response against the tumor	Adenovirus-based oncolytic therapy
Immune modulators	Administration of immunomodulatory agents (e.g., immune stimulants or suppressors) to regulate the immune response against NB	Interferon-alpha (IFN- α)
Combination therapies	Integration of multiple immunotherapy approaches to maximize the chances of successful NB treatment	CAR T-cells with checkpoint inhibitors

therapeutic approaches capable of targeting NB cells, scientists have managed to induce a novel immune response in tumors that otherwise appear to escape detection by the natural immune system. These immunotherapies, which utilize synthetic immune recognition, are frequently based on monoclonal antibodies (Carazas et al., 2021; Varadé et al., 2021). Monoclonal antibodies designed to target the disialoganglioside GD2, commonly overexpressed on most NB cells, have brought about a transformative impact on the treatment of NB (Hung and Alice, 2019). They have led to a remarkable increase in event-free survival rates, as high as 20%.

Furthermore, synthetic recognition agents can be further developed from antibody derivatives, including CAR-T cells and antibody-drug conjugates. These innovative therapeutic approaches have demonstrated promising results in pre-clinical and early clinical trials, signifying a significant advancement in immunotherapy. TGF-β regulates cellular activities, including controlling cell growth, proliferation, differentiation, and apoptosis. TGF-β was initially thought to inhibit cancer, but recent studies show it can also promote cancer by impairing NK cell function, leading to increased tumorigenesis, metastasis, and drug resistance, which is a promising target for immunotherapy (Slattery and Gardiner, 2019; Wang et al., 2021; Wienke et al., 2021). Table 2 comprises various immunotherapy approaches to combat NB.

4.1 Monoclonal antibodies

Monoclonal antibodies (mAbs) targeting GD2 effectively respond to high-risk NB cases. However, the outcomes and side effects vary among different kinds of anti-GD2 antibodies (Chan and Chan, 2022). One of these immunotherapies is centered on utilizing mAbs to target GD2, which is found in higher quantities than in a control group in cases of NB. GD2 is a disialoganglioside present on the outer surface of all NB cells (Tibbetts et al., 2022; Machy et al., 2023). Including monoclonal antibodies like anti-GD2 in initial and recurrent treatment strategies has significantly improved survival rates and transformed the outlook for children with HRNB (Anderson et al., 2022). Dinutuximab, a monoclonal antibody that targets GD2 found in neuroblasts, enhances survival when incorporated into the treatment plan. Furthermore, the pairing of dinutuximab with chemotherapy has proven to be highly effective in reversing recurrent disease (FDA, 2015). Monoclonal antibodies targeting GD2 enhance the outlook for HRNB, particularly in young children and older patients who undergo this treatment following multiple previous therapies, typically several months post-diagnosis (Paraboschi et al., 2021). In this study, the authors administered anti-GD2 monoclonal antibodies to infants as part of the immunotherapy protocol initiated during or immediately following induction chemotherapy. A total of 33 HRNB patients, all under 19 months old, were treated with either 3F8 (murine monoclonal antibody, 21 patients) or naxitamab (humanized-3F8, 12 patients) via intravenous infusions lasting between 30 and 90 s. Patients were also provided with analgesics and antihistamines. Two cycles of 3F8 were discontinued, one due to preexisting bradycardia and the other because of asthmatic symptoms. In the case of naxitamab, one patient initially received half the prescribed dose on day 1 due to hypotension, but subsequently received the full recommended dose. Toxicity in infants receiving naxitamab at a higher dosage was comparable to older patients. This is reassuring, as the infant HRNB patients had a high potential for cure (Kushner et al., 2023).

4.2 Immune checkpoint inhibitors (ICIs)

ICIs have transformed adult cancer treatment, like lung cancer and melanoma (Wagner and Adams, 2017). This achievement has

sparked interest in using ICIs for relapsed and resistant pediatric cancers, with three recent clinical studies assessing their effectiveness (Geoerger et al., 2020a; Geoerger et al., 2020b; Davis et al., 2020). These studies have shown disappointing results, with a low objective response rate in pediatric cancer patients when using programmed cell death protein-1 (PD1) inhibitors like pembrolizumab and nivolumab, as well as programmed cell death ligand (PD-L1) inhibitors like atezolizumab. For pembrolizumab, the objective response rate (ORR) according to RECIST v1.1 criteria for solid tumors was 5.5%. As for nivolumab and atezolizumab, no objective responses were observed among patients with solid tumors (Geoerger et al., 2020b; Davis et al., 2020). Simultaneously, reports from individual cases have demonstrated pediatric patients exhibiting a lasting positive response to immune checkpoint inhibitors, mainly when combined with other cancer-fighting medications (Ehlert et al., 2020).

4.3 Chimeric antigen receptor T (CAR T)-Cell therapy

HRNB is a prevalent childhood cancer. While most patients attain remission initially, over 50% relapse due to minimal residual disease, frequently resulting in a fatal outcome (Modak and Cheung, 2010; Tolbert and Matthay, 2018; Shohet et al., 2022). According to a recent research study, antibody therapy combined with cytokines to target minimal residual disease resulted in an approximate 20% increase in these patients' 5-year overall survival rate (Richards et al., 2018; Yu et al., 2021). Despite this progress, 1/3 of children with cancer still need more effective treatments. Inspired by successful CAR T-cell therapy for blood malignancies, several CAR T-cell treatments are now developing for NB patients (Maude et al., 2014; Maude et al., 2018). It is less effective for solid tumors like NB than leukemia, as seen in initial GD2-targeted CAR T trials for relapsed NB patients (Louis et al., 2011; Heczey et al., 2017; Heczey et al., 2020; Straathof et al., 2020). Of 42 patients with active disease across four trials treated with 14.18 or K666 GD2-CAR T-cells, only three experienced prolonged objective responses (Straathof et al., 2020). The glypican 2 (GPC2) antigen is present during the initial stages of fetal development but becomes mostly inactive in normal tissue after that (Bosse et al., 2017; Li et al., 2017; Li et al., 2021; Heitzeneder et al., 2022; Tian et al., 2022). GPC2, found in NB, is a potential immunotherapy target with much lower expression levels than GD2 and B7H3 (Heitzeneder et al., 2022). It has consequences for the CAR's efficiency because reduced antigen density is linked to decreased CAR interaction, activation, and its ability to combat tumors (Majzner et al., 2020). So, Sun and co-workers developed this therapy for children with NB. They optimized pre-clinical CAR by using interactive engineering to enhance the antitumor effect. The research indicated that among three GPC2-CAR constructs, anti-GPC2 CT3 with a CD28 hinge, CD28 transmembrane, and 4-1BB co-stimulatory domain demonstrated the most effective pre-clinical activity against NB. The authors compared the CT3.28H.BBζ chimeric antigen receptor (CAR) antitumor effectiveness to a GD2 CAR recently undergoing clinical trials with a similar CAR structure (Sun et al., 2023).

4.4 Cytokine therapy

Cytokines activate the immune system against tumors and are a promising approach in NB immunotherapy (Pistoia et al., 2011; Cavalli et al., 2020; Yu et al., 2021; Dowsey, 2023). Combining an antibody targeting GD2 (hu14.18) with interleukin-2 (IL-2) has demonstrated significant potential in pre-clinical and clinical settings for treating NB. The therapeutic benefit of IL-2 remains uncertain due to severe toxicities at higher doses and unproven efficacy at lower doses. The authors connected IL15 and IL21 to hu14.18, resulting in improved antibody-dependent cell-mediated killing in immune-competent NB models compared to hu14.18-IL2 (Nguyen et al., 2022). Migration inhibitory factor (MIF) is a versatile cytokine that plays a significant role in various diseases, including cancer. Pre-clinical and clinical research in patients with NB consistently shows that MIF possesses characteristics that promote tumor growth in NB. Levels of MIF are elevated in NB tumor tissues and cell lines, contributing to the increased aggressiveness of NB and assisting in immune evasion (Cavalli et al., 2020).

4.5 Vaccines

Cancer vaccines differ from infectious disease vaccines as they are typically therapeutic rather than preventive (Huebener et al., 2003; Bleeke et al., 2009; Komorowski et al., 2018). Only two Food Drug and Administration (FDA) approved preventive cancer vaccines exist for HPV and HBV-related cancers. Challenges in predicting antigens and achieving substantial immune responses impede the clinical progress of anticancer preventive vaccines (Ahmed, 2022). Top-notch cancer immunotherapy aims to harness the immune system's potential to eradicate cancer cells efficiently. Cancer vaccines face challenges in precisely delivering antigenic markers and adjuvants to coordinate an effective immune response. Messenger ribonucleic acid vaccines show promise in cancer treatment by prompting antigen expression in antigen-presenting cells, leading to adaptive immune responses (Webb et al., 2020; Wolfson et al., 2021).

5 Conclusion

In conclusion, the comprehensive review of NB underscores the significance of diverse therapeutic targets, including ISL1, DPYSL3, MDM2-p53, and ROR1. The exploration of these targets has provided valuable insights into potential avenues for interventions in this cancer. The outcomes of clinical trials and other therapies hold evidence for advancing precision medicine. Identifying additional valuable targets and creating more efficient therapies are imperative. Furthermore, delving into novel combinations that incorporate inhibitors addressing multiple targets in conjunction with conventional treatments remains a paramount focus for future research. This direction is poised to advance treatment protocols, enhance results, and extend the survival of high-risk NB in children. Although significant breakthroughs have been achieved in molecularly targeted therapy for NB, applying these findings to clinical disease

management has had limited success in previous years. Thus far, the sole approved treatment for pediatric patients with relapsed or refractory HRNB is anti-GD2 monoclonal antibodies. The disparity between research conducted in pre-clinical settings and its translation into clinical trials is striking. There is also a need for novel combinations to target multiple molecular targets in conjunction with traditional therapies.

6 Future perspectives

NB, a childhood cancer, is characterized by abnormal development and typically has a low mutational burden, distinguishing it from most adult malignancies. Conversely, drug testing primarily relies on conventional NB cell and mouse models. There is a need to progress towards developing new disease models by harnessing cutting-edge technologies, like 3D tissue-engineered systems and patient-derived xenograft model systems. Researchers must focus on *in vitro* and *in vivo* testing studies to develop new synthetic drugs to treat NB. Further research must be carried out on designing novel inhibitors and immunotherapy. Apart from discovering novel targets for immunotherapy and designing therapeutic interventions, researchers must also prioritize designing a combination of new small NB inhibitors with radiotherapy to inhibit the various signaling pathways. Decreasing the high dose of radiochemotherpeutic agents could be another significant approach to combat NB without toxicity and side effects. New target-based inhibitors are being used for the patients. In summary, the future of immunotherapy and novel targeted therapies in NB treatment is marked by a commitment to innovation, personalization, and collaboration. With ongoing research and technological advancements, people can expect improved outcomes and quality of life for NB patients in the years to come.

Author contributions

MZ: Data curation, Formal Analysis, Visualization, Writing–original draft. AK: Data curation, Formal Analysis, Visualization, Writing–original draft. VS: Formal Analysis, Writing–review and editing. TB: Conceptualization, Formal Analysis, Supervision, Visualization, Writing–review and editing. AS: Formal Analysis, Writing–review and editing. PW: Formal Analysis, Writing–review and editing. NS: Formal Analysis, Writing–review and editing. BK: Formal Analysis, Writing–review and editing. AK: Formal Analysis, Writing–review and editing. MKA: Formal Analysis, Writing–review and editing. MG: Formal Analysis, Writing–review and editing. BS: Formal Analysis, Supervision, Writing–review and editing. RS: Conceptualization, Formal Analysis, Supervision, Writing–review and editing. SB: Methodology, Validation, Formal Analysis, Proofing, Supervision.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Natural Science Foundation of China (32070671, and 32270690), the COVID-19 research projects of West China Hospital Sichuan University (Grant no. HX-2019-nCoV-057), and the regional innovation cooperation between Sichuan and Guangxi Provinces (2020YFQ0019).

Conflict of interest

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

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

Adam, K., Lesperance, J., Hunter, T., and Zage, P. E. (2020). The potential functional roles of NME1 histidine kinase activity in neuroblastoma pathogenesis. *Int. J. Mol. Sci.* 21 (9), 3319. doi:10.3390/ijms21093319

Advani, D., Sharma, S., Kumari, S., Ambasta, R. K., and Kumar, P. (2022). Precision oncology, signaling, and anticancer agents in cancer therapeutics. *Anti-Curr Med. Chem. Anticancer Agents* 22 (3), 433–468. doi:10.2174/1871520621666210308101029

Agaimy, A., Erlenbach-Wünsch, K., Konukiewitz, B., Schmitt, A. M., Rieker, R. J., Vieth, M., et al. (2013). ISL1 expression is not restricted to pancreatic well-differentiated neuroendocrine neoplasms, but is also commonly found in well and poorly differentiated neuroendocrine neoplasms of extrapancreatic origin. *Mod. Pathol.* 26 (7), 995–1003. doi:10.1038/modpathol.2013.40

Ahmed, A. A., Zhang, L., Reddivalla, N., and Hetherington, M. (2017). Neuroblastoma in children: update on clinicopathologic and genetic prognostic factors. *Pediatr. Hematol. Oncol.* 34 (3), 165–185. doi:10.1080/08880018.2017.1330375

Ahmed, T. (2022). Immunotherapy for neuroblastoma using mRNA vaccines. *Adv. Cancer Biol. Metastat* 4, 100033. doi:10.1016/j.adcanc.2022.100033

Alabed, Y. Z., Pool, M., Ong Tone, S., Sutherland, C., and Fournier, A. E. (2010). GSK3 beta regulates myelin-dependent axon outgrowth inhibition through CRMP4. *J. Neurosci.* 30 (16), 5635–5643. doi:10.1523/JNEUROSCI.6154-09.2010

Anderson, J., Majzner, R. G., and Sondel, P. M. (2022). Immunotherapy of neuroblastoma: facts and hopes. *Clin. Cancer Res.* 28 (15), 3196–3206. doi:10.1158/1078-0432.CCR-21-1356

Attiyeh, E. F., London, W. B., Mossé, Y. P., Wang, Q., Winter, C., Khazi, D., et al. (2005). Chromosome 1p and 11q deletions and outcome in neuroblastoma. *N. Engl. J. Med.* 353 (21), 2243–2253. doi:10.1056/NEJMoa052399

Aygun, N. (2018). Biological and genetic features of neuroblastoma and their clinical importance. *Curr. Pediatr. Rev.* 14 (2), 73–90. doi:10.2174/1573396314666180129101627

Aygun, N., and Altungoz, O. (2019). MYCN is amplified during S phase, and c-myb is involved in controlling MYCN expression and amplification in MYCN-amplified neuroblastoma cell lines. *Mol. Med. Rep.* 19 (1), 345–361. doi:10.3892/mmr.2018.9686

Baedke, J. (2013). The epigenetic landscape in the course of time: Conrad Hal Waddington's methodological impact on the life sciences. *Stud. Hist. Philosophy Sci. Part C Stud. Hist. Philosophy Biol. Biomed. Sci.* 44 (4), 756–773. doi:10.1016/j.shpsc.2013.06.001

Balint, E., and Vousden, K. (2001). Activation and activities of the p53 tumour suppressor protein. *Br. J. Cancer* 85 (12), 1813–1823. doi:10.1054/bjoc.2001.2128

Bartolucci, D., Montemurro, L., Raieli, S., Lampis, S., Pession, A., Hrelia, P., et al. (2022). MYCN impact on high-risk neuroblastoma: from diagnosis and prognosis to targeted treatment. *Cancers* 14 (18), 4421. doi:10.3390/cancers14184421

Behl, T., Sharma, A., Sharma, L., Sehgal, A., Singh, S., Sharma, N., et al. (2021). Current perspective on the natural compounds and drug delivery techniques in glioblastoma multiforme. *Cancers* 13 (11), 2765. doi:10.3390/cancers13112765

Bell, D. W. (2010). Our changing view of the genomic landscape of cancer. *J. Pathology J Patholog Soc* 220 (2), 231–243. doi:10.1002/path.2645

Ben Amar, D., Thoinet, K., Villalard, B., Imbaud, O., Costechareyre, C., Jarrosson, L., et al. (2022). Environmental cues from neural crest derivatives act as metastatic triggers in an embryonic neuroblastoma model. *Nat. Commun.* 13 (1), 2549. doi:10.1038/s41467-022-30237-3

Bhoopathi, P., Mannangatti, P., Emdad, L., Das, S. K., and Fisher, P. B. (2021). The quest to develop an effective therapy for neuroblastoma. *J. Cell Physiol.* 236 (11), 7775–7791. doi:10.1002/jcp.30384

Bleeke, M., Fest, S., Huebener, N., Landgraf, C., Schraven, B., Gaedicke, G., et al. (2009). Systematic amino acid substitutions improved efficiency of GD2-peptide mimotope vaccination against neuroblastoma. *Eur. J. Cancer* 45 (16), 2915–2921. doi:10.1016/j.ejca.2009.07.026

Bosse, K. R., Raman, P., Zhu, Z., Lane, M., Martinez, D., Heitzeneder, S., et al. (2017). Identification of GPC2 as an oncoprotein and candidate immunotherapeutic target in high-risk neuroblastoma. *Cancer Cell* 32 (3), 295–309. doi:10.1016/j.ccell.2017.08.003

Brodeur, G. M., Minturn, J. E., Ho, R., Simpson, A. M., Iyer, R., Varela, C. R., et al. (2009). Trk receptor expression and inhibition in neuroblastomas. *Clin. Cancer Res.* 15 (10), 3244–3250. doi:10.1158/1078-0432.CCR-08-1815

Cabral, J., Fernandez, E. I., Toy, B., and Secola, R. (2023). Multidisciplinary clinical care in the management of patients receiving anti-GD2 immunotherapy for high-risk neuroblastoma. *Pediatr. Drugs* 25 (1), 13–25. doi:10.1007/s40272-022-00544-9

Campbell, K., Kao, P. C., Naranjo, A., Kamijo, T., Ramanujachar, R., London, W. B., et al. (2023). Clinical and biological features prognostic of survival after relapse or progression of INRGSS stage MS pattern neuroblastoma: a report from the International Neuroblastoma Risk Group (INRG) project. *Pediatr. Blood Cancer* 70 (2), e30054. doi:10.1002/pbc.30054

Carazas, M. M. G., Pinto, J. A., and Casado, F. L. (2021). Biological bases of cancer immunotherapy. *Exp. Rev. Mol. Med.* 23, e3. doi:10.1017/erm.2021.5

Carén, H., Abel, F., Kogner, P., and Martinsson, T. (2008). High incidence of DNA mutations and gene amplifications of the ALK gene in advanced sporadic neuroblastoma tumours. *Biochem. J.* 416 (2), 153–159. doi:10.1042/bj20081834

Carvajal, L. A., and Manfredi, J. J. (2013). Another fork in the road—life or death decisions by the tumour suppressor p53. *EMBO Rep.* 14 (5), 414–421. doi:10.1038/embor.2013.25

Castro, M. V., and Lopez-Bergami, P. (2022). Cellular and molecular mechanisms implicated in the dual role of ROR2 in cancer. *Crit. Rev. Oncol. Hematol.* 170, 103595. doi:10.1016/j.critrevonc.2022.103595

Cattelani, S., Defferrari, R., Marsilio, S., Bussolari, R., Candini, O., Corradini, F., et al. (2008). Impact of a single nucleotide polymorphism in the MDM2 gene on neuroblastoma development and aggressiveness: results of a pilot study on 239 patients. *Clin. Cancer Res.* 14 (11), 3248–3253. doi:10.1158/1078-0432.CCR-07-4725

Cattelani, S., Ferrari-Amorotti, G., Soliera, A. R., Manzotti, G., Raschella, G., Calabrette, B., et al. (2013). Neuroblastoma: role of MDM2 and SNP309 as markers. *Pediatr. Cancer* 4, 19–25. doi:10.1007/978-94-007-6591-7_3

Cavalli, E., Ciurleo, R., Petralia, M. C., Fagone, P., Bella, R., Mangano, K., et al. (2020). Emerging role of the macrophage migration inhibitory factor family of cytokines in neuroblastoma. pathogenic effectors and novel therapeutic targets? *Molecules* 25 (5), 1194. doi:10.3390/molecules25051194

Cervantes-Villagrana, R. D., Albores-García, D., Cervantes-Villagrana, A. R., and García-Acevez, S. J. (2020). Tumor-induced neurogenesis and immune evasion as targets of innovative anticancer therapies. *Signal Transduct. Target Ther.* 5 (1), 99. doi:10.1038/s41392-020-0205-z

Chan, G.C.-F., and Chan, C. M. (2022). Anti-GD2 directed immunotherapy for high-risk and metastatic neuroblastoma. *Biomolecules* 12 (3), 358. doi:10.3390/biom12030358

Cheung, I. Y., Feng, Y., Gerald, W., and Cheung, N. K. V. (2008). Exploiting gene expression profiling to identify novel minimal residual disease markers of neuroblastoma. *Clin. Cancer Res.* 14 (21), 7020–7027. doi:10.1158/1078-0432.CCR-08-0541

Chicco, D., Sanavia, T., and Jurman, G. (2023). Signature literature review reveals AHCY, DPYSL3, and NME1 as the most recurrent prognostic genes for neuroblastoma. *BioData Min.* 16 (1), 7. doi:10.1186/s13040-023-00325-1

Cornel, A. M., Mimpen, I. L., and Nierkens, S. (2020). MHC class I downregulation in cancer: underlying mechanisms and potential targets for cancer immunotherapy. *Cancers* 12 (7), 1760. doi:10.3390/cancers12071760

Dave, Z. (2020). *erRORs in cellular crosstalk-Importance in development*.

Davidoff, A. M. (2021). Neonatal neuroblastoma. *Clin. Perinatol.* 48 (1), 101–115. doi:10.1016/j.clp.2020.11.006

Davis, K. L., Fox, E., Merchant, M. S., Reid, J. M., Kudgus, R. A., Liu, X., et al. (2020). Nivolumab in children and young adults with relapsed or refractory solid tumours or lymphoma (ADVL1412): a multicentre, open-label, single-arm, phase 1–2 trial. *Lancet Oncol.* 21 (4), 541–550. doi:10.1016/S1470-2045(20)30023-1

Desprez, F., Ung, D. C., Vourc'h, P., Jeanne, M., and Laumonnier, F. (2023). Contribution of the dihydropyrimidinase-like proteins family in synaptic physiology and in neurodevelopmental disorders. *Front. Neurosci.* 17, 1154446. doi:10.3389/fnins.2023.1154446

Dhatchinamoorthy, K., Colbert, J. D., and Rock, K. L. (2021). Cancer immune evasion through loss of MHC class I antigen presentation. *Front. Immunol.* 12, 636568. doi:10.3389/fimmu.2021.636568

Dowsey, M. (2023). Cytokine immunotherapy for neuroblastoma. *SciInsights* 42 (1), 787–792. doi:10.15354/si.23.re115

Du, R., Huang, C., Liu, K., Li, X., and Dong, Z. (2021). Targeting AURKA in Cancer: molecular mechanisms and opportunities for Cancer therapy. *Mol. Cancer* 20, 15–27. doi:10.1186/s12943-020-01305-3

Durinck, K., and Speleman, F. (2018). Epigenetic regulation of neuroblastoma development. *Cell Tissue Res.* 372 (2), 309–324. doi:10.1007/s00441-017-2773-y

Ehlert, K., Hansjuergens, I., Zinke, A., Otto, S., Siebert, N., Henze, G., et al. (2020). Nivolumab and dinutuximab beta in two patients with refractory neuroblastoma. *J. Immunother. Cancer* 8 (1), e000540. doi:10.1136/jitc-2020-000540

Esposito, M. R., Aveic, S., Seydel, A., and Tonini, G. P. (2017). Neuroblastoma treatment in the post-genomic era. *J. Biomed. Sci.* 24 (1), 14–16. doi:10.1186/s12929-017-0319-y

Fadaka, A. O., Sibuyi, N. R. S., Madiehe, A. M., and Meyer, M. (2020). MicroRNA-based regulation of Aurora A kinase in breast cancer. *Oncotarget* 11 (46), 4306–4324. doi:10.18632/oncotarget.27811

FDA (2015). *FDA approves first therapy for high-risk neuroblastoma*.

Filippi, L., Chiaravalloti, A., Schillaci, O., Cianni, R., and Bagni, O. (2020). Theranostic approaches in nuclear medicine: current status and future prospects. *Expert Rev. Med. Devices* 17 (4), 331–343. doi:10.1080/17434440.2020.1741348

Flavahan, W. A., Gaskell, E., and Bernstein, B. E. (2017). Epigenetic plasticity and the hallmarks of cancer. *Science* 357 (6348), eaal2380. doi:10.1126/science.aal2380

Fletcher, J. I., Ziegler, D. S., Trahair, T. N., Marshall, G. M., Haber, M., and Norris, M. D. (2018). Too many targets, not enough patients: rethinking neuroblastoma clinical trials. *Nat. Rev. Cancer* 18 (6), 389–400. doi:10.1038/s41568-018-0003-x

Fulcher, L. J., and Sapkota, G. P. (2020). Mitotic kinase anchoring proteins: the navigators of cell division. *Cell Cycle* 19 (5), 505–524. doi:10.1080/15384101.2020.1728014

Gaetano, C., Matsuo, T., and Thiele, C. J. (1997). Identification and characterization of a retinoic acid-regulated human homologue of the unc-33-like phosphoprotein gene (hUlip) from neuroblastoma cells. *J. Biol. Chem.* 272 (18), 12195–12201. doi:10.1074/jbc.272.18.12195

Gao, W., Guo, N., Yan, H., Zhao, S., Sun, Y., and Chen, Z. (2024). Mycn ameliorates cardiac hypertrophy-induced heart failure in mice by mediating the USP2/JUP/Akt/β-catenin cascade. *BMC Cardiovasc Disord.* 24 (1), 82. doi:10.1186/s12872-024-03748-8

Geoerger, B., Kang, H. J., Yalon-Oren, M., Marshall, L. V., Vezina, C., Pappo, A., et al. (2020a). Pembrolizumab in paediatric patients with advanced melanoma or a PD-L1-positive, advanced, relapsed, or refractory solid tumour or lymphoma (KEYNOTE-051): interim analysis of an open-label, single-arm, phase 1–2 trial. *Lancet Oncol.* 21 (1), 121–133. doi:10.1016/S1470-2045(19)30671-0

Geoerger, B., Zwaan, C. M., Marshall, L. V., Michon, J., Bourdeaut, F., Casanova, M., et al. (2020b). Atezolizumab for children and young adults with previously treated solid tumours, non-Hodgkin lymphoma, and Hodgkin lymphoma (iMATRIX): a multicentre phase 1–2 study. *Lancet Oncol.* 21 (1), 134–144. doi:10.1016/S1470-2045(19)30693-X

Gomez, R. L., Ibragimova, S., Ramachandran, R., Philpott, A., and Ali, F. R. (2022). Tumoral heterogeneity in neuroblastoma. *Biochim. Biophys. Acta Rev. Cancer* 1877, 188805. doi:10.1016/j.bbcan.2022.188805

Green, J. L., Kuntz, S. G., and Sternberg, P. W. (2008). Ror receptor tyrosine kinases: orphans no more. *Trends Cell Biol.* 18 (11), 536–544. doi:10.1016/j.tcb.2008.08.006

Guo, T., Wen, X. Z., Li, Z. Y., Han, H. B., Zhang, C. G., Bai, Y. H., et al. (2019). ISL1 predicts poor outcomes for patients with gastric cancer and drives tumor progression through binding to the ZEB1 promoter together with SETD7. *Cell Death Dis.* 10 (2), 33. doi:10.1038/s41419-018-1278-2

Hamzehloie, T., Mojarrad, M., Hasanzadeh Nazarabadi, M., and Shekouhi, S. (2012). The role of tumor protein 53 mutations in common human cancers and targeting the murine double minute 2–p53 interaction for cancer therapy. *Iran. J. Med. Sci.* 37 (1), 3–8.

Heczey, A., Courtney, A. N., Montalbano, A., Robinson, S., Liu, K., Li, M., et al. (2020). Anti-GD2 CAR-NKT cells in patients with relapsed or refractory neuroblastoma: an interim analysis. *Nat. Med.* 26 (11), 1686–1690. doi:10.1038/s41591-020-1074-2

Heczey, A., Louis, C. U., Savoldo, B., Dakhova, O., Durett, A., Grilley, B., et al. (2017). CAR T cells administered in combination with lymphodepletion and PD-1 inhibition to patients with neuroblastoma. *Mol. Ther.* 25 (9), 2214–2224. doi:10.1016/j.ymthe.2017.05.012

Heitzeneder, S., Bosse, K. R., Zhu, Z., Zhelev, D., Majzner, R. G., Radosevich, M. T., et al. (2022). GPC2-CAR T cells tuned for low antigen density mediate potent activity against neuroblastoma without toxicity. *Cancer Cell* 40 (1), 53–69. doi:10.1016/j.ccell.2021.12.005

Henry, C., Quadir, A., Hawkins, N. J., Jary, E., Llamosas, E., Kumar, D., et al. (2015). Expression of the novel Wnt receptor ROR2 is increased in breast cancer and may regulate both β-catenin dependent and independent Wnt signalling. *J. Cancer Res. Clin. Oncol.* 141, 243–254. doi:10.1007/s00432-014-1824-y

Horton, J. D. (2022). "Pediatric surgery, an issue of surgical clinics," in *E-Book* (New York: Elsevier Health Sciences).

Huebener, N., Lange, B., Lemmel, C., Rammensee, H. G., Strandsby, A., Wenkel, J., et al. (2003). Vaccination with minigenes encoding for novel 'self'antigens are effective in DNA-vaccination against neuroblastoma. *Cancer Lett.* 197 (1-2), 211–217. doi:10.1016/s0304-3835(03)00102-2

Hung, J.-T., and Alice, L. Y. (2019). "GD2-Targeted immunotherapy of neuroblastoma," in *Neuroblastoma* (Amsterdam, Netherlands: Elsevier), 63–78.

Irwin, M. S., Naranjo, A., Zhang, F. F., Cohn, S. L., London, W. B., Gastier-Foster, J. M., et al. (2021). Revised neuroblastoma risk classification system: a report from the Children's Oncology Group. *J. Clin. Oncol.* 39 (29), 3229–3241. doi:10.1200/JCO.21.00278

Irwin, M. S., and Park, J. R. (2015). Neuroblastoma: paradigm for precision medicine. *Pediatr. Clin.* 62 (1), 225–256. doi:10.1016/j.pcl.2014.09.015

Jacobson, J. C., Clark, R. A., and Chung, D. H. (2023). High-risk neuroblastoma: a surgical perspective. *Children* 10 (2), 388. doi:10.3390/children10020388

Janovská, P., and Bryja, V. (2017). Wnt signalling pathways in chronic lymphocytic leukaemia and B-cell lymphomas. *Br. J. Pharmacol.* 174 (24), 4701–4715. doi:10.1111/bph.13949

Jha, S. K., Brown, C., Kang, L., Diaz, E. S., Gwal, K., Alvarez, E., et al. (2023). Update on the role of imaging in staging of common pediatric abdominal tumors. *Curr. Probl. Cancer* 47, 100969. doi:10.1016/j.currproblcancer.2023.100969

Jones, D. T., Banito, A., Grünewald, T. G. P., Haber, M., Jäger, N., Kool, M., et al. (2019). Molecular characteristics and therapeutic vulnerabilities across paediatric solid tumours. *Nat. Rev. Cancer* 19 (8), 420–438. doi:10.1038/s41568-019-0169-x

Kanda, M., Nomoto, S., Oya, H., Shimizu, D., Takami, H., Hibino, S., et al. (2014). Dihydropyrimidinase-like 3 facilitates malignant behavior of gastric cancer. *J. Exp. Clin. Cancer Res.* 33 (1), 66. doi:10.1186/s13046-014-0066-9

Karlsson, O., Thor, S., Norberg, T., Ohlsson, H., and Edlund, T. (1990). Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo-and a Cys–His domain. *Nature* 344 (6269), 879–882. doi:10.1038/344879a0

Kattner, P., Strobel, H., Khoshnevis, N., Grunert, M., Bartholomae, S., Pruss, M., et al. (2019). Compare and contrast: pediatric cancer versus adult malignancies. *Cancer Metastasis Rev.* 38, 673–682. doi:10.1007/s10555-019-09836-y

Kawahara, T., Hotta, N., Ozawa, Y., Kato, S., Kano, K., Yokoyama, Y., et al. (2013). Quantitative proteomic profiling identifies DPYSL3 as pancreatic ductal adenocarcinoma-associated molecule that regulates cell adhesion and migration by stabilization of focal adhesion complex. *PLoS One* 8 (12), e79654. doi:10.1371/journal.pone.0079654

Kholodenko, I. V., Kalinovsky, D. V., Doronin, I. I., Deyev, S. M., and Kholodenko, R. V. (2018a). Neuroblastoma origin and therapeutic targets for immunotherapy. *J. Immunol. Res.* 2018, 7394268. doi:10.1155/2018/7394268

Kholodenko, I. V., Kalinovsky, D. V., Doronin, I. I., Deyev, S. M., and Kholodenko, R. V. (2018b). Neuroblastoma origin and therapeutic targets for immunotherapy. *J. Immunol. Res.* 2018, 7394268. doi:10.1155/2018/7394268

Kim, E., and Shohet, J. (2009). *Targeted molecular therapy for neuroblastoma: the ARF/MDM2/p53 axis*. Oxford: Oxford University Press, 1527–1529.

Kim, Y., Hong, M., Do, I. G., Ha, S. Y., Lee, D., and Suh, Y. L. (2015). Wnt5a, Ryk and Ror2 expression in glioblastoma subgroups. *Pathol. Res. Pract.* 211 (12), 963–972. doi:10.1016/j.prp.2015.10.001

Kitchen, M. O., Bryan, R. T., Haworth, K. E., Emes, R. D., Luscombe, C., Gommersall, L., et al. (2015). Methylation of HOXA9 and ISL1 predicts patient outcome in high-grade non-invasive bladder cancer. *PloS one* 10 (9), e0137003. doi:10.1371/journal.pone.0137003

Komorowski, M., Tisonczyk, J., Kolakowska, A., Drozdz, R., and Kozbor, D. (2018). Modulation of the tumor microenvironment by CXCR4 antagonist-armed viral oncotherapy enhances the antitumor efficacy of dendritic cell vaccines against neuroblastoma in syngeneic mice. *Viruses* 10 (9), 455. doi:10.3390/v10090455

Kushner, B. H., Modak, S., Kramer, K., Basu, E. M., Iglesias-Cardenas, F., Roberts, S. S., et al. (2023). Immunotherapy with anti-GD2 monoclonal antibody in infants with high-risk neuroblastoma. *Int. J. Cancer* 152 (2), 259–266. doi:10.1002/ijc.34233

Le Grand, M., Kimpton, K., Gana, C. C., Valli, E., Fletcher, J. I., and Kavallaris, M. (2020). Targeting functional activity of AKT has efficacy against aggressive neuroblastoma. *ACS Pharmacol. Transl. Sci.* 3 (1), 148–160. doi:10.1021/acsptsci.9b00085

Li, M., Sun, C., Bu, X., Que, Y., Zhang, L., Zhang, Y., et al. (2021a). ISL1 promoted tumorigenesis and EMT via Aurora kinase A-induced activation of PI3K/AKT signaling pathway in neuroblastoma. *Cell Death Dis.* 12 (6), 620. doi:10.1038/s41419-021-03894-3

Li, N., Fu, H., Hewitt, S. M., Dimitrov, D. S., and Ho, M. (2017). Therapeutically targeting glypican-2 via single-domain antibody-based chimeric antigen receptors and immunotoxins in neuroblastoma. *Proc. Natl. Acad. Sci.* 114 (32), E6623–E6631. doi:10.1073/pnas.1706055114

Li, N., Torres, M. B., Spetz, M. R., Wang, R., Peng, L., Tian, M., et al. (2021b). CAR T cells targeting tumor-associated exons of glypican 2 regress neuroblastoma in mice. *Cell Rep. Med.* 2 (6), 100297. doi:10.1016/j.xcrm.2021.100297

Li, P., Harris, D., Liu, Z., Liu, J., Keating, M., and Estrov, Z. (2010). Stat3 activates the receptor tyrosine kinase like orphan receptor-1 gene in chronic lymphocytic leukemia cells. *PLoS One* 5 (7), e11859. doi:10.1371/journal.pone.0011859

Louis, C. U., Savoldo, B., Dotti, G., Pule, M., Yvon, E., Myers, G. D., et al. (2011). Antitumor activity and long-term fate of chimeric antigen receptor–positive T cells in patients with neuroblastoma. *Am. J. Hematol.* 118 (23), 6050–6056. doi:10.1182/blood-2011-05-354449

Luksch, R., Castellani, M. R., Collini, P., De Bernardi, B., Conte, M., Gambini, C., et al. (2016). Neuroblastoma (Peripheral neuroblastic tumours). *Crit. Rev. Oncol. Hematol.* 107, 163–181. doi:10.1016/j.critrevonc.2016.10.001

Machy, P., Mortier, E., and Birklé, S. (2023). Biology of GD2 ganglioside: implications for cancer immunotherapy. *Front. Pharmacol.* 14, 1249929. doi:10.3389/fphar.2023.1249929

Mackall, C. L., Merchant, M. S., and Fry, T. J. (2014). Immune-based therapies for childhood cancer. *Nat. Rev. Clin. Oncol.* 11 (12), 693–703. doi:10.1038/nrclinonc.2014.177

Majzner, R. G., Rietberg, S. P., Sotillo, E., Dong, R., Vachharajani, V. T., Labanieh, L., et al. (2020). Tuning the antigen density requirement for CAR T-cell activity. *Cancer Discov.* 10 (5), 702–723. doi:10.1158/2159-8290.CD-19-0945

Manivannan, J., Tay, S. S. W., Ling, E. A., and Dheen, S. T. (2013). Dihydropyrimidinase-like 3 regulates the inflammatory response of activated microglia. *Neurosci* 253, 40–54. doi:10.1016/j.neuroscience.2013.08.023

Masiakowski, P., and Carroll, R. D. (1992). A novel family of cell surface receptors with tyrosine kinase-like domain. *J. Biol. Chem.* 267 (36), 26181–26190. doi:10.1016/s0021-9258(18)35733-8

Masiakowski, P., and Yancopoulos, G. D. (1998). The Wnt receptor CRD domain is also found in MuSK and related orphan receptor tyrosine kinases. *Curr. Biol.* 8 (12), R407. doi:10.1016/s0960-9822(98)70263-5

Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N. J., et al. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. *N. Eng. J. Cancer* 371 (16), 1507–1517. doi:10.1056/NEJMoa1407222

Maude, S. L., Laetsch, T. W., Buechner, J., Rives, S., Boyer, M., Bittencourt, H., et al. (2018). Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N. Eng. J. Cancer* 378 (5), 439–448. doi:10.1056/NEJMoa1709866

Mehrvar, A., Sadeghi, Y., Mehrvar, N., Faranoush, M., Alebouyeh, M., Roozrokh, M., et al. (2023). Prognosis, survival and management of pediatric patients with neuroblastoma: a 12-year experience from a single center study. *Acta Medica Iran.*, 275–281. doi:10.18502/acta.v61i5.13482

Menck, K., Heinrichs, S., Baden, C., and Bleckmann, A. (2021). The WNT/ROR pathway in cancer: from signaling to therapeutic intervention. *Cells* 10 (1), 142. doi:10.3390/cells10010142

Mishra, S., Kelly, K. K., Rumian, N. L., and Siegenthaler, J. A. (2018). Retinoic acid is required for neural stem and progenitor cell proliferation in the adult hippocampus. *Stem Cell Rep.* 10 (6), 1705–1720. doi:10.1016/j.stemcr.2018.04.024

Modak, S., and Cheung, N.-K. V. (2010). Neuroblastoma: therapeutic strategies for a clinical enigma. *Cancer Treat. Rev.* 36 (4), 307–317. doi:10.1016/j.ctrv.2010.02.006

Molenaar, J. J., Koster, J., Zwijnenburg, D. A., van Sluis, P., Valentijn, L. J., van der Ploeg, I., et al. (2012). Sequencing of neuroblastoma identifies chromothripsis and defects in neuritogenesis genes. *Nature* 483 (7391), 589–593. doi:10.1038/nature10910

Monclair, T., Brodeur, G. M., Ambros, P. F., Brisse, H. J., Cecchetto, G., Holmes, K., et al. (2009). The international neuroblastoma risk group (INRG) staging system: an INRG task force report. *J. Clin. Oncol.* 27 (2), 298–303. doi:10.1200/JCO.2008.16.6876

Morandi, F., Sabatini, F., Podestà, M., and Airoldi, I. (2021). Immunotherapeutic strategies for neuroblastoma: present, past and future. *Vaccines* 9 (1), 43. doi:10.3390/vaccines9010043

Mukhtar, M., Bilal, M., Rahdar, A., Barani, M., Arshad, R., Behl, T., et al. (2020). Nanomaterials for diagnosis and treatment of brain cancer: recent updates. *Chemosensors* 8 (4), 117. doi:10.3390/chemosensors8040117

Nag, S., Zhang, X., Srivenugopal, K. S., Wang, M. H., Wang, W., and Zhang, R. (2014). Targeting MDM2-p53 interaction for cancer therapy: are we there yet? *Curr. Med. Chem.* 21 (5), 553–574. doi:10.2174/0929867311320660325

Neuroblastoma, C. (1931). *Cancer association of South Africa (CANSA)*.

Newman, E. A., Abdessalam, S., Aldrink, J. H., Austin, M., Heaton, T. E., Bruny, J., et al. (2019). Update on neuroblastoma. *J. Pediatr. Surg.* 54 (3), 383–389. doi:10.1016/j.jpedsurg.2018.09.004

Nguyen, R., Zhang, X., Sun, M., Abbas, S., Seibert, C., Kelly, M. C., et al. (2022). Anti-Gd2 antibodies conjugated to IL15 and IL21 mediate potent antitumor cytotoxicity against neuroblastoma. *Clin. Cancer Res.* 28 (17), 3785–3796. doi:10.1158/1078-0432.CCR-22-0717

Nicolai, S., Pieraccioli, M., Peschiaroli, A., Melino, G., and Raschellà, G. (2015). Neuroblastoma: oncogenic mechanisms and therapeutic exploitation of necroptosis. *Cell Death Dis.* 6 (12), e2010. doi:10.1038/cddis.2015.354

Olivier, M., Hollstein, M., and Hainaut, P. (2010). TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb. Perspect. Biol.* 2 (1), a001008. doi:10.1101/cshperspect.a001008

Ong Tone, S., Dayanandan, B., Fournier, A. E., and Mandato, C. A. (2010). GSK3 regulates mitotic chromosomal alignment through CRMP4. *PLoS One* 5 (12), e14345. doi:10.1371/journal.pone.0014345

Paraboschi, I., Privitera, L., Kramer-Marek, G., Anderson, J., and Giuliani, S. (2021). Novel treatments and technologies applied to the cure of neuroblastoma. *Children* 8 (6), 482. doi:10.3390/children8060482

Park, J. R., Eggert, A., and Caron, H. (2010). Neuroblastoma: biology, prognosis, and treatment. *Hematol. Oncol. Clin. North Am.* 24 (1), 65–86. doi:10.1016/j.hoc.2009.11.011

Parrales, A., and Iwakuma, T. (2015). Targeting oncogenic mutant p53 for cancer therapy. *Front. Oncol.* 5, 288. doi:10.3389/fonc.2015.00288

Pilon, N. (2021). Treatment and prevention of neurocristopathies. *Trends Mol. Med.* 27 (5), 451–468. doi:10.1016/j.molmed.2021.01.009

Pinto, N. R., Applebaum, M. A., Volchenboum, S. L., Matthay, K. K., London, W. B., Ambros, P. F., et al. (2015). Advances in risk classification and treatment strategies for neuroblastoma. *J. Clin. Oncol.* 33 (27), 3008–3017. doi:10.1200/JCO.2014.59.4648

Pistoia, V., Bianchi, G., Borgonovo, G., and Raffaghello, L. (2011). Cytokines in neuroblastoma: from pathogenesis to treatment. *Immunotherapy* 3 (7), 895–907. doi:10.2217/imt.11.80

Ponnusamy, R., Lebedev, A. A., Pahlow, S., and Lohkamp, B. (2014). Crystal structure of human CRMP-4: correction of intensities for lattice-translocation disorder. *Acta Crystallogr. D.* 70 (6), 1680–1694. doi:10.1107/S1399004714006634

Ponzoni, M., Bachetti, T., Corrias, M. V., Brignole, C., Pastorino, F., Calarco, E., et al. (2022). Recent advances in the developmental origin of neuroblastoma: an overview. *J. Exp. Clin. Cancer Res.* 41 (1), 92–28. doi:10.1186/s13046-022-02281-w

Pudela, C., Balyasny, S., and Applebaum, M. A. (2020). Nervous system: embryonal tumors: neuroblastoma. *Atlas Genet. Cytogenet. Oncol. Haematol.* 24 (7), 284–290. doi:10.4267/2042/70771

Pugh, T. J., Morozova, O., Attiyeh, E. F., Asgharzadeh, S., Wei, J. S., Auclair, D., et al. (2013). The genetic landscape of high-risk neuroblastoma. *Nat. Genet.* 45 (3), 279–284. doi:10.1038/ng.2529

Qiu, B., and Matthay, K. K. (2022). Advancing therapy for neuroblastoma. *Nat. Rev. Clin. Oncol.* 19 (8), 515–533. doi:10.1038/s41571-022-00643-z

Quezada, M. J., and Lopez-Bergami, P. (2023). The signaling pathways activated by ROR1 in cancer. *Cell Signal* 104, 110588. doi:10.1016/j.cellsig.2023.110588

Raieli, S., Di Renzo, D., Lampis, S., Amadesi, C., Montemurro, L., Pession, A., et al. (2021). MYCN drives a tumor immunosuppressive environment which impacts survival in neuroblastoma. *Front. Oncol.* 11, 625207. doi:10.3389/fonc.2021.625207

Richards, R. M., Sotillo, E., and Majzner, R. G. (2018). CAR T cell therapy for neuroblastoma. *Front. Immunol.* 9, 2380. doi:10.3389/fimmu.2018.02380

Rickman, D. S., Schulte, J. H., and Eilers, M. (2018). The expanding world of N-MYC–driven tumors. *Cancer Discov.* 8 (2), 150–163. doi:10.1158/2159-8290.CD-17-0273

Rosslenbroich, V., Dai, L., Baader, S. L., Noegel, A. A., Gieselmann, V., and Kappler, J. (2005). Collapsin response mediator protein-4 regulates F-actin bundling. *Exp. Cell Res.* 310 (2), 434–444. doi:10.1016/j.yexcr.2005.08.005

Shohet, J. M., Nuchtern, J. G., and Foster, J. H. (2022). *Treatment and prognosis of neuroblastoma*. Waltham, MA: UpToDate.

Slattery, K., and Gardiner, C. M. (2019). NK cell metabolism and TGFβ–implications for immunotherapy. *Front. Immunol.* 10, 2915. doi:10.3389/fimmu.2019.02915

Sokol, E., and Desai, A. V. (2019). The evolution of risk classification for neuroblastoma. *Children* 6 (2), 27. doi:10.3390/children6020027

Steen, E. A., Basilaia, M., Kim, W., Getz, T., Gustafson, J. L., and Zage, P. E. (2023). Targeting the RET tyrosine kinase in neuroblastoma: a review and application of a novel selective drug design strategy. *Biochem. Pharmacol.* 216, 115751. doi:10.1016/j.bcp.2023.115751

Straathof, K., Flutter, B., Wallace, R., Jain, N., Loka, T., Depani, S., et al. (2020). Antitumor activity without on-target off-tumor toxicity of GD2–chimeric antigen receptor T cells in patients with neuroblastoma. *Sci. Transl. Med.* 12 (571), eabd6169. doi:10.1126/scitranslmed.abd6169

Sun, M., Cao, Y., Okada, R., Reyes-González, J. M., Stack, H. G., Qin, H., et al. (2023). Pre-clinical optimization of a GPC2-targeting CAR T-cell therapy for neuroblastoma. *J. Immunother. Cancer* 11 (1), e005881. doi:10.1136/jitc-2022-005881

Tan, F., Wahdan-Alaswad, R., Yan, S., Thiele, C. J., and Li, Z. (2013). Dihydropyrimidinase-like protein 3 expression is negatively regulated by MYCN and associated with clinical outcome in neuroblastoma. *Cancer Sci.* 104 (12), 1586–1592. doi:10.1111/cas.12278

Tian, M., Cheuk, A. T., Wei, J. S., Abdelmaksoud, A., Chou, H. C., Milewski, D., et al. (2022). An optimized bicistronic chimeric antigen receptor against GPC2 or CD276 overcomes heterogeneous expression in neuroblastoma. *J. Clin. Invest.* 132 (16), e155621. doi:10.1172/JCI155621

Tibbetts, R., Yeo, K. K., Muthugounder, S., Lee, M. H., Jung, C., Porras-Corredor, T., et al. (2022). Anti-disialoganglioside antibody internalization by neuroblastoma cells as a mechanism of immunotherapy resistance. *Cancer Immun.* 71 (1), 153–164. doi:10.1007/s00262-021-02963-y

Tolbert, V. P., and Matthay, K. K. (2018). Neuroblastoma: clinical and biological approach to risk stratification and treatment. *Cell Tissue Res.* 372, 195–209. doi:10.1007/s00441-018-2821-2

Tonini, G. P., and Capasso, M. (2020). Genetic predisposition and chromosome instability in neuroblastoma. *Cancer Metastasis Rev.* 39, 275–285. doi:10.1007/s10555-020-09843-4

Unguru, Y., Joffe, S., Fernandez, C. V., and Yu, A. L. (2013). Ethical issues for control-arm patients after revelation of benefits of experimental therapy: a framework modeled in neuroblastoma. *J. Clin. Oncol.* 31 (5), 641–646. doi:10.1200/JCO.2012.47.1227

Vanichapol, T., Chutipongtanate, S., Anurathapan, U., and Hongeng, S. (2018). Immune escape mechanisms and future prospects for immunotherapy in neuroblastoma. *Biomed. Res. Int.* 2018, 1812535. doi:10.1155/2018/1812535

Van Maerken, T., Speleman, F., Vermeulen, J., Lambertz, I., De Clercq, S., De Smet, E., et al. (2006). Small-molecule MDM2 antagonists as a new therapy concept for neuroblastoma. *Cancer Res.* 66 (19), 9646–9655. doi:10.1158/0008-5472.CAN-06-0792

Varadé, J., Magadán, S., and González-Fernández, Á. (2021). Human immunology and immunotherapy: main achievements and challenges. *Cell Mol. Immunol.* 18 (4), 805–828. doi:10.1038/s41423-020-00530-6

Veal, G. J., Tweddle, D. A., Visser, J., Errington, J., Buck, H., Marange, J., et al. (2021). Pharmacokinetics and safety of a novel oral liquid formulation of 13-cis retinoic acid in children with neuroblastoma: a randomized crossover clinical trial. *Cancers* 13 (8), 1868. doi:10.3390/cancers13081868

Veschi, V., Verona, F., and Thiele, C. J. (2019). Cancer stem cells and neuroblastoma: characteristics and therapeutic targeting options. *Front. Endocrin* 10, 782. doi:10.3389/fendo.2019.00782

Voicu, V., Tataru, C. P., Toader, C., Covache-Busuioc, R. A., Glavan, L. A., Bratu, B. G., et al. (2023). Decoding neurodegeneration: a comprehensive review of molecular mechanisms, genetic influences, and therapeutic innovations. *Int. J. Mol. Sci.* 24 (16), 13006. doi:10.3390/ijms241613006

Wagner, L. M., and Adams, V. R. (2017). Targeting the PD-1 pathway in pediatric solid tumors and brain tumors. *Onco Targets Ther.* 10, 2097–2106. doi:10.2147/OTT.S124008

Wang, M., Zhai, X., Li, J., Guan, J., Xu, S., Li, Y., et al. (2021). The role of cytokines in predicting the response and adverse events related to immune checkpoint inhibitors. *Front. Immunol.* 12, 670391. doi:10.3389/fimmu.2021.670391

Webb, E. R., Lanati, S., Wareham, C., Easton, A., Dunn, S. N., Inzhelevskaya, T., et al. (2020). Immune characterization of pre-clinical murine models of neuroblastoma. *Sci. Rep.* 10 (1), 16695. doi:10.1038/s41598-020-73695-9

Wienke, J., Dierselhuis, M. P., Tytgat, G. A. M., Künkele, A., Nierkens, S., and Molenaar, J. J. (2021). The immune landscape of neuroblastoma: challenges and opportunities for novel therapeutic strategies in pediatric oncology. *Eur. J. Cancer* 144, 123–150. doi:10.1016/j.ejca.2020.11.014

Wolfson, B., Franks, S. E., and Hodge, J. W. (2021). Stay on target: reengaging cancer vaccines in combination immunotherapy. *Vaccines (Basel)* 9 (5), 509. doi:10.3390/vaccines9050509

Woo, C. W., Tan, F., Cassano, H., Lee, J., Lee, K. C., and Thiele, C. J. (2008). Use of RNA interference to elucidate the effect of MYCN on cell cycle in neuroblastoma. *Pediatr blood cancer* 50 (2), 208–212. doi:10.1002/pbc.21195

Xue, C., Haber, M., Flemming, C., Marshall, G. M., Lock, R. B., MacKenzie, K. L., et al. (2007). p53 determines multidrug sensitivity of childhood neuroblastoma. *Cancer Res.* 67 (21), 10351–10360. doi:10.1158/0008-5472.CAN-06-4345

Yu, A. L., Gilman, A. L., Ozkaynak, M. F., Naranjo, A., Diccianni, M. B., Gan, J., et al. (2021). Long-term follow-up of a phase III study of ch14. 18 (dinutuximab)+ cytokine immunotherapy in children with high-risk neuroblastoma: COG study ANBL0032. *Clin. Cancer Res.* 27 (8), 2179–2189. doi:10.1158/1078-0432.CCR-20-3909

Yu, J., Chen, L., Cui, B., Widhopf, G. F., Shen, Z., Wu, R., et al. (2016). Wnt5a induces ROR1/ROR2 heterooligomerization to enhance leukemia chemotaxis and proliferation. *J. Clin. Invest.* 126 (2), 585–598. doi:10.1172/JCI83535

Zhang, Q., Huang, R., Ye, Y., Guo, X., Lu, J., Zhu, F., et al. (2018a). Temporal requirements for ISL1 in sympathetic neuron proliferation, differentiation, and diversification. *Cell Death Dis.* 9 (2), 247. doi:10.1038/s41419-018-0283-9

Zhang, Q., Jiang, X., Ye, Y., Liao, H., and Zhu, F. (2019). Collaborative ISL1/GATA3 interaction in controlling neuroblastoma oncogenic pathways overlapping with but distinct from MYCN. *Theranostics* 9 (4), 986–1000. doi:10.7150/thno.30199

Zhang, S., Chen, L., Cui, B., Chuang, H. Y., Yu, J., Wang-Rodriguez, J., et al. (2012b). ROR1 is expressed in human breast cancer and associated with enhanced tumor-cell growth. *PloS one* 7 (3), e31127. doi:10.1371/journal.pone.0031127

Zhang, S., Chen, L., Wang-Rodriguez, J., Zhang, L., Cui, B., Frankel, W., et al. (2012a). The onco-embryonic antigen ROR1 is expressed by a variety of human cancers. *Am. J. Pathol.* 181 (6), 1903–1910. doi:10.1016/j.ajpath.2012.08.024

Zhang, Y., Wang, L., Gao, P., Sun, Z., and Lu, Y. (2018b). ISL1 promotes cancer progression and inhibits cisplatin sensitivity in triple-negative breast cancer cells. *Int. J. Mol. Med.* 42 (5), 2343–2352. doi:10.3892/ijmm.2018.3842

Zhang, Y., Wu, W., Sun, Q., Ye, L., Zhou, D., and Wang, W. (2021). linc-ROR facilitates hepatocellular carcinoma resistance to doxorubicin by regulating TWIST1-mediated epithelial-mesenchymal transition. *Mol. Med. Rep.* 23 (5), 340–349. doi:10.3892/mmr.2021.11979

Zhou, S., Zhang, M., Zhou, C., Wang, W., Yang, H., and Ye, W. (2020). The role of epithelial-mesenchymal transition in regulating radioresistance. *Crit. Rev. Oncol. Hematol.* 150, 102961. doi:10.1016/j.critrevonc.2020.102961

frontiers | Frontiers in Cell and Developmental Biology

TYPE Review
PUBLISHED 03 June 2024
DOI 10.3389/fcell.2024.1394140

OPEN ACCESS

EDITED BY
Ajinkya Revandkar,
Massachusetts General Hospital Cancer Center, United States

REVIEWED BY
Jelena Milosevic,
Massachusetts General Hospital and Harvard Medical School, United States
Muhammad Shahzad Ali,
LifeSensors, United States

*CORRESPONDENCE
Ruipeng Song,
song.ruipeng@163.com

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

RECEIVED 01 March 2024
ACCEPTED 17 May 2024
PUBLISHED 03 June 2024

CITATION
Li M, Li J, Zhang S, Zhou L, Zhu Y, Li S, Li Q, Wang J and Song R (2024), Progress in the study of autophagy-related proteins affecting resistance to chemotherapeutic drugs in leukemia.
Front. Cell Dev. Biol. 12:1394140.
doi: 10.3389/fcell.2024.1394140

Progress in the study of autophagy-related proteins affecting resistance to chemotherapeutic drugs in leukemia

Meng Li[1†], Jing Li[2†], Shiming Zhang[3†], Linghan Zhou[1], Yuanyuan Zhu[1], Shen Li[4], Qiong Li[5], Junjie Wang[6] and Ruipeng Song[7*]

[1]Nursing Department, The Third People's Hospital of Henan Province, Zhengzhou, China, [2]Department of Pathophysiology, Sepsis Translational Medicine Key Laboratory of Hunan Province, Xiangya School of Medicine, Central South University, Changsha, Hunan, China, [3]Clinical College, Xiamen Medical University, Xiamen, Fujian, China, [4]Rehabilitation Department, Henan Institute of Massage, Luoyang, Henan, China, [5]Nursing Department, Xinxiang Medical University, Xinxiang, China, [6]Plastic Surgery, The Third People's Hospital of Henan Province, Zhengzhou, China, [7]Endocrinology Department, The Third People's Hospital of Henan Province, Zhengzhou, China

Leukemia is a life-threatening malignant tumor of the hematopoietic system. Currently, the main treatment modalities are chemotherapy and hematopoietic stem cell transplantation. However, increased drug resistance due to decreased sensitivity of leukemia cells to chemotherapeutic drugs presents a major challenge in current treatments. Autophagy-associated proteins involved in autophagy initiation have now been shown to be involved in the development of various types of leukemia cells and are associated with drug resistance. Therefore, this review will explore the roles of autophagy-related proteins involved in four key autophagic processes: induction of autophagy and phagophore formation, phagophore extension, and autophagosome formation, on the development of various types of leukemias as well as drug resistance. Autophagy may become a promising therapeutic target for treating leukemia.

KEYWORDS

autophagy-associated proteins, leukaemia, drug tolerance, chemotherapeutic drugs, review

1 Introduction

Leukemia is a disease in which the normal physiological activity of the bone marrow is impeded by the overproduction of immature white blood cells in the bone marrow and blood tissues, resulting in abnormal hematopoietic function and destruction of organs. It is a life-threatening malignant tumor of the hematopoietic system. Clinically, there are four main types of leukemia, namely, acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML). Currently, the more common treatments for leukemia are chemotherapy and hematopoietic stem cell transplantation. Recently, the advent of targeted drugs has mitigated several leukemias to some extent. For example, 80%–90% of patients with

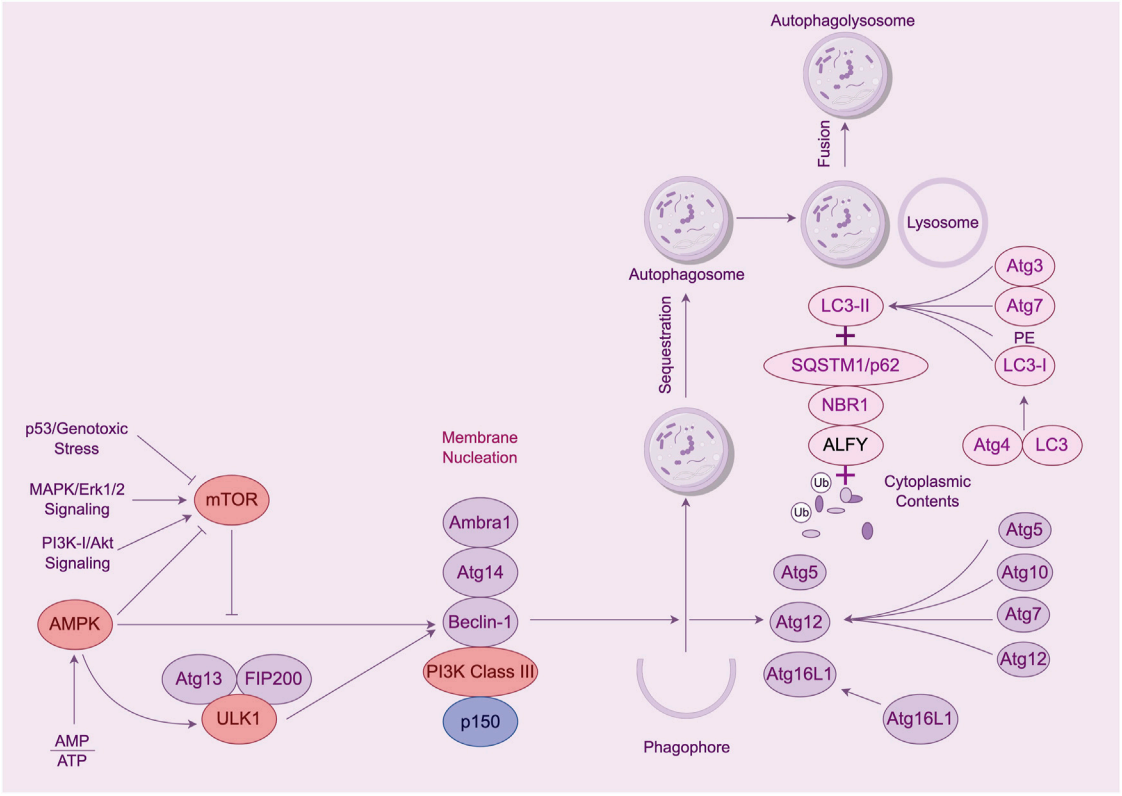


FIGURE 1
The process of cellular autophagy and its key proteins. Note: The mTOR kinase is a key molecule in autophagosome induction. mTOR activation pathways such as Akt and MAPK signaling pathways inhibit autophagy, and pathways that negatively regulate mTOR, such as AMPK and p53 signaling pathways, promote autophagy. mTOR kinases are the only core proteins of the autophagosome signaling pathway that have a serine/threonine kinase activity. Prior to autophagy lysosome assembly autophagy signaling is mediated through the activation of the ULK complex composed of ULK1, FIP200, and Atg13. The ULK1 complex *in vivo* serves as a bridge connecting the upstream nutrient or energy-receptor mTOR and AMPK to the formation of downstream autophagosomes. AMPK activates the phosphorylation of ULK1 thereby facilitating the assembly of the ULK1 complex to initiate autophagy. Class Lll PI3K complex includes Beclin-1, Atg14, p150, and Ambra1, all of which are required for the induction of autophagy. The Atg genes control autophagosome formation through the Atg12-Atg5 and LC3-ll complexes. Atg12 binds to Atg5 in a ubiquitin-like reaction that requires Atg7 and Atg10, which are E1-and E2-like enzymes, respectively. coupling. The Atg12-Atg5 linker then reacts noncovalently with Atg16 to form a larger complex. The C-terminal cup of LC3/Atg8 is proteolytically cleaved by the Atg4 protease to generate the cytoplasmic LC3-l.LC3-l is also coupled to phosphatidylethanolamine (PE) in a ubiquitin-like reaction, a reaction that requires both Atg7 and Atg3. A lipid form of LC3, known as LC3-ll, adsorbs to the autophagosome membrane. This links LC3 to autophagic vesicles. The presence of LC3 in autophagosomes and its conversion to the low-migratory form, LC3-ll, is used as an indicator of the onset of autophagy.

acute promyelocytic leukemia (APL) are cured following the use of trans-retinoic acid and arsenic trioxide (Lo-Coco et al., 2013), and ponatinib, a third-generation drug of tyrosine kinase inhibitor (TKI) therapy, offers a treatment option for CML patients with the Bcr-Abl T315I mutation (Breccia et al., 2018). Some subtypes of leukemia remain poorly treated, especially AML. AML is primarily characterized by complex and dynamic genomic instability, AML patients under 60 years of age have a better prognosis and cure rates approaching 35%–40%, only 5%–15% of patients over 60 years of age are in remission (Döhner et al., 2015). AML is prone to relapse and drug resistance, which may be due to mutations in genes associated with epigenetic modifications (TET2, IDH1 and IDH2, DNMT3A, ASXL1, WT1, EZH2), genes associated with dysregulation of DNA repair (TP53, NPM1), and genes associated with defects in cell cycle inhibition and differentiation (NPM1, CEBPA, TP53, and GATA2).

Cellular autophagy is a process by which cells encapsulate parts of their cytoplasm and organelles in a double membrane structure and fuse them with lysosomes for degradation and recycling. The purpose of cellular autophagy is to eliminate excess or defective cellular components, renew cellular structures, provide energy and raw materials, and protect cell and tissue function. Cellular autophagy is a conserved cellular process that is associated with cell and tissue regeneration, aging, and diseaseTranslated with www.DeepL.com/Translator (free version). Which is categorized into three main types: macroautophagy, microautophagy, and chaperone-mediated autophagy. All three of these different forms of autophagy ultimately deliver the phagocytosed material to the lysosome for degradation and recycling (Yang and Klionsky, 2010). The process of autophagy is broadly divided into the following four stages: induction of autophagy, assembly and formation of autophagosomes, fusion of autophagosomes with lysosomes, and degradation and recirculation of autophagosome contents (Li X. et al., 2020). The discovery of autophagy-related proteins has led to a better understanding of the molecular mechanisms of

TABLE 1 The process of autophagy and its involved proteins.

Stage of autophagy	Key players/Proteins	Function of each protein	References
Autophagy Initiation	ULK1 complex (ULK1, ATG13, FIP200, ATG101), mTORC1	ULK1: Initiates autophagy by phosphorylation of the autophagy machinery; phosphorylated by AMPK or mTORC1 ATG13:Mediates the junction of the interaction between ULK1 and FIP200; enhances ULK1 kinase activity, phosphorylated by mTORC1 FIP200: Component of the ULK1 complex; scaffolding role (ULK1/2 and ATG13)	Mizushima (2010), Jung et al. (2009), Hara et al. (2008), Ganley et al. (2009), Lee et al. (2020)
Phagophore Formation	Beclin-1, VPS34, ATG14, VPS15 (PI3K complex)	Beclin-1: Promotes PI3K1-C1 complex assembly; regulates VSP34; ULK1/AMPK phosphorylation sites; promotes autophagy VPS34: Promotes PI3KC3-C1 complex formation, a phosphorylation site for ULK1, and stabilizes the ULK1 complex ATG14: ULK1 phosphorylation site; targets PI3K3-C1 to autophagosome formation sites; contributes to phagosome expansion VPS15: Serine/threonine kinase; VPS34 regulatory protein	Backer (2008), Hu et al. (2021), Peng et al. (2013), Can et al. (2011)
Phagophore Extension	ATG12–ATG5-ATG16L1 complex, LC3/ATG8 (and PE conjugation)	ATG12–ATG5-ATG16L1 complex: ATG5 directly binds membranes, and this membrane binding is negatively regulated by ATG12 but activated by ATG16; membrane binding of the ATG12-ATG5-ATG16L1 complex is required to efficiently promote ATG8 esterification (conversion of LC3-l to LC3-ll) LC3/ATG8: Exists in two forms, LC3-1 and LC3-II; involved in the formation of autophagosome membranes, binds to PE on the surface of autophagosome membranes, and can be used as a labeling molecule for autophagosomes	Dooley et al. (2014), Tanida et al. (2004), Piya et al. (2016)
Autophagosome Formation	ATG2, WIPI1-4, ATG9	ATG2:ATG2 is part of the ATG9/ATG12-WIPI complex, which is essential for ATG9 recruitment to expand extended autophagosomes WIPI1-4:WIPI 1-4 is part of the ATG2-WIPI complex, which is important for ATG9 recruitment to autophagosomes, binds to PI3P, which is required for retrograde transport of ATG9, and to ATG2 ATG9: Transmembrane protein; interacts with ATG2-WlPl complex; shuttles between PAS and peripheral organelles to deliver lipids/factors during phagophore expansion, and self-interaction	Jang et al. (2017b), Dooley et al. (2014), Li et al. (2021)

autophagy regulation. For example, the ULK1 complex is involved in the formation of phagocytic vesicles and controls the extension of phagocytic vesicles and the formation of autophagosomes (Mizushima, 2010), and Beclin1, a core subunit in the PI3KC3 complex, interacts with another core subunit, VPS34, to activate VPS34 kinase activity to regulate the size and number of autophagosomes (Backer, 2008).

Autophagy is a process by which cells degrade themselves. See Figure 1 and Table 1. It is important for balancing sources of energy and coping with nutritional stress during critical periods of development. Also autophagy and autophagy-related proteins are involved in the development of leukemia (Piya et al., 2016; Hu et al., 2018; Pei et al., 2018). This article focuses on a review of the genes and proteins involved in the autophagy process in association with leukemia.

2 Autophagy initiation and phagophore formation

Autophagy can be induced by a variety of intra- and extracellular factors. For example, mild uncoupling of oxidative phosphorylation can be influenced by mitochondria-targeted cations thereby inducing autophagy (Lyamzaev et al., 2018), and autophagy can be induced when there are fewer mTOR kinases localized to the lysosome and the activity of mTORC1 is reduced (Hertel et al., 2022). Stimulated by these intra- and extracellular factors, the pre-autophagic structure (PAS), as a structure that can recruit autophagy-associated proteins (Atg), recruits almost all autophagy-associated proteins. Among them, ULK1 complex and PI3K complex target PAS in a hierarchical manner and participate in the formation and assembly of autophagosomes (Mizushima et al.,

TABLE 2 The role of proteins in autophagy in leukemia.

Protein	Mechanism of action in leukemia	Signaling pathways and related proteins	Specific types of leukemia	References
ULK1	Initiates autophagy, aiding leukemia cell survival in nutrient-poor conditions. Linked to chemoresistance and poor prognosis	AMPK activates ULK1 under stress; mTORC1 inhibits it under nutrient-rich conditions. Involved in the AMPK/mTOR signaling pathway	Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL)	Mizushima (2010), Jung et al. (2009), Jang et al. (2017a), Ianniciello et al. (2021), Ianniciello and Helgason (2022)
mTORC1	Promotes cell growth and proliferation by inhibiting autophagy. Upregulated activity is linked to therapy resistance	Regulates ULK1 through phosphorylation. Part of the PI3K/AKT/mTOR pathway	Acute Lymphoblastic Leukemia (ALL), AML	Ganley et al. (2009), He et al. (2016), Yu et al. (2020), Bosnjak et al. (2014))
TIGAR	Reduces ROS, shifts metabolism, indirectly modulating autophagy and contributing to resistance	Operates downstream of p53, affecting glycolysis and the pentose phosphate pathway	AML, particularly in relation to metabolic reprogramming	Hu et al. (2021), Li et al. (2021)
p62/SQSTM1	Links autophagy to the ubiquitin-proteasome system; its accumulation activates survival pathways, impacting proliferation and survival	Interacts with LC3 and ubiquitinated substrates; involved in NRF2 signaling pathway activation	AML, ALL, CLL, especially where autophagy is impaired	Hu et al. (2018), Yuan et al. (2015), Wang et al. (2023)

2011). The ULK1 complex is mainly composed of ATG13, FIP200, and ATG101. The complex further binds to itself to generate the PAS scaffold complex. Subsequently, the PIK3 complex coalesces onto the PAS, binds to the ATG13 interaction of the PAS via ATG14L, and participates in the formation of phagolysosomes. The ULK1 protein and Beclin-1 protein play a key role in the autophagy process of leukemia.

2.1 ULK1 protein

ULK1 is a serine/threonine protein kinase that plays a crucial role in the initiation of autophagy. In most cells, the absence of ULK1 disrupts autophagy. In one study, downregulation of ULK1 expression led to the inhibition of autophagy (Chan et al., 2007). During the onset of autophagy, ULK1 binds to three proteins, ATG13, FIP200 and ATG101, to form a complex with each other (Hosokawa et al., 2009; Jung et al., 2009). This complex has a role in activating autophagy (Hara et al., 2008), where ATG13 or FIP200 increases the activity and stability of ULK1 (Ganley et al., 2009). The formation of a complex between ATG13 and FIP200 provides structural support for ULK1 and helps to maintain the stability of the complex to prevent its degradation. At the same time, ATG13 and FIP200 contribute to the subcellular localization of ULK1 and can directly regulate the activity of ULK1. ULK1 has been shown to be involved in the generation of its autophagy in many diseases, for example, in pancreatic cancer, NEDD4L can interact with ULK1 to reduce ULK1 expression to inhibit autophagy and mitochondrial metabolism, which in turn inhibits the survival of pancreatic cancer cells (Lee et al., 2020). Another study demonstrated that upregulation of ULK1 in Jurkat cells and $CD4^+$ T cells after being infected by HIV induced autophagy for defense against HIV invasion (Wang et al., 2012). A growing number of studies have found that ULK1 can influence leukemia development by regulating autophagy in various types of leukemia cells. See Table 2.

2.1.1 Role of ULK1 protein on AML autophagy

Patients with AML are highly susceptible to developing resistance to chemotherapy drugs. In recent years, studies have found that ULK1 can induce autophagy production in AML to increase patient sensitivity to chemotherapeutic agents, thereby reducing drug resistance. FLT3 inhibitors can be used to target FLT3-ITD + AML, but acquired resistance occurs rapidly in most patients (Tarver et al., 2020). FLT3 inhibitors can overcome AML resistance to FLT3 inhibitors by inducing autophagy production through the AKT-mTORC1-ULK1 axis with the help of ATG3 (Koschade et al., 2022). Combination chemotherapy with cytarabine/anthracycline can lead to complete remission in some patients, but relapse associated with drug resistance remains a common cause of treatment failure. Anthracycline-based Zoerythromycin (DNR) can induce autophagy production through the AMPK-ULK1 signaling pathway, which inhibits DNR resistance thereby increasing DNR drug sensitivity in AML (Qiu et al., 2020). The AMPK-ULK1 signaling pathway can induce autophagy to increase the sensitivity of leukemia stem cells (LSC) to BET inhibitors in AML (Jang et al., 2017a; Jang et al., 2017b). Also, ULK1 can interact with proteins or genes to activate autophagy in AML. NPM1 mutations are the most common genetic alteration in AML. The most common type NPM1 mutation are type-A (NPM1-mA), which counts for70%-80% cases. NPM1-mA can neutralize ULK1 in AML. It also positively regulates ULK1 expression and maintains ULK1 stability. It was noted that NPM1-mA enhanced TRAF6-dependent ubiquitination and further maintained ULK1 stability through miR-146a, which effectively activated autophagy to promote AML cell survival (Tang et al., 2021). In another study, knockdown of ULK1 downregulated the MCL1 gene; damaging leukemia cells by impairing mitochondrial function and downregulating CD44-xCT, resulting in ROS mitigation of DNA damage and promotion of apoptosis (Bhattacharya et al., 2023). Caspase-3 is an important regulator of AML autophagy, and it can promote autophagy in AML cells by interacting with ULK1 (Man et al., 2017).

2.1.2 Role of ULK1 protein on CML autophagy

Tyrosine kinase inhibitors (TKIs) are the mainstay of treatment for chronic myelogenous leukemia (CML) today. However, leukemia stem cells (LSC) that maintain tiny residual disease (MRD) foci will rely on basic metabolic processes to resist drug treatment (Ianniciello et al., 2021). Recent studies have found that

inhibition of ULK1 expression in LSC can stress-induce LSC differentiation, causing it to be sensitive to TKI treatment (Ianniciello and Helgason, 2022). The increase in LSC sensitivity is driven by the inhibition of autophagy, increased mitochondrial respiration, and loss of quiescence caused by ULK1 deletion (Ianniciello et al., 2021). Imatinib, as the first targeted drug capable of inhibiting BCR-ABL kinase activity for the treatment of CML, is still resistant to imatinib in some CML patients (Druker et al., 2006). Resistance due to BCR-ABL point mutations is a major barrier to TKI treatment of CML. It has been demonstrated that BIIB021 can promote apoptosis in imatinib-resistant CML cells by inducing autophagy through the Akt-mTOR-ULK1 pathway (He et al., 2016). GCA was identified as a key factor regulating resistance to imatinib in CML. GCA promoted TRAF6 ubiquitination ligase allowing ubiquitination of ULK1 lys63, and the result of this ubiquitination activated autophagy in CML cells, which modulated CML resistance to imatinib (Han et al., 2019). Meanwhile, ULK1 can also affect CML resistance to imatinib by inducing autophagy through the ceRNA pathway. Circ-0009910 can regulate ULK1-induced autophagy via sponge miR-34a-5p thereby promoting CML resistance to imatinib (Cao et al., 2020).

2.1.3 Role of ULK1 protein on other diseases autophagy

The advent of targeted therapies has led to a fundamental change in the treatment of chronic lymphocytic leukemia. MRT68921 has potent cytotoxicity against CLL cells as a ULK1 inhibitor that disrupts autophagy and causes cell cycle G2 blockade in CLL cells. Also, in combination with venetoclax, it enhanced cysteine enzyme-dependent cytotoxicity (Avsec et al., 2021). This suggests that autophagy inhibitors have some potential for the treatment of CLL. Similarly, enhanced autophagy helps leukemia. Myelodysplastic syndromes (MDS) have a very high risk of transformation into AML (Corey et al., 2007), and increased expression of sperm-associated antigen 6 (SPAG6) has been detected in patients with AML transformed by MDS and in patients with new-onset AML (Steinbach et al., 2006). Upon knockdown of SPAG6, the AMPK/mTOR/ULK1 signaling pathway in SKM-1 cells was activated thereby inducing autophagy, which ultimately led to increased apoptosis in SKM-1 cells (Zhang M. et al., 2020). This shows the potential of activating autophagy to treat leukemia. ULK1 can also interact with plant extracts to play a role in acute leukemia. Pomegranate, the main phenolic compound in pomegranate peel, andrographis paniculata can induce autophagy production in acute leukemia by up-regulating ULK1 expression (Subkorn et al., 2021). In another study, sesquiterpenes likewise upregulated ULK1 expression open to activate cellular autophagy (Deesrisak et al., 2021). In both studies, activation of autophagy improved the effectiveness of treating leukemia.

2.2 Beclin-1 protein

Beclin one is a novel Bcl-2-homology (BH)-3 structural domain protein, one of the first autophagy effectors identified (Liang et al., 1999). Beclin-1 functions as a metamorphic regulator of the PI3KC3 complex. In the initiation of cellular autophagy, Beclin-1 often forms a complex with PI3KC3, the second important autophagy signaling complex that continues to induce the onset of autophagy after the role of the ULK1 complex (Funderburk et al., 2010). In cancer autophagy, the interaction of Beclin-1 with JAK2 is triggered by IL-6, which allows JAK2 to phosphorylate Beclin-1 at the Y333 site. This process promotes the formation of the PI3KC3 complex thereby activating autophagy in colon cancer (CRC) cells (Hu et al., 2021). Beclin-1 can be activated by the PI3K/Atk signaling pathway and plays a role in inducing autophagy in hepatocellular carcinoma cells by being regulated by BCL2L10 (He et al., 2019). In myocardial ischemia-reperfusion injury, ischemia preconditioned (IPC)-treated rat cells showed suppression of Beclin-1-dependent excessive autophagy, which reduced myocardial ischemia-in-perfusion injury-induced cell death (Peng et al., 2013). From the above studies, it can be found that Beclin-1 is involved in the development of autophagy in different diseases, and there is no exception in leukemia. More and more studies have shown that Beclin-1-induced autophagy plays a role in leukemia.

2.2.1 Role of Beclin-1 protein on ALL autophagy

The development of drug resistance remains a major challenge in the treatment of acute lymphoblastic leukemia (ALL). How to improve the sensitivity of ALL patients to drugs is the key to treating ALL. Glucocorticoids are widely cited for the treatment of ALL, but unintermittent use can lead to the development of resistance. It has been reported that roughly 20% of children with ALL are resistant to glucocorticoids, and even up to 70% of children with recurrent ALL are resistant to glucocorticoids (Inaba and Pui, 2010). miRNAs have a wide range of roles in leukemia, among which, miR-145 enhances the sensitivity of ALL cell lines to glucocorticoids, which is achieved by promoting the expression of Beclin-1 and Bax genes and inhibiting the expression of Bcl-2 genes to induce autophagy and apoptosis production (Long et al., 2020). The first-generation tyrosine kinase inhibitor (TKI) imatinib (IM) can be used not only for the treatment of CML, but has also been widely used in patients with Ph(+) ALL. Imatinib resistance in Ph(+) ALL cells is mediated by the hnRNPK/Beclin-1 signaling pathway. hnRNPK can bind to Beclin-1 in Ph(+) ALL, and upregulation of hnRNPK promotes the generation of autophagic vesicles in Ph(+) ALL cells, which enhances the resistance of Ph(+) ALL cells to imatinib (Zhang J. et al., 2022). Bortezomib is a proteasome inhibitor that promotes its therapeutic effects when combined with autophagy inhibitors. This is due to the fact that bortezomib promotes the formation of Beclin-1/PI3KC3 complex and activates autophagy in ALL cells, which ultimately leads to a decrease in the toxic effect of bortezomib on ALL cells (Wang et al., 2015).

Bafilomycin A1, known for its specific inhibition of the V-ATPase, plays a critical role in autophagy by preventing the acidification of various organelles, including lysosomes. This inhibition disrupts the fusion between autophagosomes and lysosomes, a key step in the degradation of autophagic cargo, making it a valuable tool for studying autophagic flux. At high doses, it is commonly used to block this fusion or inhibit lysosomal activity crucial for late-stage autophagy (Lee et al., 2020).

Recent research has demonstrated the dual role of bafilomycin A1 in targeting both autophagy and apoptosis pathways. In pediatric B-cell acute lymphoblastic leukemia (B-ALL), low concentrations of bafilomycin A1 were shown to effectively induce apoptosis in primary cells from patients, highlighting its potential as an anticancer agent. Moreover, toxicity evaluation in mice indicated that doses up to 10 mg/kg were well tolerated, with higher doses showing signs of liver toxicity (Lee et al., 2020).

This specificity in inhibiting V-ATPase and its consequential blockade of autophagosome-lysosome fusion, coupled with its ability to activate apoptosis, underscores the therapeutic potential of bafilomycin A1 in cancer treatment. By manipulating autophagy pharmacologically, bafilomycin A1, along with other autophagy inhibitors, could improve clinical outcomes in leukemia and other cancers by enhancing the activity of anticancer agents (Lee et al., 2020). Beclin-1 can also bind to Bcl-2, which is induced by bafilomycin A1 (Bafilomycin A1), further inhibiting autophagy and promoting apoptosis in ALL cells (Yuan et al., 2015).

The evidence highlights a critical linkage between Beclin-1-mediated autophagy and the efficacy of therapeutic agents in acute lymphoblastic leukemia (ALL), particularly influencing drug tolerance. This connection underscores the necessity for nuanced treatment strategies that consider autophagy's dual role in enhancing drug sensitivity and resistance, pointing toward the potential of autophagy modulation as a complementary approach in ALL therapy.

2.2.2 Role of Beclin-1 protein on other diseases autophagy

Beclin-1's influence extends beyond autophagy regulation, impacting therapeutic outcomes and drug resistance mechanisms in acute promyelocytic leukemia (APL) and chronic myeloid leukemia (CML). In APL, the autophagy pathway activated by Beclin-1 has shown an inhibitory effect on the therapeutic efficacy of bortezomib, a proteasome inhibitor. Specifically, Beclin-1 knockdown in APL cells led to reduced autophagy, enhancing bortezomib's apoptotic effect on NB4 cell lines (Jiang et al., 2021). This suggests that autophagy modulation might enhance the sensitivity of APL cells to bortezomib, providing a strategic approach to overcome drug resistance.

The interaction between Beclin-1 and the BCR-ABL oncogene in CML unveils another layer of complexity in autophagy's role in leukemia. BCR-ABL, known for its constitutive tyrosine kinase activity, promotes leukemogenesis and drug resistance. Beclin-1's engagement with BCR-ABL not only triggers autophagy but also targets BCR-ABL for degradation via autophagic mechanisms, facilitated by the co-localization with p62/SQSTM1 in autolysosomes. This process potentially diminishes the oncogenic influence of BCR-ABL and enhances the efficacy of tyrosine kinase inhibitors (TKIs) (Huang et al., 2019; Yu et al., 2020).

The strategic degradation of BCR-ABL through Beclin-1 mediated autophagy suggests a novel therapeutic pathway to mitigate TKI resistance, a prevalent challenge in CML treatment. Enhancing autophagy or specifically augmenting the Beclin-1 and BCR-ABL interaction could serve as a therapeutic strategy to decrease BCR-ABL levels, thus improving TKI treatment outcomes.

This intricate relationship between Beclin-1 and oncogenic proteins in leukemia underlines the critical role of autophagy in cancer biology, offering insights into novel therapeutic targets. Further investigation into Beclin-1's specific mechanisms of action and its interactions with oncogenes like BCR-ABL could unlock new avenues for treatment strategies aimed at leveraging autophagy modulation to combat drug resistance in leukemia.

For a deeper understanding, the following references provide comprehensive insights.

- The inhibitory effect of Beclin-1 on bortezomib in APL cells suggests a nuanced approach to autophagy modulation could improve therapeutic outcomes.
- The interaction between Beclin-1 and BCR-ABL in CML highlights the potential of targeting autophagy pathways to enhance TKI efficacy and overcome drug resistance (Huang et al., 2019; Yu et al., 2020).

Exploring these pathways offers a promising direction for enhancing leukemia treatment efficacy and addressing the challenge of drug resistance through the modulation of autophagy.

3 Phagophore extension and autophagosome formation

The ATG-related protein family plays a major role in the extension of phagolysosomes as well as the formation of autophagosomes. This process is mainly mediated by the ATG12-ATG5 coupling system and the ATG8-LC3 coupling system. ATG12-ATG5 will form an oversized complex with ATG16 (Dooley et al., 2014), and this complex will eventually bind to treated LC3, lipidating LC3 (Tanida et al., 2004), allowing phagophore extension and closure. Eventually, in the presence of the two coupled systems, a closed bilayer membrane structure is formed. The mature autophagosome thus arises (Suzuki et al., 2013).

3.1 ATG-related proteins

Autophagy-related (ATG) proteins are central to the autophagy process, a critical cellular mechanism for degrading and recycling cytoplasmic components to maintain cellular health and respond to stress. Among the array of ATG proteins, ATG5, ATG7, and ATG10 play pivotal roles in the conjugation processes essential for the formation and maturation of autophagosomes. ATG5 is part of a conjugate with ATG12, facilitated by ATG7 (acting as an E1-like enzyme) and ATG10 (an E2-like enzyme), crucial for the expansion of the autophagosome membrane. The ATG8-LC3 system, another ubiquitin-like conjugation mechanism, further assists in the autophagosome's expansion and cargo recruitment, with LC3 being a well-recognized marker for autophagy.

Leukemia, particularly acute myeloid leukemia (AML), demonstrates the complexity of autophagy's role in cancer. In AML, the autophagic process, mediated by ATG proteins, may offer a double-edged sword—promoting cell survival in some contexts while enabling therapeutic targeting in others. The dysregulation of autophagy, either through enhanced or

diminished activity of ATG proteins, can influence leukemia cell fate, affecting responses to chemotherapy and targeted therapies. This highlights the potential of targeting autophagy pathways as a therapeutic strategy in leukemia, underscoring the need for further research to understand the nuanced roles of ATG proteins in mediating autophagy within this specific disease context.

This background underscores the significance of ATG proteins not only in the fundamental process of autophagy but also in the broader implications for disease progression and treatment strategies in leukemia.

The formation process of mature autophagosomes is mainly associated with two coupling systems, ATG12-ATG5 and ATG8-LC3. Meanwhile this process is involved by various ATG proteins, for example, ATG7 and ATG10 act as E1-and E2-like enzymes, respectively, mediating the coupling process of ATG12 and AGT5 (Nakatogawa, 2013). In studies of solid tumors, ATG5 is involved in drug resistance of gastric cancer cells by regulating autophagy (Ge et al., 2014); in glioblastoma (GBM) ATG5 can be mediated by PAK1 to produce phosphorylation to promote autophagosome production to achieve hypoxia-induced autophagy (Feng et al., 2021); ATG7 can be modulated by Celastroal, an active substance extracted from Ranunculus ternatus in rectal cancer, which is achieved by the inhibition of Nur77 expression by Celastroal, and the simultaneous elevation of ATG7 expression promoted autophagy in rectal cancer cells (Zhang W. et al., 2022). Drug resistance in gastrointestinal mesenchymal stromal tumor (GIST) is associated with the activation of autophagy. It was noted that circ-CCS could downregulate ATG10 by targeting miR-197-3P, and the autophagy-promoting effect of circ-CCS on mesenchymal tumor cells was reversed after knockdown of miR-197-3p (Sui et al., 2022). The studies listed above illustrate that ATG-related proteins play a role in solid cancers in relation to autophagy. In leukemia, especially acute myeloid leukemia, ATG-related proteins play a role by mediating autophagy.

3.1.1 Role of ATG-related protein on AML autophagy

ATG-related proteins are widely used in drugs for the treatment of AML. Decitabine (DAC) not only has the effect of inhibiting the methylation of DNA, but also promotes the formation of autophagosomes in AML cells by down-regulating the expression of TIGAR, which leads to the upregulation of ATG3, ATG5, LC3, and Beclin-1 proteins and the downregulation of p62 (Li et al., 2021). ATG7 can be regulated by EVI1 to induce autophagy in myeloid leukemia. This modulation protects myeloid leukemia cells and reduces the efficacy of drugs (Niu et al., 2020). Cytarabine is susceptible to resistance in the treatment of AML (Dombret and Gardin, 2016). However, inhibition of cellular autophagy *in vitro* can increase the sensitivity of AML cells to cytarabine (Bosnjak et al., 2014). It has been suggested that miR-143 can enhance cytotoxicity induced by cytarabine by targeting ATG7 and ATG2B-dependent autophagy (Zhang H. et al., 2020). Treatment with cytarabine activates leukemia initiating cell (LIC) activity. When ATG7 defects can promote elevated mitochondrial activity, reactive oxygen species production, and apoptosis, this enhances the therapeutic effect of cytarabine (Sumitomo et al., 2016). Knockdown of ATG7 promotes autophagy in AML cells and inhibits autophagy and chemoresistance, which contributes to an increase in overall survival of AML patients (Piya et al., 2016). ATG5-dependent autophagy promotes the development of AML, while knockdown of ATG5 improves AML sensitivity to chemotherapeutic agents (Liu et al., 2016; Wang et al., 2023), ATG5 also mediates the potential differentiation capacity of AML MSCs and the cell cycle distribution, which leads to autophagy and improves AML chemosensitivity (Li Y. et al., 2020).

3.1.2 Role of ATG-related protein on other diseases autophagy

The role of ATG-associated protein-mediated autophagy in CML can be manifested in imatinib resistance. lncRNA OIP5-ASI can promote CML autophagy-associated imatinib resistance through the miR-30e-5p/ATG12 axis (Dai et al., 2021). Imatinib can also promote Beclin-1 and ATG5 expression to induce CML autophagy (Can et al., 2011). Currently, in studies on myelodysplastic syndromes, ATG3-mediated autophagy was found to have an inhibitory effect on the survival of MDS cells, and upregulation of ATG3 expression in MDS cells promoted Akt-mTOR-dependent autophagy, which inhibited the proliferation as well as promoted apoptosis of MDS cells (Wang et al., 2014; Zhuang et al., 2016).

4 Conclusion

Autophagy as an evolutionarily conserved catabolic process in cells. Various autophagy-related proteins involved in autophagy play key roles in the development of various types of leukemias. The proteins mainly play a role in the two processes of autophagy initiation and mature autophagosome formation in leukemia, mainly ULK1 complex, Beclin-1 protein, and ATG-related proteins. Autophagy mediated by these proteins plays a role in the treatment of different types of leukemia as well as drug resistance.

Today, the availability of several drugs and new treatments has prolonged or saved the lives of many leukemia patients. However, the resistance of leukemia patients to chemotherapeutic drugs has become the main reason for the refractory treatment and relapse of leukemia. It is certain that more and more studies have confirmed that autophagy mediated by autophagy-associated proteins can reduce the resistance of leukemia cells to certain chemotherapeutic drugs in order to increase the sensitivity of the cells to the drugs. This suggests that autophagy can promote the treatment of leukemia. Therefore, in the near future, preventive promotion of autophagy inducers or inhibitors in combination with modulation of autophagy activity, based on leukemogenesis and different phenotypes, could serve as a potential anti-leukemia therapy.

In summary, autophagy mediated by autophagy-associated proteins has different degrees of effects on the treatment of various leukemias, mainly in the fight against drug resistance. However, the mechanism of resistance to many therapeutic drugs and autophagy is still unclear, and further studies are needed to understand how autophagy contributes to the development and treatment of leukemia, and to provide more evidence on how autophagy mediated by autophagy-associated proteins can

improve the sensitivity of various types of leukemias to therapeutic drugs.

Author contributions

ML: Conceptualization, Funding acquisition, Validation, Writing–original draft. JL: Writing–review and editing, Conceptualization, Validation. SZ: Formal Analysis, Project administration, Writing–original draft. LZ: Methodology, Writing–original draft. YZ: Resources, Validation, Writing–original draft. SL: Project administration, Writing–original draft. QL: Funding acquisition, Project administration, Resources, Writing–original draft. JW: Conceptualization, Data curation, Writing–review and editing. RS: Project administration, Validation, Visualization, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors wish to thank Xinxiang Medical University for technical support.

Conflict of interest

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

Publisher's note

References

Avsec, D., Jakoš Djordjevič, A. T., Kandušer, M., Podgornik, H., Škerget, M., and Mlinarič-Raščan, I. (2021). Targeting autophagy triggers apoptosis and complements the action of venetoclax in chronic lymphocytic leukemia cells. *Cancers (Basel)* 13 (18), 4557. doi:10.3390/cancers13184557

Backer, J. M. (2008). The regulation and function of class III PI3Ks: novel roles for Vps34. *Biochem. J.* 410 (1), 1–17. doi:10.1042/BJ20071427

Bhattacharya, S., Piya, S., Ma, H., Sharma, P., Zhang, Q., Baran, N., et al. (2023). Targeting unc51-like autophagy activating kinase 1 (ULK1) overcomes adaptive drug resistance in acute myelogenous leukemia. *Mol. Cancer Res.* 21 (6), 548–563. doi:10.1158/1541-7786.MCR-22-0343

Bosnjak, M., Ristic, B., Arsikin, K., Mircic, A., Suzin-Zivkovic, V., Perovic, V., et al. (2014). Inhibition of mTOR-dependent autophagy sensitizes leukemic cells to cytarabine-induced apoptotic death. *PLoS One* 9, e94374. doi:10.1371/journal.pone.0094374

Breccia, M., Abruzzese, E., Castagnetti, F., Bonifacio, M., Gangemi, D., Sorà, F., et al. (2018). Ponatinib as second-line treatment in chronic phase chronic myeloid leukemia patients in real-life practice. *Ann. Hematol.* 97 (9), 1577–1580. doi:10.1007/s00277-018-3337-2

Can, G., Ekiz, H. A., and Baran, Y. (2011). Imatinib induces autophagy through BECLIN-1 and ATG5 genes in chronic myeloid leukemia cells. *Hematology* 16 (2), 95–99. doi:10.1179/102453311X12902908412039

Cao, H. X., Miao, C. F., Sang, L. N., Huang, Y. M., Zhang, R., Sun, L., et al. (2020). Circ_0009910 promotes imatinib resistance through ULK1-induced autophagy by sponging miR-34a-5p in chronic myeloid leukemia. *Life Sci.* 243, 117255. doi:10.1016/j.lfs.2020.117255

Chan, E. Y., Kir, S., and Tooze, S. A. (2007). siRNA screening of the kinome identifies ULK1 as a multidomain modulator of autophagy. *J. Biol. Chem.* 282, 25464–25474. doi:10.1074/jbc.M703663200

Corey, S. J., Minden, M. D., Barber, D. L., Kantarjian, H., Wang, J. C. Y., and Schimmer, A. D. (2007). Myelodysplastic syndromes: the complexity of stem-cell diseases. *Nat. Rev. Cancer* 7 (2), 118–129. doi:10.1038/nrc2047

Dai, H., Wang, J., Huang, Z., Zhang, H., Wang, X., Li, Q., et al. (2021). LncRNA OIP5-AS1 promotes the autophagy-related imatinib resistance in chronic myeloid leukemia cells by regulating miR-30e-5p/ATG12 Axis. *Technol. Cancer Res. Treat.* 20, 15330338211052150. doi:10.1177/15330338211052150

Deesrisak, K., Chatupheeraphat, C., Roytrakul, S., Anurathapan, U., and Tanyong, D. (2021). Autophagy and apoptosis induction by sesamin in MOLT-4 and NB4 leukemia cells. *Oncol. Lett.* 21 (1), 32. doi:10.3892/ol.2020.12293

Döhner, H., Weisdorf, D. J., and Bloomfield, C. D. (2015). Acute myeloid leukemia. *New Eng. J. Med.* 373 (12), 1136–1152. doi:10.1056/NEJMra1406184

Dombret, H., and Gardin, C. (2016). An update of current treatments for adult acute myeloid leukemia. *Blood* 127, 53–61. doi:10.1182/blood-2015-08-604520

Dooley, H. C., Razi, M., Polson, H. E., Girardin, S. E., Wilson, M. I., and Tooze, S. A. (2014). WIPI2 links LC3 conjugation with PI3P, autophagosome formation, and pathogen clearance by recruiting Atg12–5-16L1. *Mol. Cell.* 55 (2), 238–252. doi:10.1016/j.molcel.2014.05.021

Druker, B. J., Guilhot, F., O'Brien, S. G., Gathmann, I., Kantarjian, H., Gattermann, N., et al. (2006). Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N. Engl. J. Med.* 355, 2408–2417. doi:10.1056/NEJMoa062867

Feng, X., Zhang, H., Meng, L., Song, H., Zhou, Q., Qu, C., et al. (2021). Hypoxia-induced acetylation of PAK1 enhances autophagy and promotes brain tumorigenesis via phosphorylating ATG5. *Autophagy* 17 (3), 723–742. doi:10.1080/15548627.2020.1731266

Funderburk, S. F., Wang, Q. J., and Yue, Z. (2010). The Beclin 1-VPS34 complex—at the cross-roads of autophagy and beyond. *Trend Cell Biol.* 20, 355–362. doi:10.1016/j.tcb.2010.03.002

Ganley, I. G., Lam du, H., Wang, J., Ding, X., Chen, S., and Jiang, X. (2009). ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. *J. Biol. Chem.* 284, 12297–12305. doi:10.1074/jbc.M900573200

Ge, J., Chen, Z., Huang, J., Yuan, W., Deng, Z., and Chen, Z. (2014). Upregulation of autophagy-related gene-5 (ATG-5) is associated with chemoresistance in human gastric cancer. *PLoS One* 9 (10), e110293. doi:10.1371/journal.pone.0110293

Han, S. H., Korm, S., Han, Y. G., Choi, S. Y., Kim, S. H., Chung, H. J., et al. (2019). GCA links TRAF6-ULK1-dependent autophagy activation in resistant chronic myeloid leukemia. *Autophagy* 15 (12), 2076–2090. doi:10.1080/15548627.2019.1596492

Hara, T., Takamura, A., Kishi, C., Iemura, S. I., Natsume, T., Guan, J. L., et al. (2008). FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. *J. Cell Biol.* 181, 497–510. doi:10.1083/jcb.200712064

He, J., Deng, L., Liu, H., Chen, T., Chen, S., Xia, S., et al. (2019). BCL2L10/BECN1 modulates hepatoma cells autophagy by regulating PI3K/AKT signaling pathway. *Aging (Albany NY)* 11 (2), 350–370. doi:10.18632/aging.101737

He, W., Ye, X., Huang, X., Lel, W., You, L., Wang, L., et al. (2016). Hsp90 inhibitor, BIIB021, induces apoptosis and autophagy by regulating mTOR-Ulk1 pathway in imatinib-sensitive and -resistant chronic myeloid leukemia cells. *Int. J. Oncol.* 48 (4), 1710–1720. doi:10.3892/ijo.2016.3382

Hertel, A., Alves, L. M., Dutz, H., Tascher, G., Bonn, F., Kaulich, M., et al. (2022). USP32-regulated LAMTOR1 ubiquitination impacts mTORC1 activation and autophagy induction. *Cell Rep.* 41 (10), 111653. doi:10.1016/j.celrep.2022.111653

Hosokawa, N., Sasaki, T., Iemura, S., Natsume, T., Hara, T., and Mizushima, N. (2009). Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy* 5, 973–979. doi:10.4161/auto.5.7.9296

Hu, F., Song, D., Yan, Y., Huang, C., Shen, C., Lan, J., et al. (2021). IL-6 regulates autophagy and chemotherapy resistance by promoting BECN1 phosphorylation. *Nat. Commun.* 12 (1), 3651. doi:10.1038/s41467-021-23923-1

Hu, X., Mei, S., Meng, W., Xue, S., Jiang, L., Yang, Y., et al. (2018). CXCR4-mediated signaling regulates autophagy and influences acute myeloid leukemia cell survival and drug resistance. *Cancer Lett.* 425, 1–12. doi:10.1016/j.canlet.2018.03.024

Huang, X., Li, Y., Shou, L., Li, L., Chen, Z., Ye, X., et al. (2019). The molecular mechanisms underlying BCR/ABL degradation in chronic myeloid leukemia cells promoted by Beclin1-mediated autophagy. *Cancer Manag. Res.* 11, 5197–5208. doi:10.2147/CMAR.S202442

Ianniciello, A., and Helgason, G. V. (2022). Targeting ULK1 in cancer stem cells: insight from chronic myeloid leukemia. *Autophagy* 18 (7), 1734–1736. doi:10.1080/15548627.2022.2041152

Ianniciello, A., Zarou, M. M., Rattigan, K. M., Scott, M., Dawson, A., Dunn, K., et al. (2021). ULK1 inhibition promotes oxidative stress-induced differentiation and sensitizes leukemic stem cells to targeted therapy. *Sci. Transl. Med.* 13 (613), eabd5016. doi:10.1126/scitranslmed.abd5016

Inaba, H., and Pui, C. H. (2010). Glucocorticoid use in acute lymphoblastic leukaemia. *Lancet Oncol.* 11, 1096–1106. doi:10.1016/S1470-2045(10)70114-5

Jang, J. E., Eom, J. I., Jeung, H. K., Cheong, J. W., Lee, J. Y., Kim, J. S., et al. (2017a). Targeting AMPK-ULK1-mediated autophagy for combating BET inhibitor resistance in acute myeloid leukemia stem cells. *Autophagy* 13 (4), 761–762. doi:10.1080/15548627.2016.1278328

Jang, J. E., Eom, J. I., Jeung, H. K., Cheong, J. W., Lee, J. Y., Kim, J. S., et al. (2017b). AMPK-ULK1-Mediated autophagy confers resistance to BET inhibitor JQ1 in acute myeloid leukemia stem cells. *Clin. Cancer Res.* 23 (11), 2781–2794. doi:10.1158/1078-0432.CCR-16-1903

Jiang, L., Zhao, Y. M., and Yang, M. Z. (2021). Inhibition of autophagy enhances apoptosis induced by bortezomib in AML cells. *Oncol. Lett.* 21 (2), 109. doi:10.3892/ol.2020.12370

Jung, C. H., Jun, C. B., Ro, S. H., Kim, Y. M., Otto, N. M., Cao, J., et al. (2009). ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol. Biol. Cell* 20, 1992–2003. doi:10.1091/mbc.e08-12-1249

Koschade, S. E., Klann, K., Shaid, S., Vick, B., Stratmann, J. A., Thölken, M., et al. (2022). Translatome proteomics identifies autophagy as a resistance mechanism to on-target FLT3 inhibitors in acute myeloid leukemia. *Leukemia* 36 (10), 2396–2407. doi:10.1038/s41375-022-01678-y

Lee, D. E., Yoo, J. E., Kim, J., Kim, S., Kim, S., Lee, H., et al. (2020). NEDD4L downregulates autophagy and cell growth by modulating ULK1 and a glutamine transporter. *Cell Death Dis.* 11 (1), 38. doi:10.1038/s41419-020-2242-5

Li, L., Liu, W., Sun, Q., Zhu, H., Hong, M., and Qian, S. (2021). Decitabine downregulates TIGAR to induce apoptosis and autophagy in myeloid leukemia cells. *Oxid. Med. Cell Longev.* 2021, 8877460. doi:10.1155/2021/8877460

Li, X., He, S., and Ma, B. (2020a). Autophagy and autophagy-related proteins in cancer. *Mol. Cancer* 19 (1), 12. doi:10.1186/s12943-020-1138-4

Li, Y., Jiang, Y., Cheng, J., Ma, J., Li, Q., and Pang, T. (2020b). ATG5 regulates mesenchymal stem cells differentiation and mediates chemosensitivity in acute myeloid leukemia. *Biochem. Biophys. Res. Commun.* 525 (2), 398–405. doi:10.1016/j.bbrc.2020.02.091

Liang, X. H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., et al. (1999). Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* 402, 672–676. doi:10.1038/45257

Liu, Q., Chen, L., Atkinson, J. M., Claxton, D. F., and Wang, H. G. (2016). Atg5-dependent autophagy contributes to the development of acute myeloid leukemia in an MLL-AF9-driven mouse model. *Cell Death Dis.* 7 (9), e2361. doi:10.1038/cddis.2016.264

Lo-Coco, F., Avvisati, G., Vignetti, M., Thiede, C., Orlando, S. M., Iacobelli, S., et al. (2013). Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. *N. Engl. J. Med.* 369, 111–121. doi:10.1056/NEJMoa1300874

Long, S., Ren, D., Zhong, F., Niu, Y., Qin, X., Mu, D., et al. (2020). Reversal of glucocorticoid resistance in Acute Lymphoblastic Leukemia cells by miR-145. *PeerJ* 8, e9337. doi:10.7717/peerj.9337

Lyamzaev, K. G., Tokarchuk, A. V., Panteleeva, A. A., Mulkidjanian, A. Y., Skulachev, V. P., and Chernyak, B. V. (2018). Induction of autophagy by depolarization of mitochondria. *Autophagy* 14 (5), 921–924. doi:10.1080/15548627.2018.1436937

Man, N., Tan, Y., Sun, X. J., Liu, F., Cheng, G., Greenblatt, S. M., et al. (2017). Caspase-3 controls AML1-ETO-driven leukemogenesis via autophagy modulation in a ULK1-dependent manner. *Blood* 129 (20), 2782–2792. doi:10.1182/blood-2016-10-745034

Mizushima, N. (2010). The role of the Atg1/ULK1 complex in autophagy regulation. *Curr. Opin. Cell Biol.* 22 (2), 132–139. doi:10.1016/j.ceb.2009.12.004

Mizushima, N., Yoshimori, T., and Ohsumi, Y. (2011). The role of Atg proteins in autophagosome formation. *Annu. Rev. Cell Dev. Biol.* 27, 107–132. doi:10.1146/annurev-cellbio-092910-154005

Nakatogawa, H. (2013). Two ubiquitin-like conjugation systems that mediate membrane formation during autophagy. *Essays Biochem.* 55, 39–50. doi:10.1042/bse0550039

Niu, Y., Yang, X., Chen, Y., Jin, X., Li, L., Guo, Y., et al. (2020). EVI1 induces autophagy to promote drug resistance via regulation of ATG7 expression in leukemia cells. *Carcinogenesis* 41 (7), 961–971. doi:10.1093/carcin/bgz167

Pei, S., Minhajuddin, M., Adane, B., Khan, N., Stevens, B. M., Mack, S. C., et al. (2018). AMPK/FIS1-mediated mitophagy is required for self-renewal of human AML stem cells. *Cell Stem Cell* 23 (1), 86–100. doi:10.1016/j.stem.2018.05.021

Peng, W., Liu, Y., Xu, W. J., and Xia, Q. H. (2013). Role of Beclin 1-dependent autophagy in cardioprotection of ischemic preconditioning. *J. Huazhong Univ. Sci. Technol. Med. Sci.* 33 (1), 51–56. doi:10.1007/s11596-013-1070-6

Piya, S., Kornblau, S. M., Ruvolo, V. R., Mu, H., Ruvolo, P. P., McQueen, T., et al. (2016). Atg7 suppression enhances chemotherapeutic agent sensitivity and overcomes stroma-mediated chemoresistance in acute myeloid leukemia. *Blood* 128 (9), 1260–1269. doi:10.1182/blood-2016-01-692244

Qiu, L., Zhou, G., and Cao, S. (2020). Targeted inhibition of ULK1 enhances daunorubicin sensitivity in acute myeloid leukemia. *Life Sci.* 243, 117234. doi:10.1016/j.lfs.2019.117234

Steinbach, D., Schramm, A., Eggert, A., Onda, M., Dawczynski, K., Rump, A., et al. (2006). Identification of a set of seven genes for the monitoring of minimal residual disease in pediatric acute myeloid leukemia. *Clin. Cancer Res.* 12, 2434–2441. doi:10.1158/1078-0432.CCR-05-2552

Subkorn, P., Norkaew, C., Deesrisak, K., and Tanyong, D. (2021). Punicalagin, a pomegranate compound, induces apoptosis and autophagy in acute leukemia. *PeerJ* 9, e12303. doi:10.7717/peerj.12303

Sui, S., Ma, F., Mi, L., Gao, L., Yu, W., Li, M., et al. (2022). Circ-CCS enhances autophagy during imatinib resistance of gastrointestinal stromal tumor by regulating miR-197-3p/ATG10 signaling. *J. Cancer Res. Ther.* 18 (5), 1338–1345. doi:10.4103/jcrt.jcrt_625_22

Sumitomo, Y., Koya, J., Nakazaki, K., Kataoka, K., Tsuruta-Kishino, T., Morita, K., et al. (2016). Cytoprotective autophagy maintains leukemia-initiating cells in murine myeloid leukemia. *Blood* 128 (12), 1614–1624. doi:10.1182/blood-2015-12-684696

Suzuki, K., Akioka, M., Kondo-Kakuta, C., Yamamoto, H., and Ohsumi, Y. (2013). Fine mapping of autophagy-related proteins during autophagosome formation in *Saccharomyces cerevisiae*. *J. Cell Sci.* 126 (Pt 11), 2534–2544. doi:10.1242/jcs.122960

Tang, Y., Tao, Y., Wang, L., Yang, L., Jing, Y., Jiang, X., et al. (2021). NPM1 mutant maintains ULK1 protein stability via TRAF6-dependent ubiquitination to promote autophagic cell survival in leukemia. *FASEB J.* 35 (2), e21192. doi:10.1096/fj.201903183RRR

Tanida, I., Ueno, T., and Kominami, E. (2004). LC3 conjugation system in mammalian autophagy. *Int. J. Biochem. Cell Biol.* 36 (12), 2503–2518. doi:10.1016/j.biocel.2004.05.009

Tarver, T. C., Hill, J. E., Rahmat, L., Perl, A. E., Bahceci, E., Mori, K., et al. (2020). Gilteritinib is a clinically active FLT3 inhibitor with broad activity against FLT3 kinase domain mutations. *Blood Adv.* 4, 514–524. doi:10.1182/bloodadvances.2019000919

Wang, H. L., Li, J. N., Kan, W. J., Xu, G. Y., Luo, G. H., Song, N., et al. (2023). Chloroquine enhances the efficacy of chemotherapy drugs against acute myeloid leukemia by inactivating the autophagy pathway. *Acta Pharmacol. Sin.* 44 (11), 2296–2306. doi:10.1038/s41401-023-01112-8

Wang, L., Song, J., Zhang, J., Zhu, C., Ma, Y., and Xu, X. (2014). Lentiviral vector-mediate ATG3 overexpression inhibits growth and promotes apoptosis of human SKM-1 cells. *Mol. Biol. Rep.* 41 (4), 2093–2099. doi:10.1007/s11033-014-3058-0

Wang, X., Gao, Y., Tan, J., Devadas, K., Ragupathy, V., Takeda, K., et al. (2012). HIV-1 and HIV-2 infections induce autophagy in Jurkat and CD4+ T cells. *Cell Signal* 24, 1414–1419. doi:10.1016/j.cellsig.2012.02.016

Wang, Z., Zhu, S., Zhang, G., and Liu, S. (2015). Inhibition of autophagy enhances the anticancer activity of bortezomib in B-cell acute lymphoblastic leukemia cells. *Am. J. Cancer Res.* 5 (2), 639–650.

Yang, Z., and Klionsky, D. J. (2010). Mammalian autophagy: core molecular machinery and signaling regulation. *Curr. Opin. Cell Biol.* 22, 124–131. doi:10.1016/j.ceb.2009.11.014

Yu, C., Gorantla, S. P., Müller-Rudorf, A., Müller, T. A., Kreutmair, S., Albers, C., et al. (2020). Phosphorylation of BECLIN-1 by BCR-ABL suppresses autophagy in chronic myeloid leukemia. *Haematologica* 105 (5), 1285–1293. doi:10.3324/haematol.2018.212027

Yuan, N., Song, L., Zhang, S., Lin, W., Cao, Y., Xu, F., et al. (2015). Bafilomycin A1 targets both autophagy and apoptosis pathways in pediatric B-cell acute lymphoblastic leukemia. *Haematologica* 100 (3), 345–356. doi:10.3324/haematol.2014.113324

Zhang, H., Kang, J., Liu, L., Chen, L., Ren, S., and Tao, Y. (2020b). MicroRNA-143 sensitizes acute myeloid leukemia cells to cytarabine via targeting ATG7-and ATG2B-dependent autophagy. *Aging (Albany NY)* 12 (20), 20111–20126. doi:10.18632/aging.103614

Zhang, J., Liu, X., Yin, C., and Zong, S. (2022a). hnRNPK/Beclin1 signaling regulates autophagy to promote imatinib resistance in Philadelphia chromosome-positive acute lymphoblastic leukemia cells. *Exp. Hematol.* 108, 46–54. doi:10.1016/j.exphem.2022.01.004

Zhang, M., Luo, J., Luo, X., and Liu, L. (2020a). SPAG6 silencing induces autophagic cell death in SKM-1 cells via the AMPK/mTOR/ULK1 signaling pathway. *Oncol. Lett.* 20 (1), 551–560. doi:10.3892/ol.2020.11607

Zhang, W., Wu, Z., Qi, H., Chen, L., Wang, T., Mao, X., et al. (2022b). Celastrol upregulated ATG7 triggers autophagy via targeting Nur77 in colorectal cancer. *Phytomedicine* 104, 154280. doi:10.1016/j.phymed.2022.154280

Zhuang, L., Ma, Y., Wang, Q., Zhang, J., Zhu, C., Zhang, L., et al. (2016). Atg3 overexpression enhances bortezomib-induced cell death in SKM-1 cell. *PLoS One* 11 (7), e0158761. doi:10.1371/journal.pone.0158761

frontiers | Frontiers in Cell and Developmental Biology

TYPE Original Research
PUBLISHED 06 June 2024
DOI 10.3389/fcell.2024.1379435

OPEN ACCESS

EDITED BY
Sambhavi Animesh,
Massachusetts General Hospital and Harvard Medical School, United States

REVIEWED BY
Karan Singh,
New York University, United States
Abhishek Bhardwaj,
New York University, United States

*CORRESPONDENCE
Xiaodong Wang,
xiaodongw75@yahoo.com

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

RECEIVED 31 January 2024
ACCEPTED 02 May 2024
PUBLISHED 06 June 2024

CITATION
Fu S, Dai Y, Zhang P, Zheng K, Cao G, Xu L, Zhong Y, Niu C and Wang X (2024), Extrachromosomal circular DNA (eccDNA) characteristics in the bile and plasma of advanced perihilar cholangiocarcinoma patients and the construction of an eccDNA-related gene prognosis model.
Front. Cell Dev. Biol. 12:1379435.
doi: 10.3389/fcell.2024.1379435

Extrachromosomal circular DNA (eccDNA) characteristics in the bile and plasma of advanced perihilar cholangiocarcinoma patients and the construction of an eccDNA-related gene prognosis model

Shijie Fu†, Yuyang Dai†, Pengjun Zhang, Kanglian Zheng, Guang Cao, Liang Xu, Yujie Zhong, Chuanxin Niu and Xiaodong Wang*

Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Interventional Oncology, Peking University Cancer Hospital and Institute, Beijing, China

Extrachromosomal DNAs (eccDNAs) frequently carry amplified oncogenes. This investigation aimed to examine the occurrence and role of eccDNAs in individuals diagnosed with advanced perihilar cholangiocarcinoma (pCCA) who exhibited distinct prognostic outcomes. Five patients with poor survival outcomes and five with better outcomes were selected among patients who received first-line hepatic arterial infusion chemotherapy from June 2021 to June 2022. The extracted eccDNAs were amplified for high-throughput sequencing. Genes associated with the differentially expressed eccDNAs were analyzed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. The differentially expressed bile eccDNA-related genes were used to construct a prognostic model. Across all 10 patients, a total of 19,024 and 3,048 eccDNAs were identified in bile and plasma, respectively. The concentration of eccDNA detected in the bile was 9-fold higher than that in plasma. The chromosome distribution of the eccDNAs were similar between bile and matched plasma. GO and KEGG pathway analyses showed enrichment in the mitogen-activated protein kinase (MAPK) and Wnt/β-catenin pathways in patients with poor survival outcomes. According to the prognostic model constructed by eccDNA-related genes, the high-risk group of cholangiocarcinoma patients displayed significantly shorter overall survival ($p < 0.001$). Moreover, the degree of infiltration of immunosuppressive cells was higher in patients in the high-risk group. In conclusion, EccDNA could be detected in bile and plasma of

Abbreviations: eccDNA, extrachromosomal circular DNAs; GO, gene ontology; HAIC, hepatic arterial infusion chemotherapy; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase; OS, overall survival; pCCA, perihilar cholangiocarcinoma; RAP1, Ras-related protein 1; GSEA, gene set enrichment analysis; GSVA, gene set variation analysis; PCA, principal component analysis; LASSO Cox, analysis, least absolute shrinkage, and selection operator Cox analysis; ssGSEA, single-sample gene set analysis.

pCCA patients, with a higher concentration. A prognostic model based on eccDNA-related genes showed the potential to predict the survival and immune microenvironment of patients with cholangiocarcinoma.

KEYWORDS

perihilar cholangiocarcinoma, extrachromosomal DNAs, hepatic arterial infusion chemotherapy, immune response, prognosis

1 Introduction

1. Based on the anatomical location, cholangiocarcinoma (CCA) is typically classified into intrahepatic CCA, perihilar CCA (pCCA), and distal CCA (Rizvi et al., 2018). Approximately half of all cholangiocarcinoma (CCA) cases are attributed to pCCA, predominantly located in the perihilar bile duct (Banales et al., 2016). Gemcitabine and cisplatin (CisGem) remain the first-line systemic chemotherapy options for advanced pCCA, with a 5-year overall survival (OS) rate of approximately 5% and a median overall survival duration of less than a year (Palmieri et al., 2020). We have demonstrated the safety and efficacy of hepatic arterial infusion chemotherapy (HAIC) with oxaliplatin and 5-fluorouracil in a prospective phase II trial, with a median progression-free survival (PFS) duration of 12.2 months and a median overall survival (OS) duration of 20.5 months (Wang et al., 2017). However, the survival benefits of HAIC for pCCA vary. At present, the molecular mechanisms governing the differential prognoses observed in pCCA patients who undergo HAIC are incompletely understood.
2. Extrachromosomal DNAs (eccDNAs) have a closed circular structure and are separate from the 22 human linear autosome pairs and pair of sex-determining chromosomes (Shibata et al., 2012). Initially described as double minutes, eccDNAs could be detected in children with malignant tumors over half a century ago (Cox et al., 1965). Next-generation sequencing studies have revealed that eccDNAs exist in healthy human somatic tissues (Møller et al., 2018a) and maternal and fetal plasma (Sin et al., 2020). In cancers, eccDNAs frequently carry oncogenes, leading to genetic heterogeneity and promoting tumor progression (Turner et al., 2017). EccDNAs can participate in oncogenesis by driving oncogenic genome remodeling in neuroblastoma (Koche et al., 2020). In addition, oncogenic eccDNAs can operate as mobile enhancers, thereby increasing chromosomal transcription to support the progression of tumors (Zhu et al., 2021). Sequencing data obtained from a substantial population of cancer patients has demonstrated that eccDNAs correlate with the amplification of oncogenes in various cancer types (Kim et al., 2020). However, the presence and function of eccDNAs remain unknown in advanced pCCA. Recent cancer research on eccDNAs has mainly centered on tumor cell lines, tissue samples and blood samples. Knowledge of eccDNAs in bile samples remains limited. This study investigated eccDNAs in bile and plasma samples obtained from patients with advanced pCCA and the potential value of eccDNA as a survival biomarker in pCCA patients receiving HAIC.

2 Materials and methods

2.1 Subjects

This study was approved by the institutional review board of Peking University Cancer Hospital (approval protocol number: 2021KT144). A retrospective review of the Hospital Information System identified 31 advanced pCCA patients who received first-line HAIC treatment from June 2021 to June 2022 at our center. Bile or plasma samples were obtained from 18 patients prior to treatment. According to complete survival follow-up data, all patients died, with a survival distribution as follows: OS < 6 months (n = 7), 6 months < OS < 12 months (n = 4), and OS > 12 months (n = 7). We randomly selected five patients among seven with a better prognosis (OS > 12 months; Group A) and five patients among seven with a poor prognosis (OS < 6 months; Group B) from the biobank for this study.

2.2 Data collection and processing

The Cancer Genome Atlas Program (TCGA) database (https://portal.gdc.cancer.gov/) was used to download the cholangiocarcinoma (TCGA-CHOL) RNA-seq dataset (Chang et al., 2013) from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) to obtain the GSE107943 cholangiocarcinoma dataset (Edgar, Domrachev, and Lash, 2002). The cholangiocarcinoma E-MTAB-6389 dataset was obtained from ArrayExpress (https://www.ebi.ac.uk/biostudies/arrayexpress) (Sarkans et al., 2021). The gene expression values of the TCGA-CHOL and GSE107943 datasets were then normalized to $\log_2$ (TPM+1).After sorting the dataset and excluding the data with incomplete prognostic information and normal sample data, the merged cohort (n = 142) was created by combining the TCGA-CHOL (n = 36), E-MTAB-6389 (n = 76), and GSE107943 (n = 30) dataset cohorts using the R package "inSilicoMerging". Furthermore, batch effects were removed using the R package "combat" (Supplementary Material S6).

2.3 Treatment and follow-up

HAIC was performed as previously described (Hu et al., 2019). The HAIC treatment, referred to as "3cir-OFF," consisted of administering

TABLE 1 Summary of patient baseline characteristics.

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Age (Y)	56	68	34	53	42	92	58	70	54	73
Sex	Female	Male	Female	Female	Male	Male	Male	Male	Male	Male
HBV infection	None	Yes	None	None	None	None	None	Yes	None	None
Child-Pugh class	B	B	B	B	B	A	B	A	B	B
CEA (ng/mL)	2.45	4.06	1.23	1.93	1.04	4.42	5.44	1.99	2.04	6.68
CA19-9 (U/mL)	1978	2,614	2003	330	1,508	94.94	246.3	1,080	11.29	7,401
Total bilirubin (μmol/L)	93.6	100	97.75	134.1	68.8	31.9	78.4	74	108.4	147.4
ECOG performance status	0	0	1	0	0	0	1	1	0	1
Extent of disease	N0M0	N1M0	N2M1	N0M0	N1M0	N0M0	N0M0	N0M0	N0M0	N0M0
HAIC cycles	2	2	2	2	2	4	6	6	6	6
PFS (months)	1.6	2.2	1.9	2.0	1.8	10.0	12.0	19.0	25.0	6.0+*
OS (months)	5.5	3.0	2.5	6.0	2.0	25.0	14.0	25.0	30.5	22.5
Bile sample	Yes	Yes	Yes	Yes	None	Yes	Yes	Yes	Yes	Yes
Plasma sample	Yes	None	None	Yes	Yes	Yes	None	Yes	Yes	Yes
Treatment response	PD	PD	PD	PD	PD	PR	PR	SD	PR	PR
Group	B	B	B	B	B	A	A	A	A	A

*: Based on the date of last imaging, the PFS, of P10 was recorded as 6+ months.
CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; HAIC: hepatic arterial infusion chemotherapy; PFS: progression-free survival; OS: overall survival; PR: partial response; SD: stable disease; PD: progressive disease; HBV: hepatitis B virus; ECOG, eastern cooperative oncology group.

oxaliplatin (40 mg/m2 for 2 h), 5-fluorouracil (800 mg/m2 for 22 h), and intravenous folinic acid (200 mg/m2) over a period of three consecutive days, repeated every three or 4 weeks. Response Evaluation Criteria in Solid Tumors (RECIST) (version 1.1) were utilized to assess treatment response. Overall survival (OS) was calculated from the initiation of HAIC until death. Progression-free survival (PFS) was determined from the start of HAIC until either tumor progression or death, whichever occurred first.

2.4 Bile and blood samples

Bile samples (15 mL) were obtained by percutaneous transhepatic cholangial drainage (PTCD). Peripheral blood samples (10 mL) were obtained by venipuncture within 7 days before the first cycle of the HAIC procedure and collected into anticoagulant tubes. The fresh bile and peripheral blood samples were centrifuged at 2,000 rpm for 10 min; next, the supernatant was stored in a refrigerator at −80°C. All patients signed informed consent documentation for clinical specimen collection before PTCD and HAIC treatment.

2.5 DNA preparation and eccDNA sequencing

CloudSeq Biotech Inc. (Shanghai, China) sequenced eccDNA according to previously established experimental procedures (Møller et al., 2018a). DNA extraction from either bile or plasma samples was performed using the QIAamp Circulating Nucleic Acid Kit (QIAGEN Sciences, Inc., Germantown, MD, USA). Subsequently, 25 ng of bile or plasma DNA was treated with 1 μL of Plasmid-Safe ATP-dependent DNase (Epicenter, Madison, WI, USA) within a 50-μL reaction system and maintained at 37°C for 5 min. Afterward, column purification was performed using the MinElute Reaction Cleanup Kit (QIAGEN Sciences, Inc.) to remove any linear DNA. EccDNA isolated from the bile or plasma samples was enriched using the Nextera XT DNA Library Preparation Kit (Illumina, Inc., San Diego, CA, USA). The resulting DNA libraries were then sequenced using an Illumina NovaSeq 6000 platform.

2.6 Sequencing analysis of eccDNA

Q30 quality control was conducted on the paired-end reads. Subsequently, circle-map software (v1.1.4) was used to identify eccDNA, while samtools (v0.2) was used to retrieve the raw soft-clipped read counts at the breakpoint. EdgeR was used to conduct between-samples statistical analyses, counts per million (CPM) was used to standardize the detected split read, and the subsequent difference analysis was conducted according to standardized data (Robinson et al., 2010). Differential analysis was conducted using DESeq2 on standardized data, and the differential genes in the group were screened according to the calculated fold change values ($p < 0.05$, fold change >2.0). Genes linked to these differentially expressed eccDNAs were used to perform GO and KEGG pathway enrichment analyses.

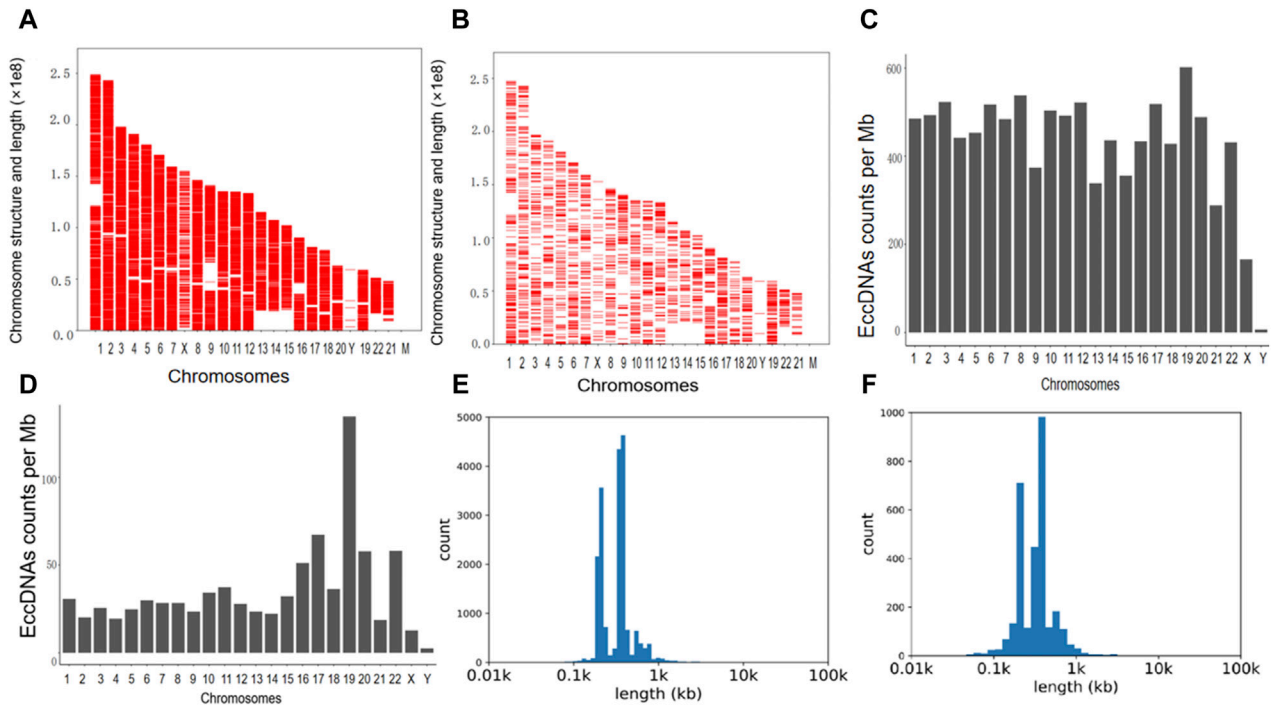


FIGURE 1
The presence and length distribution of eccDNA in bile and plasma. **(A,B)** The presence of eccDNAs in the bile and corresponding plasma samples is illustrated in the Venn diagram. **(C,D)** The frequency of eccDNAs in bile and plasma samples. **(E,F)** Length distribution of eccDNA at ≤ 100,000 bp in bile and plasma. eccDNAs, extrachromosomal circular DNAs.

2.7 Kaplan–Meier (K–M) curve plotting

The "survival" and "survminer" R packages were utilized to generate K–M curves to illustrate the disparity in prognosis among distinct cohorts of patients categorized as high-risk and low-risk.

2.8 ROC curve plotting

ROC curves were generated using the R packages "survival," "survminer," and "timeROC" to assess the model's predictive ability for 1-, 3-, and 5-year survival.

2.9 Analysis of immune characteristics

The analysis of enrichment scores for 29 immune-related gene sets in each sample was performed using the R packages "GSEABase" and "GSVA". Subsequently, the "limma" and "ggpubr" packages were used to identify and illustrate the immune gene sets that differed between the high- and low-risk groups.

2.10 Function enrichment analysis

Gene set enrichment analysis (GSEA) was conducted using version 3.0 of the GSEA software obtained from the GSEA website. Relevant pathways and molecular mechanisms were assessed by acquiring gene sets from the Molecular Signatures database. The gene set size ranged from 5 to 5000, with 1,000 resampling iterations. A p-value <0.05 and FDR <0.25 were considered to indicate significance. GSVA analysis was performed using the R packages "GSEABase" and "GSVA" to calculate the enrichment score for each gene per sample to obtain the enrichment score matrix. Further analysis and visualization of the gene sets that displayed differences between the high- and low-risk groups were conducted using the "limma" and "pheatmap" packages.

2.11 Statistical analysis

Differently expressed eccDNAs between two groups were identified by t-test. A p-value <0.05 was considered to indicate significance. All of the analyses were performed using SPSS v.23.0 software (IBM Corp, Armonk, NY, USA).

3 Result

3.1 Patient characteristics

Table 1 presents the baseline traits of the individuals and their PFS and OS outcomes. Notably, patients in group A exhibited a considerably longer OS than those in group B [25 months (95% CI: 22.8–27.2 months) vs. 3 months (95% CI: 1.9–4.1 months), p = 0.002]. The PFS of patients in group A was also longer than that of

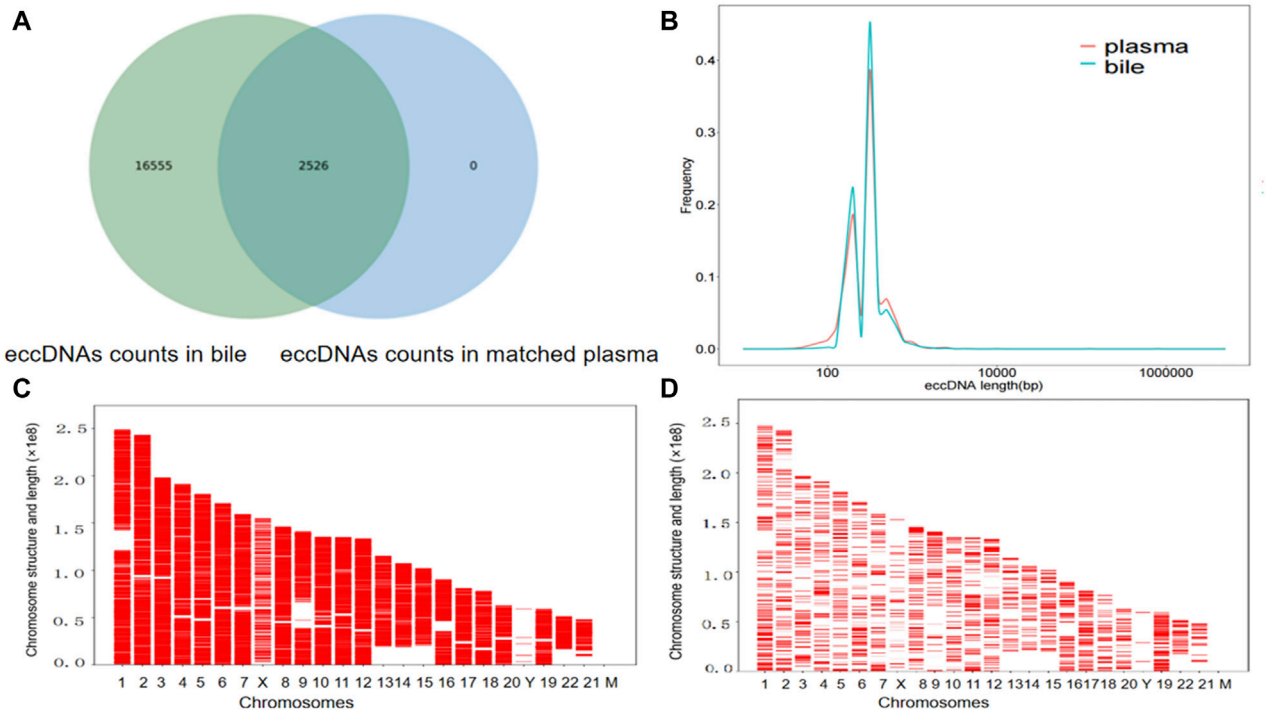


FIGURE 2
The presence of eccDNAs in bile and matched plasma samples. **(A)** Venn diagram showing the eccDNAs in the bile and matched plasma samples. **(B)** Length distribution of eccDNAs in the bile and matched plasma samples. **(C)** Distribution of eccDNAs in the 23 pairs of chromosomes in six bile samples. **(D)** A comparative examination of the distribution of eccDNAs across the 23 pairs of chromosomes in the six plasma samples corresponding to the bile samples.

patients in group B [19 months (95% CI: 4–34 months) vs. 1.9 months (95% CI: 1.7–2.1 months), $p = 0.002$].

3.2 Genome-wide detection of eccDNAs in bile and plasma samples

The cleaned reads obtained through high-throughput sequencing were aligned with the human genome (UCSC hg19) to identify eccDNAs in the bile and plasma samples. In total, 19,024 eccDNAs were annotated to the 23 pairs of chromosomes in bile samples, while seven plasma samples contained 3,048 eccDNAs. Analysis of the genomic distribution of eccDNAs revealed their presence in all 23 pairs of chromosomes, as shown in Figures 1A, B. Notably, eccDNAs originating from mitochondria were excluded before high-throughput sequencing and, hence, were not detected. The frequency of eccDNAs per Mb was relatively consistent across each chromosome in both bile and plasma samples, as depicted in Figures 1C, D. Notably, the length distribution of eccDNAs in bile samples ranged from 38 to 5,351,028 bp, with a prominent peak observed at approximately 550 bp (Figure 1E). Similarly, plasma samples exhibited a length distribution ranging from 38 to 883,052 bp, with a peak also observed at 550 bp (Figure 1F). These findings demonstrate that eccDNAs are prevalent in both bile and plasma samples from patients with pCCA.

3.3 Comparison of eccDNA distribution patterns between the bile and matched plasma samples

For the six patients (patients 1, 4, 6, 8, 9, and 10) with both bile and matched plasma samples, overlap comparisons showed that out of 19,081 eccDNAs, 16,555 eccDNAs were exclusively observed in bile samples, 2,625 were identified in both bile and plasma samples, and no eccDNAs were exclusively detected in plasma samples; the level of eccDNA in the bile was 9-fold higher than in the plasma (Figure 2A). In the group comparison of these six individuals, similar characteristics were observed in the length distribution of bile samples and corresponding plasma samples, including features such as the peak location and length range (Figure 2B). The length distribution for each matched sample also showed similar peak locations (Supplementary Material S1). In addition, the chromosome distribution of the eccDNAs was similar between the bile and matched plasma in these six patients (Figures 2C, D).

3.4 Investigation into the disparity in eccDNA expression in the bile between group A and group B

We identified 2,195 eccDNAs exhibiting differential expression patterns according to the screening criteria (p-value <0.05 and |LogFC| > 1). As shown in Figure 3A, compared to group A, there

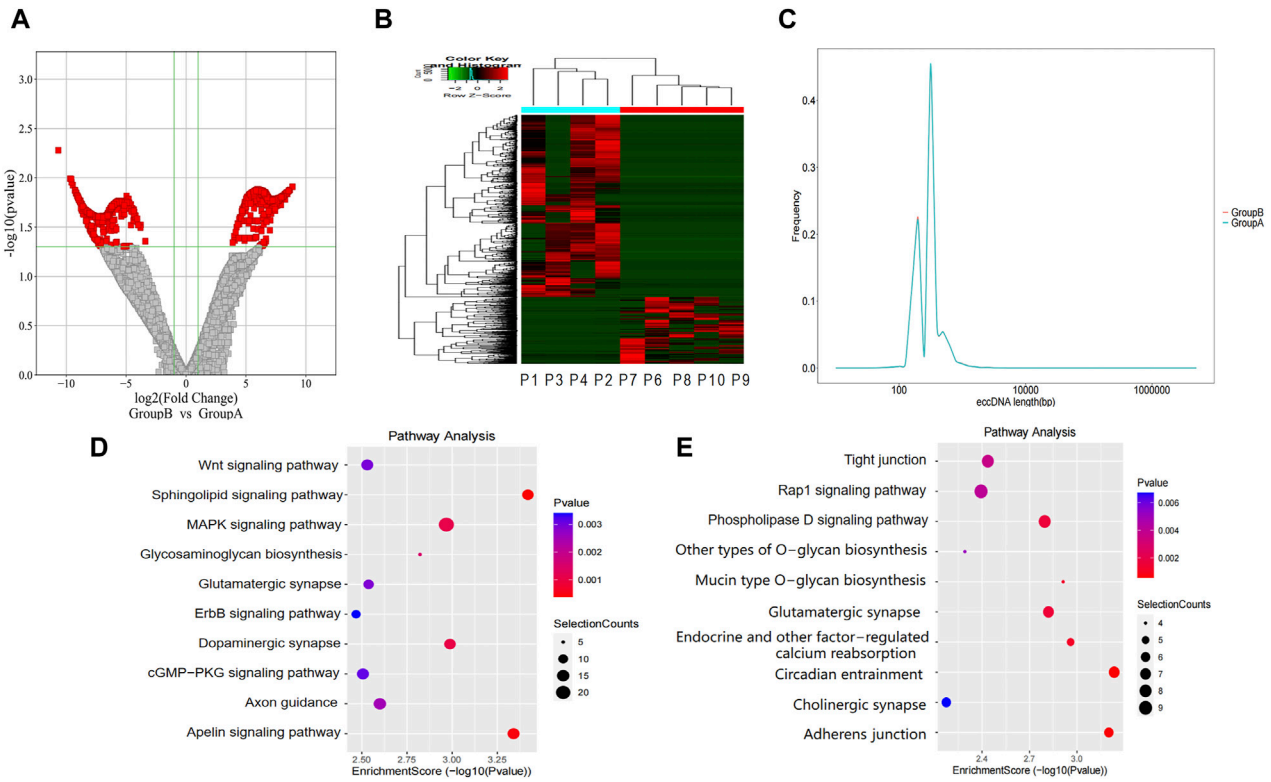


FIGURE 3
Differentially expressed eccDNAs in bile between group A and group B. **(A,B)** Scatter and cluster plots showing the differentially expressed eccDNAs in bile samples from patients with different prognoses. **(C)** Length distribution of eccDNAs in bile samples from patients with different prognoses. **(D)** KEGG pathway analysis of the upregulated eccDNAs in bile samples from patients with a poor prognosis. **(E)** KEGG pathway analysis of the upregulated eccDNAs in bile samples from patients with a better prognosis. KEGG, Kyoto Encyclopedia of Genes and Genomes.

were 1,602 upregulated eccDNAs and 593 downregulated eccDNAs in group B. A hierarchical clustering approach was employed to confirm the consistency of differently expressed eccDNAs in group B (n = 4) and group A (n = 5) (Figure 3B). Most of these candidate eccDNAs were observed either in group A or group B. The distribution in terms of length indicated comparable peak positions and ranges of eccDNAs among patients in distinct groups (Figure 3C). We conducted a GO analysis to investigate the functionalities of the genes linked to the differentially expressed eccDNAs. Regarding genes associated with upregulated eccDNAs in group B, the dominant biological process was cell adhesion, the main molecular function was catalytic activity, and the main cellular component was cell junction (Supplementary Material S2). Regarding genes associated with upregulated eccDNAs in group A, the dominant biological process was the modulation of chemical synaptic transmission, the main molecular function was cytoskeletal protein binding, and the main cellular component was cell protection (Supplementary Material S3). KEGG pathway analysis revealed that the genes associated with the upregulated eccDNAs in group B are involved in sphingolipid signaling, apelin signing, and tumor-related signaling pathways, such as the MAPK and WNT signaling pathways (Figure 3D). Genes associated with the upregulated eccDNAs in group A were involved in circadian entrainment and adherens junctions (Figure 3E).

3.5 Identification of the differentially expressed eccDNAs in the plasma between group A and group B

The level of eccDNA expression in plasma samples in groups A and B was compared as described for the bile samples. Compared with group A, there were 358 upregulated eccDNAs and 41 downregulated eccDNAs in group B (Figures 4A, B). The length distribution also showed similar peak locations and length spans of eccDNAs in the different groups (Figure 4C). GO analysis indicated that regarding genes associated with upregulated eccDNAs in group B, the dominant biological process was the regulation of postsynaptic membrane neurotransmitter receptor levels, the main molecular function was transmembrane receptor protein phosphatase activity, and the main cellular component was the cytoplasm (Supplementary Material S4). Regarding genes associated with upregulated eccDNAs in group A, the dominant biological process was protein homotetramerization, the main molecular function was amino acid transmembrane transporter activity, and the main cellular component was nuclear matrix (Supplementary Material S5). KEGG pathway analysis revealed that the genes associated with the upregulated eccDNAs in group B are involved in gonadotropin-releasing hormone (GnRH) secretion and the WNT signaling pathway (Figure 4D). Genes associated with the upregulated eccDNAs in

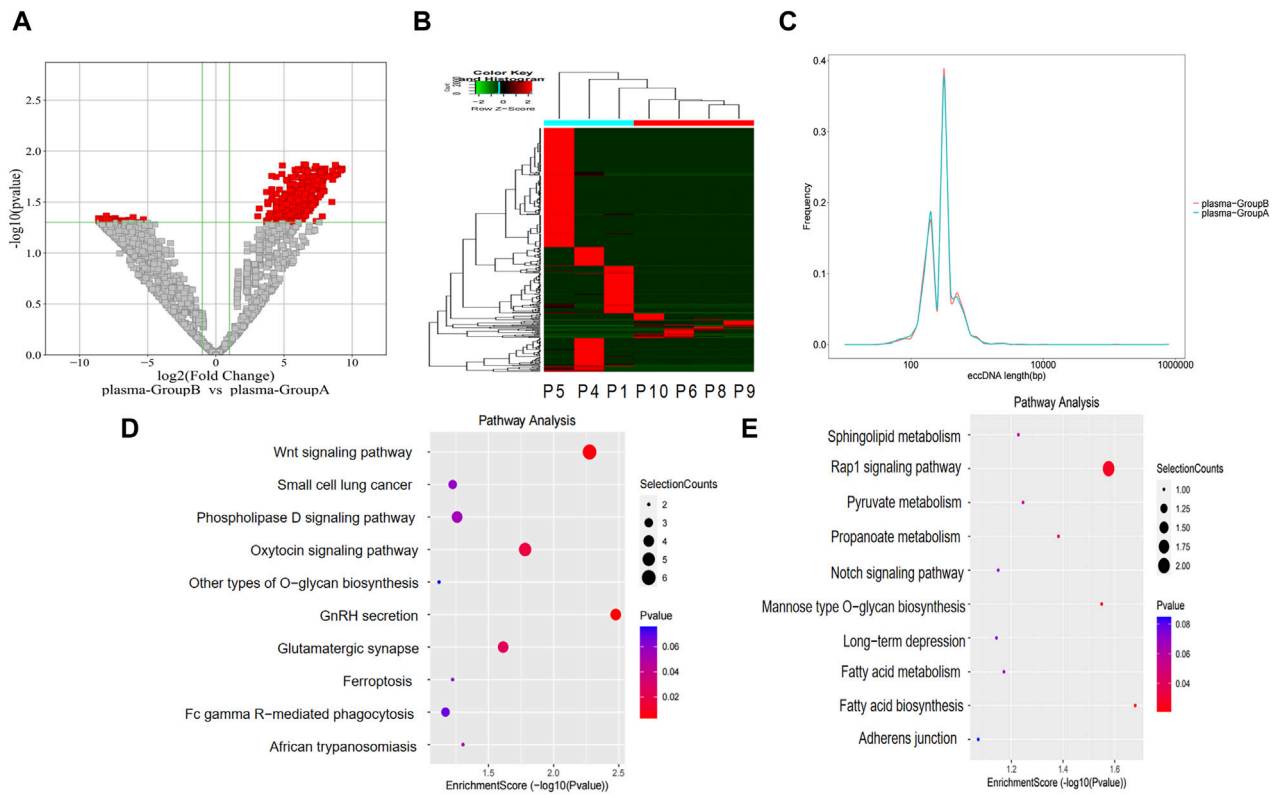


FIGURE 4
Differentially expressed eccDNAs in plasma between group A and group B. **(A,B)** Scatter and cluster plots showing the differentially expressed eccDNAs in plasma samples from patients with different prognoses. **(C)** Length distribution of eccDNAs in plasma samples from patients with different prognoses. **(D)** KEGG pathway analysis of the upregulated eccDNAs in the plasma samples from patients with a poor prognosis. **(E)** KEGG pathway analysis of the upregulated eccDNAs in the plasma samples from patients with a better prognosis.

group A are involved in the Ras-related protein 1 (RAP1) signaling pathway (Figure 4E).

3.6 Construction of an eccDNA-related gene prognostic model for cholangiocarcinoma

First, the difference in annotated eccDNA genes in the bile of patients in group B and group A was screened. Then, the merged cohort (n = 142) was randomly divided into a training cohort (n = 102) and a validation cohort (n = 40) at a 7:3 ratio. Univariate Cox regression analysis was performed on differentially expressed eccDNA-related genes in the training cohort to screen out genes related to cholangiocarcinoma patient prognosis. Among these differentially expressed eccDNA-related genes, those with risk coefficients >1 were regarded as risk genes, and those with risk coefficients <1 were considered protective genes. The risk gene overlap with the upregulated eccDNA-related genes in the bile of patients with poor prognosis after HAIC was assessed, and a total of 13 genes were obtained. After overlapping the protective genes with the upregulated eccDNA-related genes in the bile of patients with better prognosis, 60 genes were obtained (Figure 5A). Figure 5B shows the univariate Cox analysis results of the 73 eccDNA genes (p-value <0.05). LASSO Cox regression analysis was further used in the training cohort, a 10-fold cross-validation method was adopted, and 16 genes were identified according to the optimal λ value (Figure 5C). Finally, multivariate Cox regression analysis and stepwise regression methods were used to construct the prognostic model. The model genes ALDH3B2, RACGAP1, SH3PXD2A, PDE4D, SCAPER and STX18 were obtained. Each patient's risk score was calculated according to the multivariate Cox regression risk coefficient and gene expression values as follows = 0.245*ALDH3B2 + 0.473*RACGAP1 + 0.664*SH3PXD2A + 0.541*PDE4D − 0.677*SCAPER − 0.483*STX18. The patients were divided into high-risk and low-risk groups according to the median risk score (Figure 5D). Principal component analysis (PCA) showed that this model could better classify patients into these two groups (Figure 5E).

3.7 Identification of the risk characteristics and the predictive power of the model

In the training (n = 102) (Figure 6A), validation (n = 40) (Figure 6B), TCGA-CHOL (n = 36) (Figure 6C), E-MTAB-6389 (n = 76) (Figure 6D), and GEO107943 (n = 30) (Figure 6E) cohorts, the OS prognosis in low-risk patients was significantly better than that in high-risk patients. Furthermore, the low-risk group demonstrated better disease-free survival (DFS) outcomes than the high-risk group (Figure 6F). ROC curves were plotted

10.3389/fcell.2024.1379435

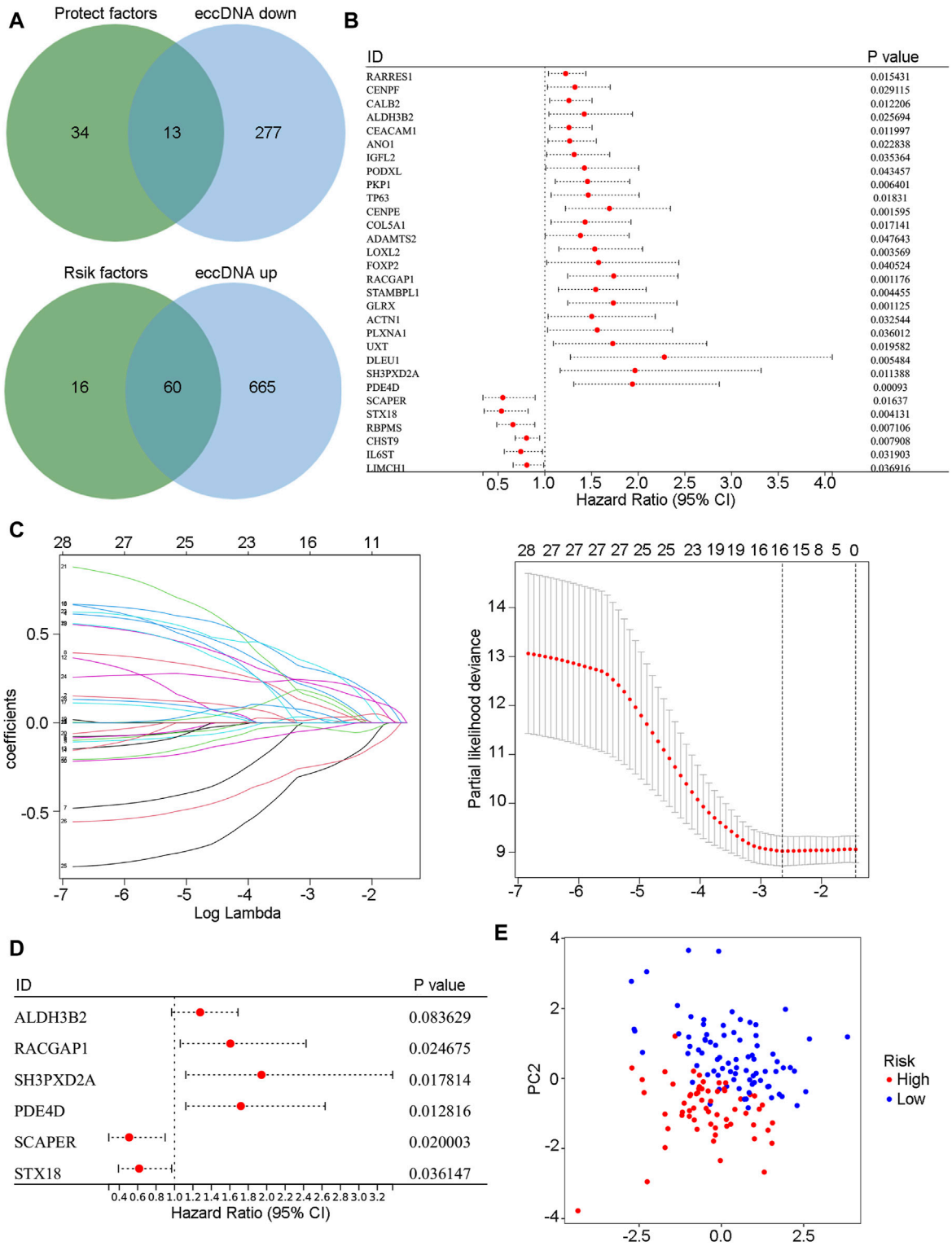


FIGURE 5
The prognosis model was constructed based on eccDNA-related genes. **(A)** The Venn diagram shows the intersection of risk genes with highly expressed eccDNA genes in the bile of group B patients and the intersection of protective genes with low-expressed eccDNA genes in the bile of group B patients. **(B)** Univariate Cox regression results of intersecting genes in the merged cohort (p-value <0.05). **(C)** The intersecting genes were further analyzed by LASSO-Cox regression analysis. **(D)** Model genes were selected by stepwise regression multivariate Cox regression analysis. **(E)** Model genes were used to conduct a PCA of the merged cohort. PCA, principal component analysis.

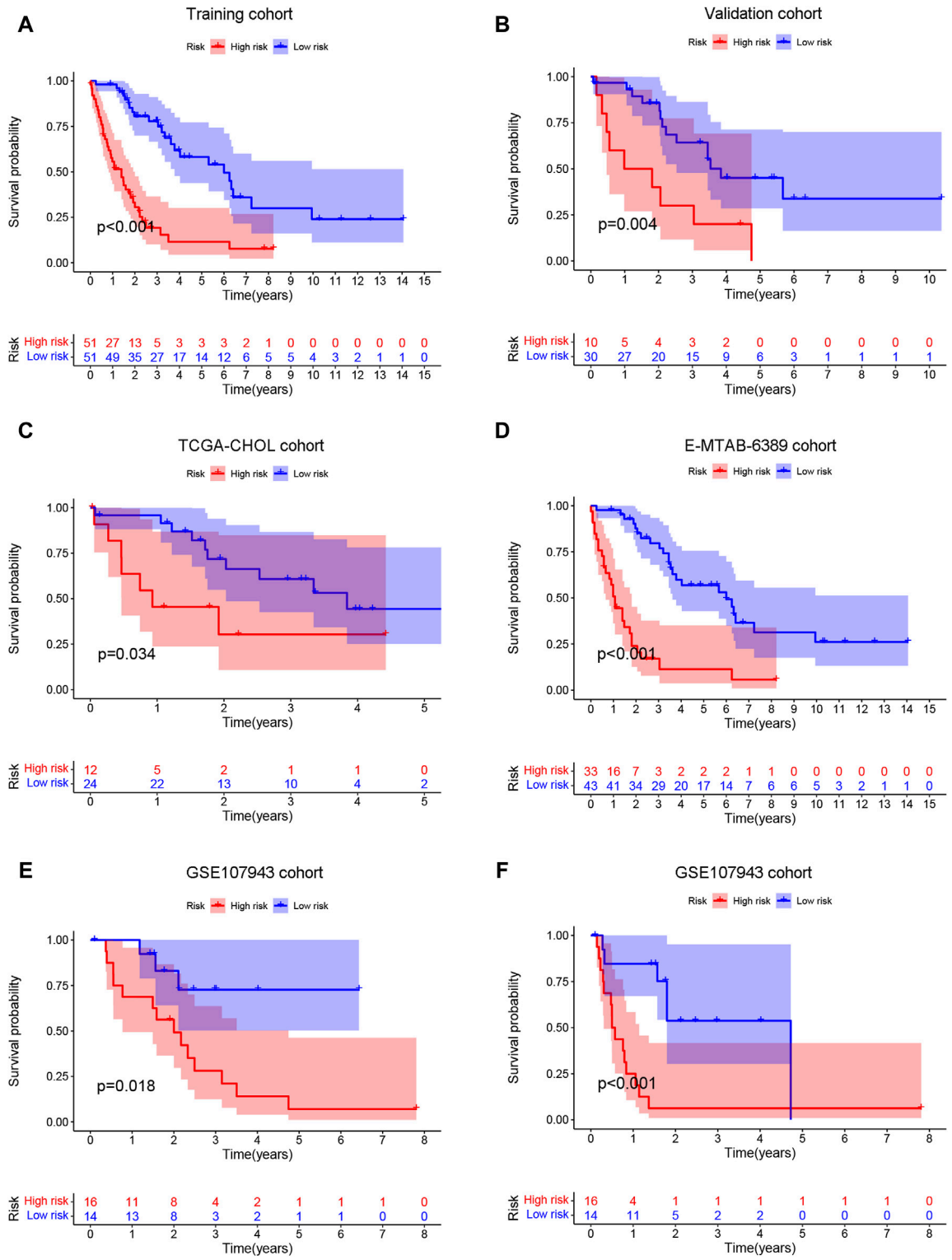


FIGURE 6
Survival prediction ability of prognostic models. The patients were categorized into high- and low-risk groups according to the risk score median. Then, the training **(A)**, validation **(B)**, TCGA-CHOL **(C)**, E-MTAB-6389 **(D)**, and GSE107943 **(E)** cohorts were employed to generate overall survival K–M survival curves. **(F)** The GSE107943 cohort was also utilized to construct a disease-free survival K–M curve.

for OS and DFS at 1-, 3-, and 5-year intervals to assess the predictive capability of the model, revealing a high level of diagnostic efficacy. In the training cohort, the AUC for overall survival was 0.809 at 1 year, 0.884 at 3 years, and 0.837 at 5 years (Figure 7A). The validation cohort displayed AUC values of 0.874, 0.776, and 0.865 for 1-, 3-, and 5-year intervals,

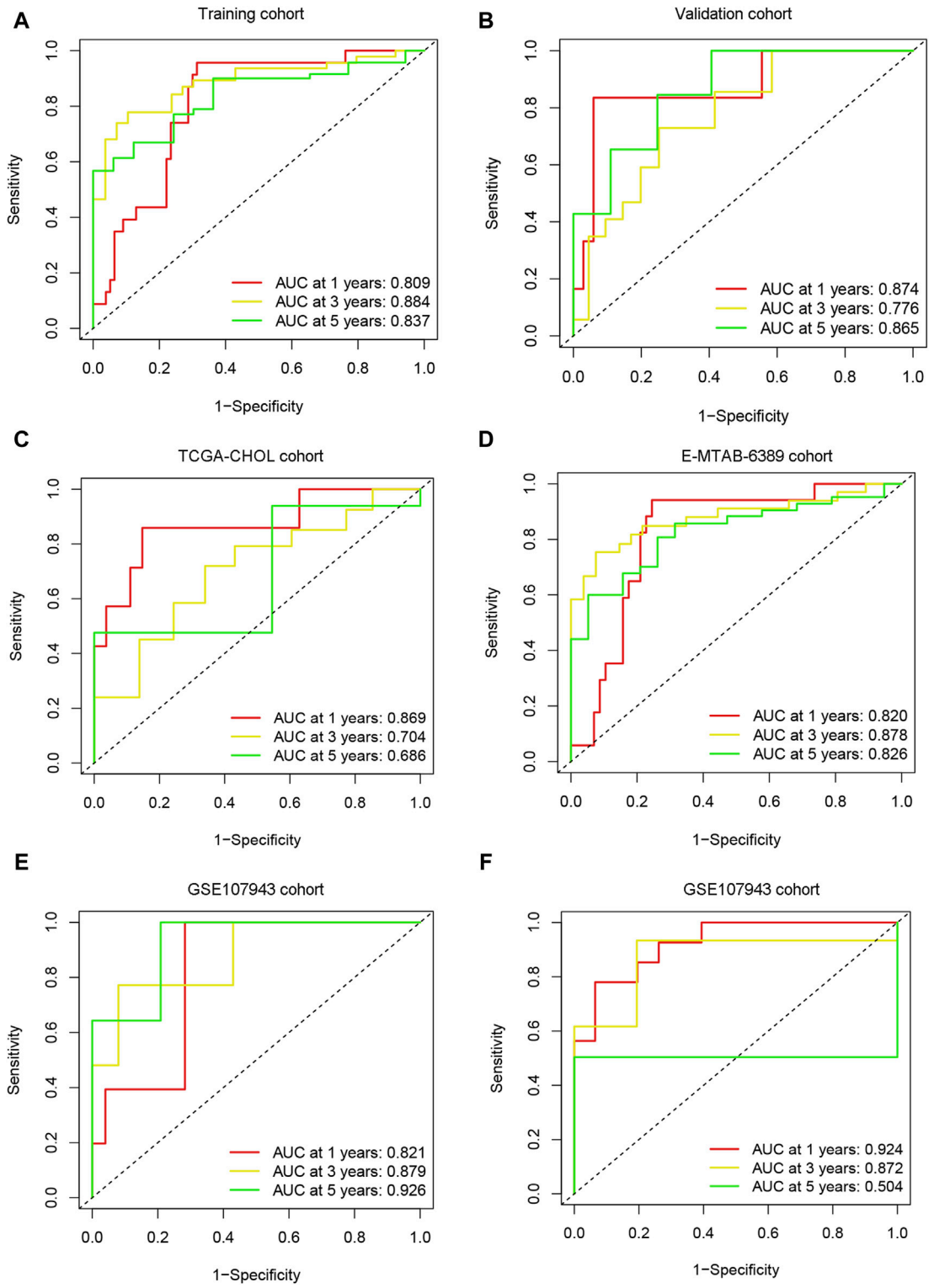


FIGURE 7
Model predictive performance. ROC analysis was performed on the risk scores in the training **(A)**, validation **(B)**, TCGA-CHOL **(C)**, E-MTAB-6389 **(D)**, and GSE107943 **(E)** cohorts, and the AUC value was calculated for 1-, 3-, and 5-year overall survival. **(F)** ROC analysis was performed for risk scores using the GSE107943 cohort, and AUC values were calculated for disease-free survival at 1, 3, and 5 years.

respectively (Figure 7B). The AUCs for the OS outcomes in the TCGA-CHOL, GSE107943, and E-MTAB-6389 datasets are shown in Figures 7C–E. Within the GSE107943 cohort, the AUC values for disease-free survival at 1, 3, and 5 years were 0.924, 0.872, and 0.504, respectively (Figure 7F). The lower AUC value for 5-year DFS is due to the limited number of patients with

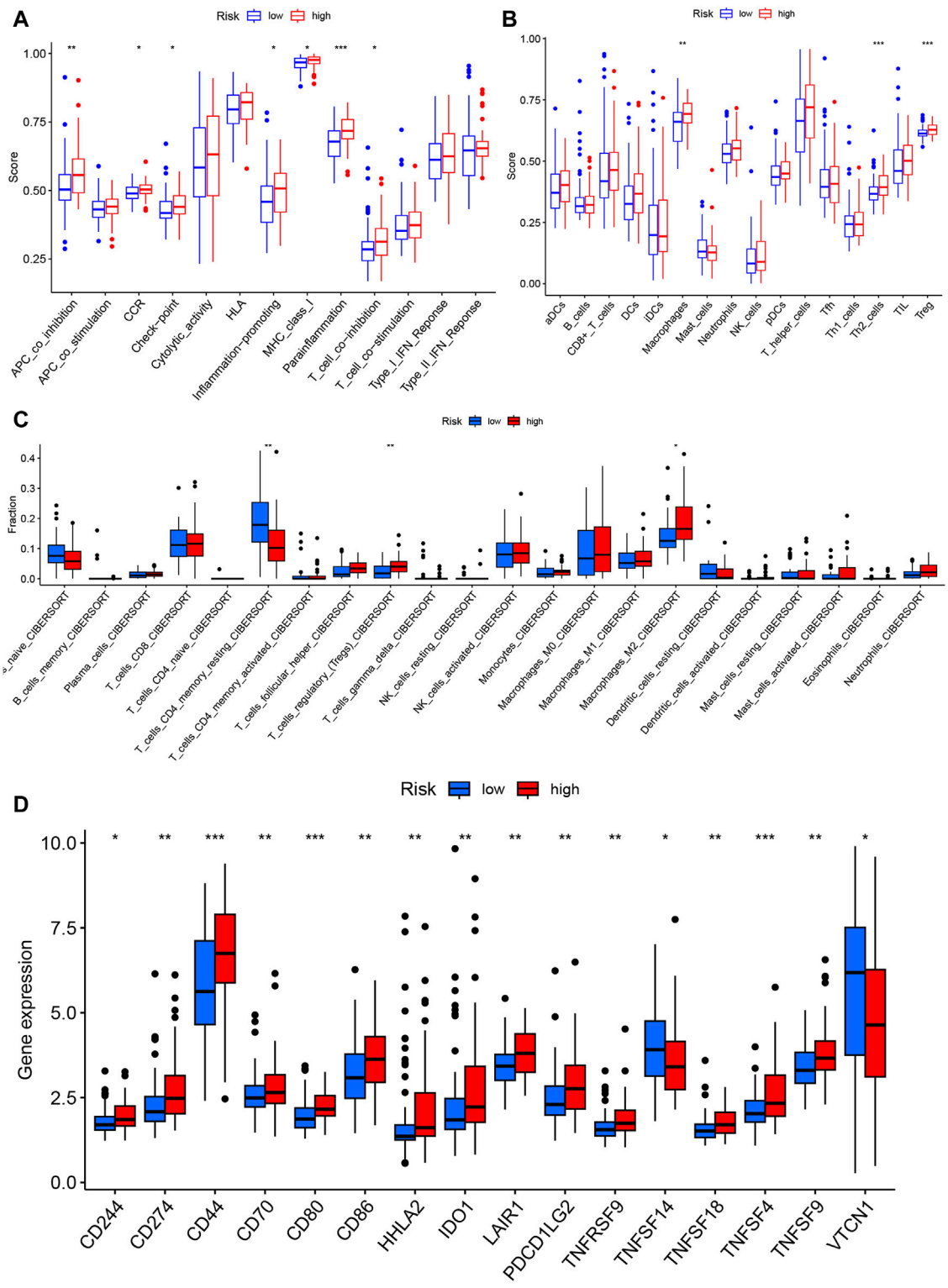


FIGURE 8
Immune characteristics of the prognostic model. Single-sample gene set analysis (ssGSEA) was used to calculate immune function and immune cell gene set enrichment scores for each sample to determine differential immune function **(A)** and immune cell infiltration **(B)** in the high- and low-risk groups. **(C)** The CIBERSORT algorithm was used to evaluate differential immune cell infiltration between the high- and low-risk groups. **(D)** Differential expression of immune checkpoint genes between the high- and low-risk groups.

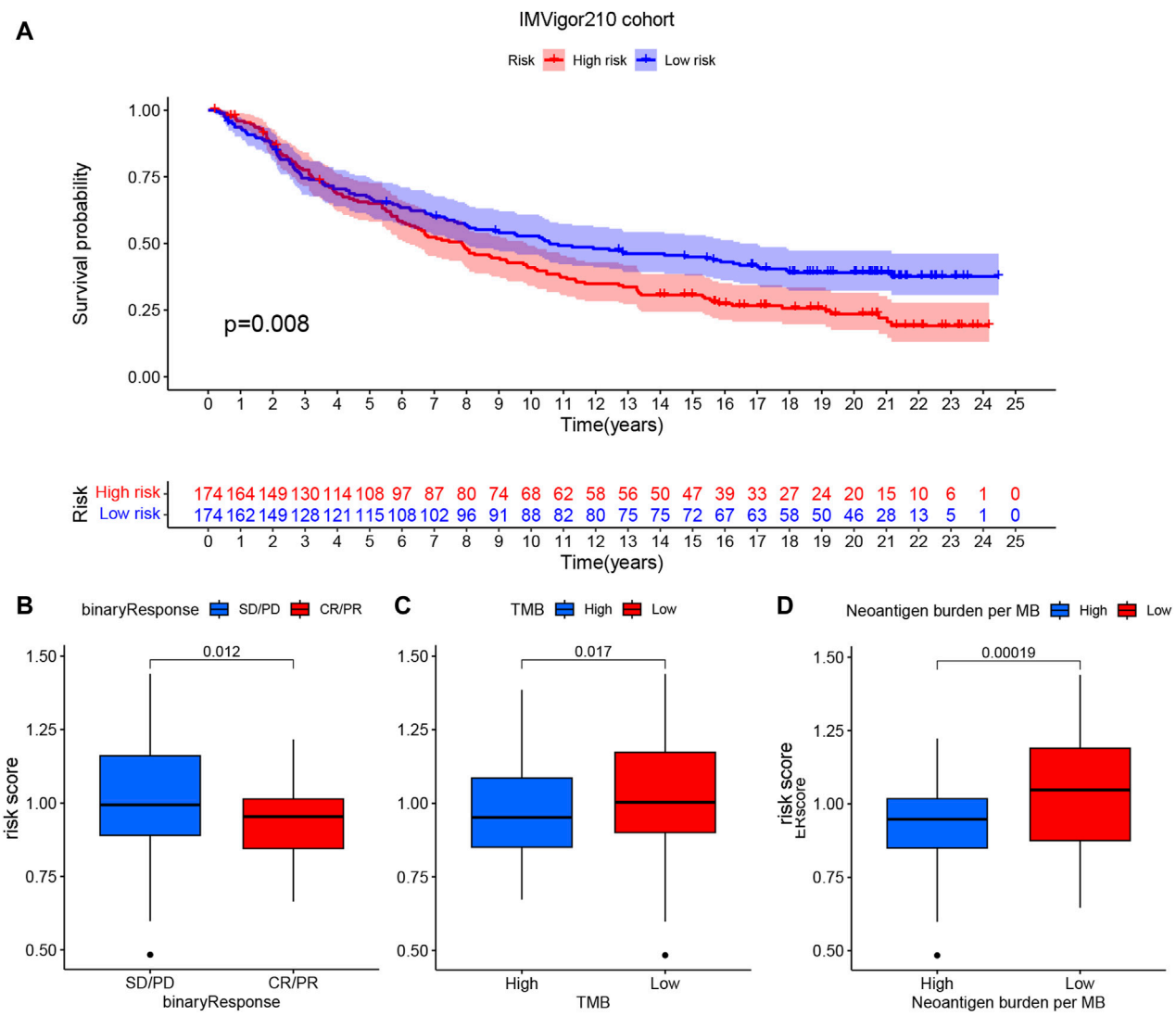


FIGURE 9
Potential impact of prognostic models on PD-L1 immunotherapy. **(A)** Survival curve indicating prognostic differences among patients categorized into high- and low-risk groups in the cohort receiving anti-PD-L1 treatment. **(B)** Connection between risk score and response to anti-PD-L1 treatment, **(C)** tumor mutational burden and **(D)** neoantigen TMB.

DFS outcomes exceeding 5 years. Overall, we demonstrated that the model could predict cholangiocarcinoma patient OS and DFS outcomes.

3.8 Immune characteristics of the prognostic model

The ssGSEA algorithm was used to score the immune function and infiltration of immune cells in the high-risk and low-risk groups. The enrichment scores of APC coinhibition, immune checkpoint, and T-cell coinhibition were higher in the high-risk group (Figure 8A). Furthermore, the high-risk group showed greater infiltration of macrophages, Th2 cells, and Treg cells (Figure 8B). The CIBERSORT algorithm was also employed to analyze immune cell infiltration, and the high-risk group demonstrated greater Treg cell and M2-type macrophage infiltration (Figure 8C). Additionally, the expression of 29 immune checkpoints was examined in different risk populations: CD244, CD274, CD44, CD80, CD80, CD86, HHLA2, IDO1, LAIR1, PDCD1LG2, TNFRSF9, TNFSF14, TNFSF18, TNFSF4, CD244, CD274, CD44, CD80, CD86, HHLA2, IDO1, PDCD1LG2, TNFRSF9, TNFSF14, TNFSF18, and TNFSF4. TNFSF9 was highly expressed in the high-risk group, whereas VTCN1 was highly expressed in the low-risk group (Figure 8D). These findings indicate that the tumor microenvironment in the high-risk group is more immunosuppressive. An analysis was conducted to investigate the predictive potential of the model in immunotherapy using the IMVigor210 dataset, which comprises patients with bladder cancer undergoing anti-PD-L1 treatment. A model incorporating six specific eccDNA genes was established using

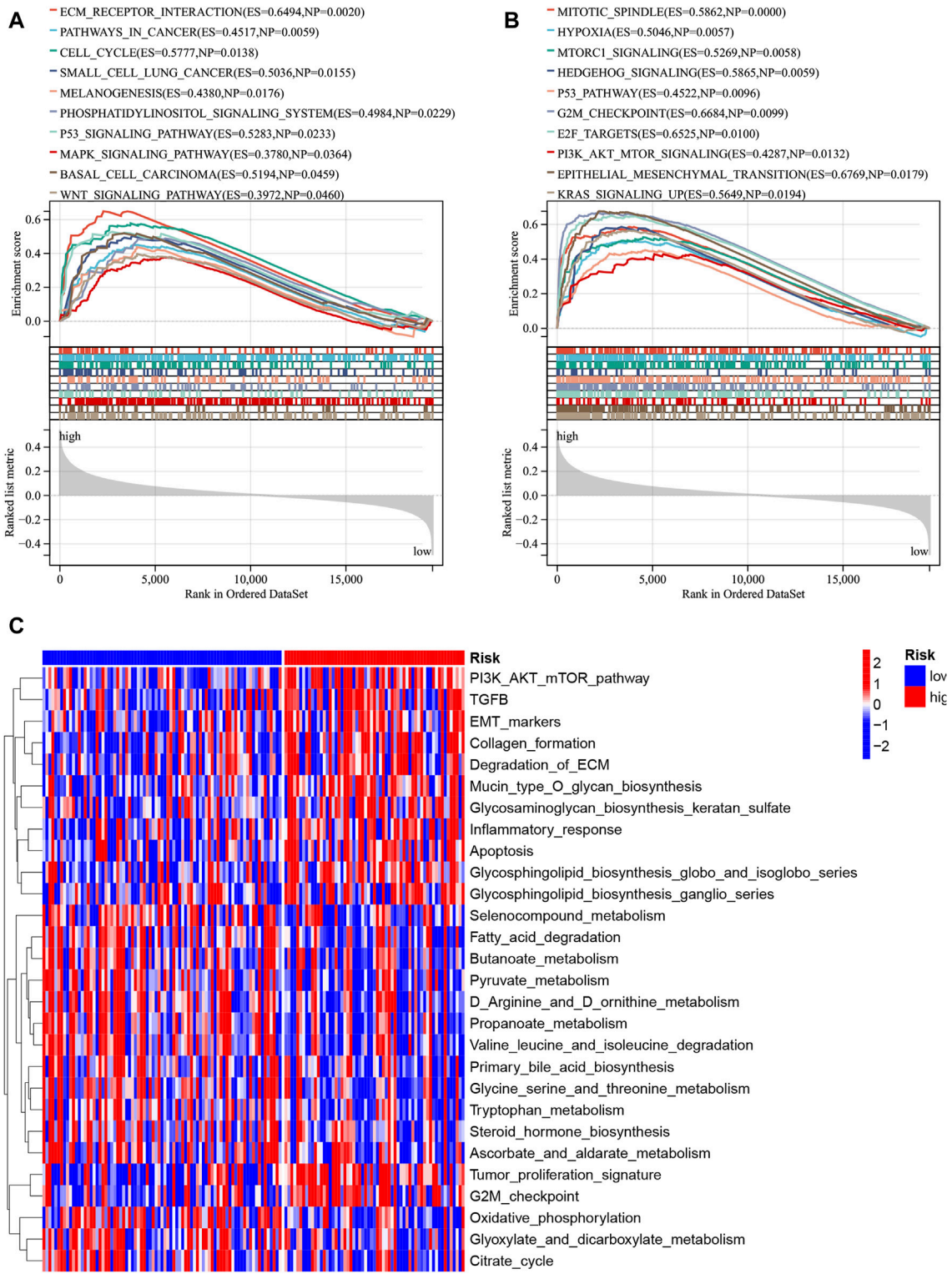


FIGURE 10
Functional enrichment disparities among the high- and low-risk groups were assessed. The enrichment analysis for the KEGG gene set **(A)** and hallmark gene set **(B)** was conducted via GSEA. Moreover, the disparity in enriched pathways between the high- and low-risk groups was examined utilizing GSVA **(C)**.

this cohort. The findings indicated that individuals in the low-risk category who underwent anti-PD-L1 treatment exhibited a better prognosis (Figure 9A), and those who attained CR/PR following anti-PD-L1 therapy displayed reduced risk scores (Figure 9B). There was a positive correlation between the TMB and neoantigen TMB with the patient's risk score, as

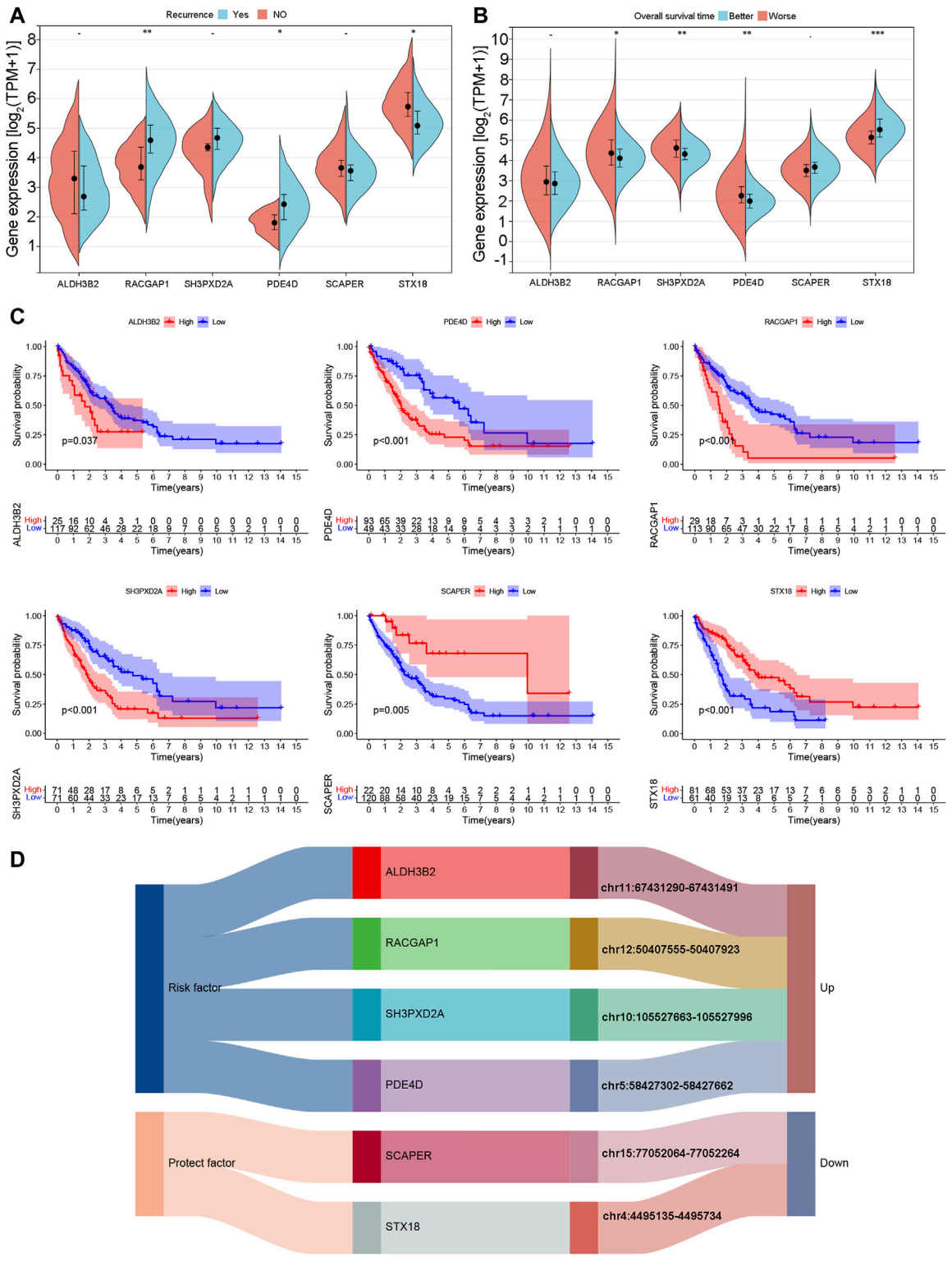


FIGURE 11
Expression and prognostic characteristics of model genes. **(A)** Expression of model genes in samples with and without recurrent disease. **(B)** Expression of model genes in better and poor overall survival outcome groups. **(C)** K–M curves of model genes according to overall survival outcomes. **(D)** Correspondence between model genes and eccDNA locations.

demonstrated in Figures 9C, D. Based on these findings, it can be inferred that the 6-gene eccDNA model distinguishes disparate immune microenvironments in high-risk versus low-risk groups. Additionally, this model could provide valuable insights guiding the administration of immune checkpoint inhibitors in treatment approaches.

3.9 Functional enrichment analysis of the high-risk and low-risk groups

In the merged cohort, GSEA was employed to identify differing molecular functions and pathways between the high- and low-risk groups using the KEGG signaling pathway and hallmark gene set. The high-risk group displayed increased enrichment in numerous molecular pathways associated with cancer, including cancer pathway, small cell lung cancer, and Wnt signaling pathway (Figure 10A). Moreover, the high-risk group exhibited a high enrichment of gene sets linked to tumor cell proliferation (HALLMARK_MITOTIC_SPINDLE and HALLMARK_G2M_CHECKPOINT) and metastasis (HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION), as indicated in Figure 10B. Gene set variation analysis (GSVA) was also employed to assess the degree of enrichment of cancer-related pathways in each sample and analyze pathway differences. The results demonstrated a significant increase in the enrichment of oncogene sets (Tumor_proliferation_signature, PI3K_AKT_mTOR_pathway and EMT_markers) in the high-risk group (Figure 10C). Collectively, these findings suggest that high-risk patients show an enrichment of multiple cancer-promoting pathways that contribute to a poor prognosis.

3.10 The characteristics of ALDH3B2, RACGAP1, SH3PXD2A, PDE4D, SCAPER, and STX18 in cholangiocarcinoma

The expression of ALDH3B2, RACGAP1, SH3PXD2A, PDE4D, SCAPER, and STX18 was analyzed in the TCGA-CHOL cohort. Samples from patients who experienced recurrence exhibited elevated expression of the risk genes RACGAP1 and PDE4D and reduced expression of the protective gene STX18 (Figure 11A). Patients were categorized into two groups according to the median OS duration: the better prognosis and poor prognosis groups. High expression of the risk genes PDE4D, RACGAP1, and SH3PXD2A was observed in the poor prognosis group, whereas the better prognosis group exhibited high expression of the protective gene STX18 (Figure 11B). The K–M curves of the six genes were plotted for the merged cohort. The analysis revealed that higher expression levels of risk genes, such as ALDH3B2, PDE4D, RACGAP1, and SH3PXD2A, were associated with a worse patient prognosis. Lower expression levels of the protective genes SCAPER and STX18 were also linked to a worse patient prognosis (Figure 11C). Figure 11D shows the correlation between the six eccDNA genes and eccDNA locations. The eccDNAs corresponding to four risk genes were highly expressed in the poor prognosis group (group B), while the eccDNAs corresponding to two protective genes were expressed at low levels in group B.

4 Discussion

In clinical practice, performing a core needle biopsy to obtain pCCA tissues is challenging because of its particular perihilar anatomical site and periductal infiltration growth pattern along the bile duct wall. There is a clinical need for reliable circulating biomarkers to predict treatment response and survival outcomes for pCCA patients. EccDNA has higher stability than linear DNA because it is circular, extrachromosomal, and exonuclease-resistant (Gaubatz and Flores, 1990). The present study first showed that eccDNA can be successfully detected in the bile and plasma of pCCA patients, with more eccDNAs detected in the bile than in the plasma. Almost all eccDNAs detected in the plasma could also be detected in the bile, possibly due to the special location and characteristics of pCCA. Moreover, there was good consistency in the length and chromosome distribution features between eccDNAs in bile and matched plasma samples.

This study examined the prognostic significance of differentially expressed eccDNAs in pCCA patients. The investigation revealed a strong correlation between the expression of eccDNAs and patient prognosis. Furthermore, an analysis of genes associated with the increased levels of eccDNAs in patients with poor survival demonstrated enrichment in the MAPK pathway (in bile samples) and WNT pathway (in both bile and plasma samples). These two pathways have been extensively implicated in the progression of cancer and are known to contribute to unfavorable clinical outcomes (Taciak et al., 2018; Kciuk et al., 2022). The upregulated eccDNAs enriched in the MAPK pathway in patients with poor survival outcomes originated from cancer-related genes, such as AKT3 and MAPK. Several studies have elucidated AKT3-specific oncogenic roles (Cristiano et al., 2006; Grabinski et al., 2014; Madhunapantula and Robertson, 2017). The eccDNA located on chr19:3018721-3019021, originating from the TLE-2 gene, was upregulated and enriched in the WNT pathway in both bile and plasma samples from patients with poor survival outcomes. This finding indicates the value of TLE-2 in eccDNAs for predicting poor survival outcomes. However, to date, few studies have investigated the relationship between the expression of TLE and the development of solid tumors (Dayyani et al., 2008; Ramasamy et al., 2016). The detailed function of the MAPK and WNT pathways in pCCA and eccDNA enrichment in these pathways requires further exploration to determine whether they are prognosis-related or efficacy-related factors for pCCA patients.

A prognostic model (including ALDH3B2, RACGAP1, SH3PXD2A, PDE4D, SCAPER and STX18) was constructed for cholangiocarcinoma patients according to the differentially expressed eccDNA-related genes in the bile to further analyze the function of eccDNA annotated genes and the regulation of cholangiocarcinoma. The model showed a relatively accurate ability to predict survival in public datasets and could distinguish cholangiocarcinoma patients' different immune microenvironments. This prognostic model was consistent with previous studies on these genes in hepatobiliary malignancies. ALDH3B2 expression was significantly upregulated in perihilar cholangiocarcinoma tissues, which was also correlated with tumor stage in pCCA patients. ALDH3B2 promotes the proliferation and metastasis of cholangiocarcinoma by regulating ITGB1 expression (Wang et al., 2021). RACGAP1 is a suppressor gene in hepatocellular carcinoma (HCC), and its expression can predict early HCC recurrence. In addition, silencing RACGAP1 can inhibit HCC cell migration and invasion (Wang, Ooi, and Hui, 2011). PDE4D overexpression increases the dephosphorylation and activity of YAP, promoting the growth of hepatocellular carcinoma cells *in vitro* and *in vivo* (Ren et al., 2022). SH3PXD2A and STX18 promote or inhibit the development of other cancers,

consistent with our prognostic model. SH3PXD2A forms a complex with ULK1 and MTOR, and the ULK1-SH3PXD2a-MMP14 axis upregulates the aggressiveness of ovarian cancer (Lin et al., 2023). Downregulation of STX18 can significantly enhance the growth of MCF-7 breast cancer cells (Bassett et al., 2008). SCAPER can bind cyclin A/Cdk2 in the endoplasmic reticulum. Overexpression of SCAPER transports cyclin A of the nucleus, delays G2/M phase transition, and prevents cyclin A from interacting with Cdk (Tsang and Dynlacht, 2008).

As eccDNA was more abundant in bile than in peripheral blood, eccDNA-related genes in bile were applied to construct the prognostic model. Moreover, as mentioned above, previous studies have revealed that the six genes included in the prognostic model can promote or inhibit the progression of cholangiocarcinoma (or other cancers), confirming that these genes play a role in cancer progression and prognosis. In addition, GSEA enrichment analysis based on the prognostic model showed that several cancer-promoting molecular pathways were more enriched in the high-risk group, including the MAPK and WNT signaling pathways, consistent with the KEGG enrichment analysis of upregulated bile eccDNAs in pCCA patients with worse survival outcomes. These results indicate that the model based on bile eccDNA-related genes is reliable, and bile eccDNAs might serve as important survival biomarkers for pCCA patients.

Our research has several limitations. First, because this was a single-center study based on several bile and plasma samples, the results require further validation in a large cohort of pCCA patients. Second, the high-throughput sequencing results were not validated by traditional PCR or Sanger sequencing. Finally, although we found enrichment of eccDNA-associated genes in the MAPK and WNT pathways, we did not further explore their molecular mechanisms in pCCA.

5 Conclusion

In summary, this study demonstrates that eccDNAs can be detected in the bile and plasma of pCCA patients and demonstrates similar length distribution ranges and peak values. There were substantially more eccDNAs detected in the bile than in the plasma, and the eccDNAs detected in the bile overlapped and comprised all the eccDNAs detected in the plasma. The enrichment of eccDNAs in the WNT and MAPK pathways might be associated with a poor prognosis. In addition, the prognostic model of cholangiocarcinoma constructed using differentially expressed eccDNA-related genes can predict patient survival times, differentiate the immune microenvironment of different patients, and predict immunotherapy efficacy. The value of bile eccDNAs as potential liquid biomarkers of survival in pCCA patients requires further investigation.

Data availability statement

The data presented in the study are deposited in the Sequence Read Archive (SRA) repository, accession number: PRJNA1113366.

Ethics statement

The studies involving humans were approved by the institutional review board of Peking University Cancer Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SF: Conceptualization, Data curation, Formal Analysis, Methodology, Resources, Supervision, Validation, Writing–original draft, Writing–review and editing. YD: Conceptualization, Formal Analysis, Methodology, Validation, Writing–original draft, Writing–review and editing. PZ: Investigation, Writing–review and editing. KZ: Investigation, Writing–original draft. GC: Formal Analysis, Writing–review and editing. LX: Investigation, Writing–review and editing. YZ: Data curation, Writing–review and editing. CN: Data curation, Writing–original draft. XW: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was funded by the National Natural Science Foundation of China (No. 82172039), Beijing Hospitals Authority Clinical Medicine Development of Special Funding Support (No. ZYLX202117), and Beijing Natural Science Foundation (No. 7212198).

Acknowledgments

We express our gratitude to LetPub (www.letpub.com) for providing linguistic support during the manuscript's preparation.

Conflict of interest

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

Publisher's note

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2024.1379435/full#supplementary-material

SUPPLEMENTARY MATERIAL S1
Length distribution of eccDNAs in the bile and matched plasma samples. Supplementary Figures S1–S6: Length distribution of eccDNAs in the bile and matched plasma samples of patients 1, 4, 6, 8, 9, and 10. eccDNAs, extrachromosomal circular DNAs.

SUPPLEMENTARY MATERIAL S2
GO analysis of bile eccDNAs. Cellular components, molecular functions, and biological processes associated with the upregulated eccDNAs in the bile of group B.

SUPPLEMENTARY MATERIAL S3
GO analysis of bile eccDNAs. Cellular components, molecular functions, and biological processes associated with the upregulated eccDNAs in the bile of group A.

SUPPLEMENTARY MATERIAL S4
GO analysis of plasma eccDNAs. Cellular components, molecular functions, and biological processes associated with the upregulated eccDNAs in the plasma of group B.

SUPPLEMENTARY MATERIAL S5
GO analysis of plasma eccDNAs. Cellular components, molecular functions, and biological processes associated with the upregulated eccDNAs in the plasma of group A.

SUPPLEMENTARY MATERIAL S6
(A) The UMAP shows that the samples within each data set are clustered together before batch effect removal, indicating a batch effect between different data sets. **(B)** After batch effect removal, the samples in each data set are mutually clustered, which is not limited to clustering within the respective data set, indicating that batch effect removal is optimal.

References

Banales, J. M., Cardinale, V., Carpino, G., Marzioni, M., Andersen, J. B., Invernizzi, P., et al. (2016). Expert consensus document: cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat. Rev. Gastroenterol. Hepatol.* 13 (5), 261–280. doi:10.1038/nrgastro.2016.51

Bassett, T., Harpur, B., Poon, H. Y., Kuo, K. H., and Lee, C. H. (2008). Effective stimulation of growth in MCF-7 human breast cancer cells by inhibition of syntaxin18 by external guide sequence and ribonuclease P. *Cancer Lett.* 272 (1), 167–175. doi:10.1016/j.canlet.2008.07.014

Chang, K., Creighton, C. J., Davis, C., Donehower, L., Drummond, J., Wheeler, D., et al. (2013). The cancer genome Atlas pan-cancer analysis project. *Nat. Genet.* 45 (10), 1113–1120. doi:10.1038/ng.2764

Cox, D., Yuncken, C., and Spriggs, A. I. (1965). Minute chromatin bodies in malignant tumours of childhood. *Lancet* 286 (7402), 55–58. doi:10.1016/s0140-6736(65)90131-5

Cristiano, B. E., Chan, J. C., Hannan, K. M., Lundie, N. A., Marmy-Conus, N. J., Campbell, I. G., et al. (2006). A specific role for AKT3 in the genesis of ovarian cancer through modulation of G(2)-M phase transition. *Cancer Res.* 66 (24), 11718–11725. doi:10.1158/0008-5472.Can-06-1968

Dayyani, F., Wang, J. F., Yeh, J. R. J., Ahn, E. Y., Tobey, E., Zhang, D.Er, et al. (2008). Loss of TLE1 and TLE4 from the del(9q) commonly deleted region in AML cooperates with AML1-ETO to affect myeloid cell proliferation and survival. *Blood* 111 (8), 4338–4347. doi:10.1182/blood-2007-07-103291

Edgar, R., Domrachev, M., and Lash, A. E. (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res.* 30 (1), 207–210. doi:10.1093/nar/30.1.207

Hu, J. G., Zhu, Xu, Wang, X. D., Cao, G., Wang, X., and Yang, R. J. (2019). Evaluation of percutaneous unilateral trans-femoral implantation of side-hole port-catheter system with coil only fixed-catheter-tip for hepatic arterial infusion chemotherapy. *Cancer Imaging* 19 (1), 15. doi:10.1186/s40644-019-0202-z

Gaubatz, J. W., and Flores, S. C. (1990). Purification of eucaryotic extrachromosomal circular DNAs using exonuclease III. *Anal. Biochem.* 184 (2), 305–310. doi:10.1016/0003-2697(90)90685-3

Grabinski, N., Möllmann, K., Milde-Langosch, K., Müller, V., Schumacher, U., Brandt, B., et al. (2014). AKT3 regulates ErbB2, ErbB3 and estrogen receptor α expression and contributes to endocrine therapy resistance of ErbB2(+) breast tumor cells from Balb-neuT mice. *Cell. Signal* 26 (5), 1021–1029. doi:10.1016/j.cellsig.2014.01.018

Kciuk, M., Gielecińska, A., Budzinska, A., Mojzych, M., and Kontek, R. (2022). Metastasis and MAPK pathways. *Int. J. Mol. Sci.* 23 (7), 3847. doi:10.3390/ijms23073847

Kim, H., Nguyen, N. P., Turner, K., Wu, S., Gujar, A. D., Luebeck, J., et al. (2020). Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers. *Nat. Genet.* 52 (9), 891–897. doi:10.1038/s41588-020-0678-2

Koche, R. P., Rodriguez-Fos, E., Helmsauer, K., Burkert, M., MacArthur, I. C., Maag, J., et al. (2020). Publisher correction: extrachromosomal circular DNA drives oncogenic genome remodeling in neuroblastoma. *Nat. Genet.* 52 (4), 464. doi:10.1038/s41588-020-0598-1

Lin, C. Y., Wu, K. Y., Chi, L. M., Tang, Y. H., Huang, H. J., Lai, C. H., et al. (2023). Starvation-inactivated MTOR triggers cell migration via a ULK1-SH3PXD2A/TKS5-MMP14 pathway in ovarian carcinoma. *Autophagy* 19, 3151–3168. doi:10.1080/15548627.2023.2239633

Madhunapantula, S. V., and Robertson, G. P. (2017). Targeting protein kinase-b3 (akt3) signaling in melanoma. *Expert Opin. Ther. Targets* 21 (3), 273–290. doi:10.1080/14728222.2017.1279147

Møller, H. D., Mohiyuddin, M., Prada-Luengo, I., Reza Sailani, M., Halling, J. F., Plomgaard, P., et al. (2018b). Circular DNA elements of chromosomal origin are common in healthy human somatic tissue. *Nat. Commun.* 9 (1), 1069. doi:10.1038/s41467-018-03369-8

Møller, H. D., Mohiyuddin, M., Prada-Luengo, I., Sailani, M. R., Halling, J. F., Plomgaard, P., et al. (2018a). Circular DNA elements of chromosomal origin are common in healthy human somatic tissue. *Nat. Commun.* 9 (1), 1069. doi:10.1038/s41467-018-03369-8

Palmieri, L. J., Lavolé, J., Dermine, S., Brezault, C., Dhooge, M., Barré, A., et al. (2020). The choice for the optimal therapy in advanced biliary tract cancers: chemotherapy, targeted therapies or immunotherapy. *Pharmacol. Ther.* 210, 107517. doi:10.1016/j.pharmthera.2020.107517

Ramasamy, S., Saez, B., Mukhopadhyay, S., Ding, Da C., Ahmed, A. M., Chen, Xi, et al. (2016). Tle1 tumor suppressor negatively regulates inflammation *in vivo* and modulates NF-κB inflammatory pathway. *Proc. Natl. Acad. Sci. U. S. A.* 113 (7), 1871–1876. doi:10.1073/pnas.1511380113

Ren, H., Chen, Y., Zhou, Ao, Qi, C., Yang, X., Hua, T., et al. (2022). PDE4D binds and interacts with YAP to cooperatively promote HCC progression. *Cancer Lett.* 541, 215749. doi:10.1016/j.canlet.2022.215749

Rizvi, S., Khan, S. A., Hallemeier, C. L., Kelley, R. K., and Gores, G. J. (2018). Cholangiocarcinoma - evolving concepts and therapeutic strategies. *Nat. Rev. Clin. Oncol.* 15 (2), 95–111. doi:10.1038/nrclinonc.2017.157

Robinson, M. D., McCarthy, D. J., and Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26 (1), 139–140. doi:10.1093/bioinformatics/btp616

Sarkans, U., Füllgrabe, A., Ali, A., Athar, A., Behrangi, E., Diaz, N., et al. (2021). From ArrayExpress to BioStudies. *Nucleic Acids Res.* 49 (D1), D1502–d1506. doi:10.1093/nar/gkaa1062

Shibata, Y., Kumar, P., Layer, R., Willcox, S., Gagan, J. R., Griffith, J. D., et al. (2012). Extrachromosomal microDNAs and chromosomal microdeletions in normal tissues. *Science* 336 (6077), 82–86. doi:10.1126/science.1213307

Sin, S. T. K., Jiang, P. Y., Deng, J.En, Ji, Lu, Cheng, S. H., Dutta, A., et al. (2020). Identification and characterization of extrachromosomal circular DNA in maternal plasma. *Proc. Natl. Acad. Sci. U. S. A.* 117 (3), 1658–1665. doi:10.1073/pnas.1914949117

Taciak, B., Pruszynska, I., Kiraga, L., Bialasek, M., and Krol, M. (2018). Wnt signaling pathway in development and cancer. *J. Physiology Pharmacol.* 69 (2), 185–196. doi:10.26402/jpp.2018.2.07

Tsang, W. Y., and Dynlacht, B. D. (2008). Double identity of SCAPER: a substrate and regulator of cyclin A/Cdk2. *Cell. Cycle* 7 (6), 702–705. doi:10.4161/cc.7.6.5611

Turner, K. M., Deshpande, V., Beyter, D., Koga, T., Rusert, J., Lee, C., et al. (2017). Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. *Nature* 543 (7643), 122–125. doi:10.1038/nature21356

Wang, S. M., Ooi, L. L., and Hui, K. M. (2011). Upregulation of Rac GTPase-activating protein 1 is significantly associated with the early recurrence of human hepatocellular carcinoma. *Clin. Cancer Res.* 17 (18), 6040–6051. doi:10.1158/1078-0432.Ccr-11-0557

Wang, X. D., Hu, J. G., Cao, G., Zhu, Xu, Cui, Y., Ji, X. Q., et al. (2017). Phase II study of hepatic arterial infusion chemotherapy with oxaliplatin and 5-fluorouracil for advanced perihilar cholangiocarcinoma. *Radiology* 283 (2), 580–589. doi:10.1148/radiol.2016160572

Wang, Y., Li, K., Zhao, W., Liu, Z., Liu, J., Shi, A., et al. (2021). Aldehyde dehydrogenase 3B2 promotes the proliferation and invasion of cholangiocarcinoma by increasing Integrin Beta 1 expression. *Cell. Death Dis.* 12 (12), 1158. doi:10.1038/s41419-021-04451-8

Zhu, Y. F., Gujar, A. D., Wong, C. H., Tjong, H., Ngan, C. Y., Gong, L., et al. (2021). Oncogenic extrachromosomal DNA functions as mobile enhancers to globally amplify chromosomal transcription. *Cancer Cell.* 39 (5), 694–707.e7. doi:10.1016/j.ccell.2021.03.006

frontiers | Frontiers in Cell and Developmental Biology

TYPE Review
PUBLISHED 23 July 2024
DOI 10.3389/fcell.2024.1437951

OPEN ACCESS

EDITED BY
Ajinkya Revandkar,
Massachusetts General Hospital Cancer Center, United States

REVIEWED BY
Marwa Tantawy,
University of Florida, United States
Zhaofeng Liang,
Jiangsu University, China

*CORRESPONDENCE
Biao Qian,
qb2003_2000@163.com

RECEIVED 24 May 2024
ACCEPTED 15 July 2024
PUBLISHED 23 July 2024

CITATION
Gan L, Zheng L, Zou J, Luo P, Chen T, Zou J, Li W, Chen Q, Cheng L, Zhang F and Qian B (2024), MicroRNA-21 in urologic cancers: from molecular mechanisms to clinical implications. *Front. Cell Dev. Biol.* 12:1437951. doi: 10.3389/fcell.2024.1437951

MicroRNA-21 in urologic cancers: from molecular mechanisms to clinical implications

Lifeng Gan[1,2,3], Liying Zheng[4], Junrong Zou[2,3], Peiyue Luo[1,2,3], Tao Chen[1,2,3], Jun Zou[1,2,3], Wei Li[1,2,3], Qi Chen[1,2,3], Le Cheng[1,2,3], Fangtao Zhang[1,2,3] and Biao Qian[2,3]*

[1]The First Clinical College, Gannan Medical University, Ganzhou, Jiangxi, China, [2]Department of Urology, The First Affiliated Hospital of Gannan Medical University, Ganzhou, Jiangxi, China, [3]Key Laboratory of Urology and Andrology of Ganzhou, Ganzhou, Jiangxi, China, [4]Department of Graduate, The First Affiliated Hospital of Gannan Medical University, Ganzhou, Jiangxi, China

The three most common kinds of urologic malignancies are prostate, bladder, and kidney cancer, which typically cause substantial morbidity and mortality. Early detection and effective treatment are essential due to their high fatality rates. As a result, there is an urgent need for innovative research to improve the clinical management of patients with urologic cancers. A type of small noncoding RNAs of 22 nucleotides, microRNAs (miRNAs) are well-known for their important roles in a variety of developmental processes. Among these, microRNA-21 (miR-21) stands out as a commonly studied miRNA with implications in tumorigenesis and cancer development, particularly in urological tumors. Recent research has shed light on the dysregulation of miR-21 in urological tumors, offering insights into its potential as a prognostic, diagnostic, and therapeutic tool. This review delves into the pathogenesis of miR-21 in prostate, bladder, and renal cancers, its utility as a cancer biomarker, and the therapeutic possibilities of targeting miR-21.

KEYWORDS

miR-21, cancer, prostate, bladder, kidney, urinary system

Introduction

Cancer of the urinary system, including prostate, bladder, and kidney cancer, is a significant contributor to morbidity and mortality globally. Prostate cancer, bladder cancer, and kidney cancer are among the most prevalent cancers of the urinary system, with prostate cancer being one of the top ten causes of cancer-related deaths in men in Europe. The latest global cancer statistics from 2020 report 1,414,259 new cases of prostate cancer, accounting for 7.3% of the total new cases, and 375,304 new deaths, representing 3.8% of the total new cases. Additionally, there were 573,278 new cases of bladder cancer (3.0%) and 212,536 new deaths (2.1%), as well as 431,288 new cases of kidney cancer (2.2%) and 179,368 new deaths (1.8%) (Sung et al., 2021). Despite advancements in prognostic tools for urologic tumors, long-term outcomes remain unfavorable due to issues such as drug resistance and recurrence. Addressing these challenges necessitates further research to identify novel biomarkers for precise monitoring of urologic tumor progression and to discover therapeutic targets that can enhance survival rates. Short, single-stranded RNAs, known as microRNAs (miRNAs), are naturally occurring molecules that interact with target messenger RNAs (mRNAs) to regulate gene expression at the post-transcriptional level (Lujambio and Lowe, 2012). Research has linked abnormalities in miRNAs to various diseases, particularly cancer. Oncogenic miRNAs (onco-miRs) can drive tumor

progression, while anti-cancer miRNAs may impede cancer growth and metastasis by inhibiting oncogenes (Rupaimoole and Slack, 2017; Wang X. et al., 2021). These miRNAs can influence critical cellular processes such as cell proliferation, differentiation, and apoptosis (Maatouk and Harfe, 2006; Bueno et al., 2008; Wang and Lee, 2009). Among the numerous miRNAs associated with cancer development, miR-21 was one of the first identified as an oncogenic miRNA or "oncomiR." Changes in miR-21 expression have been linked to disruptions in epigenetic factors, as well as transcriptional and post-transcriptional regulators, either during their biogenesis or through repression, ultimately leading to an oncogenic phenotype (Fujita et al., 2008; Feng and Tsao, 2016). This review aims to explore the regulatory role and function of miRNA-21 in prostate, renal cell, and bladder cancers, focusing on highly dysregulated signaling pathways. Recent advances in miRNA-21-based cancer therapies are summarized, highlighting the significant progress made since the discovery of miRNA-21's critical regulatory function in various tumor types. Special attention is given to the newly identified role of miRNA-21 in carcinogenesis and its potential implications for the diagnosis and treatment of urological tumors, including prostate, kidney, and bladder cancers.

MicroRNA

miRs are approximately 22-nucleotide long single-stranded noncoding RNAs. The discovery of the first miRNA, lin-4, in *Caenorhabditis elegans* in 1993 marked the beginning of understanding miRNA processing and function, leading to the identification of thousands of miRNAs across species (Lee et al., 1993; Wightman et al., 1993; Ambros, 2004). miRNA genes are distributed across the genome (Rodriguez et al., 2004), with many being non-coding genes that produce miRNA as their sole transcription product. In other instances, miRNAs are situated within introns or untranslated regions (UTRs) of protein-coding genes. Transcription of miRNA host genes by RNA polymerase II yields pri-miRNA or primary miRNA transcripts (Lee et al., 2004; Bortolin-Cavaillé et al., 2009). These pri-miRNAs typically undergo splicing, capping, and polyadenylation similar to protein-coding mRNAs (Cai et al., 2004). To become mature active miRNAs, pri-miRNAs undergo two nucleic acid endonuclease processing steps (Kim et al., 2009). The RNA-binding protein DGCR8 is associated with Drosha enzymes and is necessary for pri-miRNA cleavage (Han et al., 2004). The nucleic acid endonuclease Dicer cleaves the loop region of the precursor, releasing the mature miRNA (Hutvágner et al., 2001). Similar to Drosha, Dicer is linked to RNA-binding proteins. miRNAs ultimately become part of a RISC (or miRISC). The specific composition of this protein complex is currently unknown, but it includes the crucial protein Argonaute, with four family members identified in humans (Ago1-4) (Tabara et al., 1999; Hammond et al., 2001). Argonaute directly binds mature miRNAs and targets mRNAs that are complementary to the miRNA (Lewis et al., 2003). These molecules play crucial roles in biological processes by regulating gene expression at the post-transcriptional level. By binding to messenger RNAs in the cytoplasm, miRNAs can either degrade mRNA or temporarily inhibit translation (Fabian et al., 2010). The downregulation of specific miRNAs results in the upregulation of corresponding proteins, and vice versa. Conversely, upregulation of miRNAs leads to decreased expression of target proteins. Additionally, miRNAs induce translational repression by binding to the 3′and 5′untranslated regions (UTRs) as well as the coding region of mRNAs. They also contribute to gene transcription by binding within the promoter region of genes (Broughton et al., 2016). miRNAs are estimated to regulate approximately 60% of protein-coding genes, with an average of 200 targets per miRNA (Krek et al., 2005; Friedman et al., 2009). This indicates their significant role in regulating various physiological and pathological cellular processes. miRNAs are key regulators in cell fate determination, proliferation, and cell death. They play significant roles in various metabolic pathways, including cholesterol and fatty acid metabolism, as well as pancreatic islet function and glucose metabolism (Fernández-Hernando et al., 2013). Notably, 522 virally encoded miRNAs have been discovered, with a particular emphasis on the herpesvirus family (Griffiths-Jones et al., 2006). Beyond their metabolic and viral connections, miRNAs also participate in diverse cellular functions like immune responses (Calame, 2007; Gantier et al., 2007), insulin secretion (Poy et al., 2004), neurotransmitter synthesis (Greco and Rameshwar, 2007), circadian rhythms (Cheng et al., 2007), and viral replication (Jopling et al., 2005). The biogenesis and function of miRNAs are tightly controlled processes, and dysregulation in miRNA production, availability, and target regulation has been linked to a range of human diseases, including cancer (Croce, 2009).

MiRNAs and cancer

The role of miRNAs in cancer has been extensively studied, revealing dysregulation in many cancer types and stages through various mechanisms (Croce, 2009). Chromosomal abnormalities are a known cause of miRNA dysregulation in cancer, with tumorigenesis often linked to chromosomal aberrations like deletions, amplifications, and translocations. Computational analysis has revealed that a significant number of miRNAs are located within cancer-associated genomic regions or fragile sites in both humans and mice (Calin et al., 2004; Sevignani et al., 2007). Furthermore, epigenetic factors can impact miRNA expression, with CpG island hypermethylation in promoter regions leading to heritable transcriptional silencing of tumor-suppressor genes in many cancers. This gene silencing through DNA methylation is intricately connected to histone modifications. Computational simulations have identified CpG islands proximal to numerous miRNAs. Transcription factors can stimulate the production of miRNAs by activating the transcription of pri-miRNAs (Lehmann et al., 2008). Notably, many oncogenes or tumor suppressors function as transcription factors, and various miRNA-transcription factor interactions have been observed in cancer, including with proteins like p53, c-Myc, and E2F (Dews et al., 2006; He et al., 2007; Sylvestre et al., 2007). Apart from the transcription rate of pri-miRNAs, the steady-state levels of mature miRNAs are influenced by the efficiency of processing their precursors and subsequent stability (Thomson et al., 2006). In cancer, miRNAs finely tune the expression of oncogenes and tumor suppressors in response to extracellular signals (Pagotto et al., 2022). Oncogenic miRNAs negatively suppress tumor

suppressor genes, promoting tumor development, influencing cell differentiation and proliferation timing, cell cycle exit, and regulating oncogene expression, particularly the Ras gene (Takamizawa et al., 2004; He et al., 2005; Iorio et al., 2005; Li et al., 2012; Palma et al., 2014). Tumor suppressor miRNAs inhibit cancer by regulating oncogenes and genes controlling cell differentiation or apoptosis, targeting oncogenes involved in cell differentiation, cancer invasion, apoptosis, proliferation, and metastasis (Iorio et al., 2005; Zhang et al., 2007; Iqbal et al., 2019). The main aspects of miRNA-regulated cancer biology include the following: Cell cycle regulators often act as oncogenes or tumor suppressors. The most typical example is the cell cycle inhibitor p27(Kip1). p27(Kip1) is a tumor suppressor that is expressed at low levels in some cancers. p27(Kip1) binds to Cdk2-cell cycle protein E and blocks the transition from G1 to S. p27(Kip1) is a direct target of miR-221 and -222 in glioblastoma (Gillies and Lorimer, 2007; le Sage et al., 2007)and prostate cancer cells (Galardi et al., 2007); apoptosis is an active process controlled by gene expression programs. Apoptosis is an active process controlled by a gene expression program that varies depending on the biological context. miRNAs are involved in tumorigenesis by directly targeting anti-apoptotic genes. Representative examples are miR-29b (Mott et al., 2007) and miR-34s (Bommer et al., 2007), -15a and -16 (Cimmino et al., 2005) that inhibit the anti-apoptotic genes Mcl-1 and Bcl-2, respectively; malignant tumors, unlike benign tumors, are characterized by invasiveness and metastasis. Ectopic expression of miR-125 has been reported to impair cell motility and invasion in breast cancer cell lines (Scott et al., 2007); the recruitment of the vascular system is critical for tumor cell survival, and stimulation of neovascularization by c-Myc involves downregulation of the anti-angiogenic factor Tsp-1 (platelet reactive protein-1). c-Myc inhibits Tsp-1 and related proteins through activation of miR-17-92 cluster. CTGF (connective tissue growth factor). Tsp-1 and CTGF appear to be direct targets of miR-19 and -18 in this cluster, respectively. Ectopic expression of the miR-17-92 cluster is sufficient to promote angiogenesis (Dews et al., 2006). These properties have sparked significant interest in miRNAs as prognostic markers and therapeutic targets for human tumors (Croce, 2012).

MiR-21 expression and urologic tumors

miR-21 stands out among the numerous miRNAs linked to cancer progression as one of the earliest identified oncogenic miRNAs. Positioned on chromosome 17q23.2 within the intron of the transmembrane protein 49 (TMEM49)/vesicular membrane protein 1 (VMP1) gene, miR-21 possesses a distinct and highly conserved promoter region. This region is known to be activated by activator protein 1 (AP-1), which interacts with the switch/sucrose nonfermentable (SWI/SNF) complex, as well as the Ets-associated proteins PU.1 and CCAAT/enhancer-binding protein (C/EBP). Additionally, miR-21 is influenced by nuclear factor I (NFI), serum response factor (SRF), p53, and signal transducer and activator of transcription 3 (STAT3) (Fujita et al., 2008; Feng and Tsao, 2016). miR-21 has been identified as significantly overexpressed in various human cancer types, such as breast, gastric, lung, esophageal, colorectal, biliary tract, nasopharyngeal, hepatocellular carcinomas, osteosarcomas, gliomas, leukemias, retinoblastomas, and lymphomas (Volinia et al., 2006; Wu et al., 2015). Among them is growing evidence that miR-21 functions as an oncogene. Recent literature increasingly highlights the association between urological tumors and miR-21, with numerous studies emphasizing its impact on prostate, bladder, and kidney cancers - three of the most prevalent urological malignancies. In the context of prostate cancer and miR-21 research, Guan et al. discovered that levels of miR-21 were notably higher in prostate cancer (PCa) tissues compared to adjacent noncancerous prostate tissues (Guan et al., 2016); Meanwhile, Kamla et al. illustrated that miR-21 plays a role in promoting prostate cancer stem cells (PCSC) and potentially targets apoptotic genes involved in the development of PCa, suggesting its potential as a diagnostic biomarker for the disease (Shukla et al., 2023); Furthermore, existing literature acknowledges the close association between the androgen receptor and hormone-dependent prostate cancer. Studies have also indicated that miR-21, recognized as an androgen receptor (AR)-regulated miRNA, can enhance the growth of androgen-dependent CaP and contribute to resistance to desmoplasia (Ribas et al., 2009). In the literature on bladder cancer and miR-21, Zhang et al. (2015) demonstrated that miR-21 mRNA expression was significantly reduced in bladder cancer (BC) tissues compared to normal bladder tissues. They also suggested that the upregulation of miR-21 in BC may promote tumor progression (Zhang et al., 2015). Regarding renal cell carcinoma (RCC) and miR-21, Lv et al. (2013) found that miR-21 was significantly overexpressed in RCC tissues compared to adjacent normal tissues. They observed that miR-21 inhibitors inhibited cell growth by inducing apoptosis and cell cycle arrest in S phase (Lv et al., 2013); The metastasis of clear cell renal cell carcinoma (ccRCC) may be triggered by epithelial mesenchymal transition and mesenchymal stem cells, which also contribute to the development of primary tumors. Furthermore, miR-21 overexpression was linked to the formation of ccRCC spheroids, while reducing its expression could directly inhibit the proliferation of ccRCC cells (Cao J. et al., 2016); Studies have shown that the expression of miR-21 is associated with the survival of renal cancer patients, with the expression level correlating with the 5-year survival rate and disease stage. Notably, patients with low miR-21 expression had a 100% 5-year survival rate post-surgery, while only 50% of patients with high miR-21 expression survived (Zaman et al., 2012). These findings underscore the importance of further investigating the specific mechanisms of miR-21 in urological tumors and elucidating its role in the development and progression of these malignancies.

Mechanism of MiR-21 associated with urologic cancers

Mechanisms of action associated with miR-21 in prostate cancer

In miR-21-related tumor-related studies, we have found that miR-21 can affect tumor progression both by regulating its downstream targets and by regulating its expression in tumors through related genes, thereby affecting tumor progression. On the right side of Figure 1, we summarize all current studies on

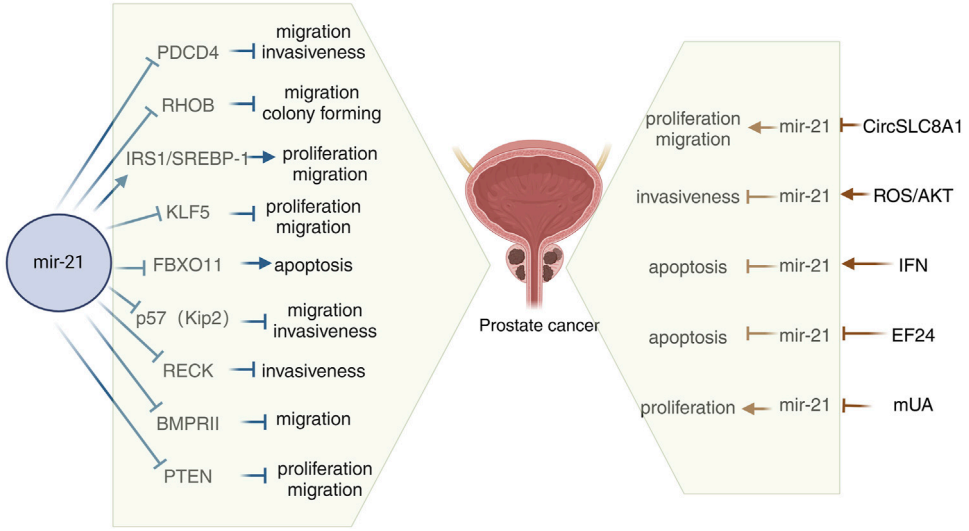


FIGURE 1
Schematic diagram of the mechanism of miR-21 in prostate cancer. mUA, Methylated urolithin A; PDCD4, Programmed cell death 4; SREBP-1, Sterol regulatory element binding protein 1; BMPRII, Bone morphogenetic protein receptor II.

the ability to influence prostate cancer progression by regulating miR-21 expression. Wang D. et al. (2021) conducted a study to assess the expression of CircSLC8A1 in human prostate cancer using qRT-PCR. Their findings indicated a significant reduction in CircSLC8A1 levels in PCa. The study also revealed that CircSLC8A1 acts as a tumor suppressor by impeding the proliferation and migration of PCa cells. Moreover, the upregulation of miR-21 in PCa suggests a potential direct interaction between miR-21 and circSLC8A1, with circSLC8A1 playing a role in inhibiting prostate cancer progression by sponging miR-21 (Wang D. et al., 2021). Jajoo et al. discovered that miR-21 plays a role in regulating the invasiveness of PC-3M-MM2 prostate cancer cells. They also found that the expression of miR-21 in these cells is influenced by high levels of reactive oxygen species (ROS) production. Previous research has demonstrated that ROS can impact invasiveness by activating Akt in prostate cancer cells, which in turn can regulate miR-21 expression. This suggests that Akt may serve as a target of ROS to modulate miR-21 levels (Kumar et al., 2008). Furthermore, Akt has been shown to directly influence miR-21 expression in prostate cancer cells (Sheth et al., 2012), indicating its potential role as a target for ROS-mediated regulation of miR-21. Overall, these findings suggest that ROS can stimulate the Akt pathway to enhance miR-21 expression (Jajoo et al., 2013). Interferons (IFN) are antiviral cytokines with profound impacts on cell functions such as proliferation, differentiation, apoptosis, and immune responses. Research on prostate cancer cells has revealed that miR-21 acts as a critical factor in suppressing IFN-induced apoptosis. Studies have indicated that the oncogenic miR-21 can be increased by IFN and that modulating miR-21 expression, particularly through miR-21 knockdown, can amplify the apoptotic effects of IFN (Yang et al., 2010). EF24, a curcumin analog, exhibits superior anticancer activity compared to curcumin. Research indicates that EF24 triggers apoptosis in ovarian, gastrointestinal, and breast cancer models (Adams et al., 2005; Liang et al., 2011; Zhu et al., 2012). Some studies have demonstrated EF24's efficacy against prostate cancer *in vivo* by inhibiting miR-21 expression and upregulating miR-21 target genes for tumor suppression (Yang et al., 2013). Urolithin, a metabolite derived from ellagic acid-derived and produced by human colonic microflora, exhibits biological activity. Research investigating the impact of methylated urolithin A (mUA) on the viability of human prostate cancer DU145 cells has revealed a decrease in miR-21 expression following mUA exposure. Additionally, mUA has been found to suppress cell viability in DU145 cells by regulating miR-21 and its downstream targets such as PTEN, Akt, and Wnt/β-catenin signaling pathways (Zhou et al., 2016) (Figure 1).

On the left side of Figure 1, we summarize all the current studies on miR-21 affecting prostate cancer progression by modulating its downstream targets. Programmed cell death 4 (PDCD4) is a known suppressor of tumorigenesis, tumor progression, and invasion, functioning both independently and in response to external stimuli. PDCD4 is recognized as the first specific translational repressor to be characterized (Hilliard et al., 2006). Studies have shown that PDCD4 protein is predominantly localized in the cytoplasm, and that lower levels of PDCD4 expression are correlated with less differentiated prostate cancer. Additionally, miR-21 has been found to enhance the growth of prostate cancer cells by reducing the expression of PDCD4 (Dong et al., 2015). Tumor hypoxia is a well-recognized factor in the progression of prostate cancer and is known to influence the expression of various miRNAs. Research has shown that hypoxia plays a crucial role in the upregulation of miR-21 in prostate tumors, leading to the downregulation of the tumor suppressor gene Ras homologue family member B (RHOB) and ultimately promoting the progression of prostate cancer (Angel et al., 2023). Sterol regulatory element binding protein 1 (SREBP-1) plays a crucial role in adipogenesis and lipid metabolism, impacting disease progression and prognosis in prostate cancer (PCa) patients. Research indicates that SREBP-1 is regulated by miR-21 at a

transcriptional level in cell cultures and mouse models, leading to increased cell proliferation, migration, and SREBP-1 levels in human PCa cells. The activation of the IRS1/SREBP-1 axis by miR-21 contributes to PCa advancement, suggesting that targeting the miR-21/SREBP-1 signaling pathway could be a promising approach to managing PCa aggressiveness (Kanagasabai et al., 2022). KLF5, a transcription factor located in the nucleus, plays a crucial role in regulating gene expression and impacting various cellular functions in cancer (Gao et al., 2015). Chen et al. discovered that miR-21, acting as an oncogene in PCa, targets KLF5 directly. Overexpression of miR-21 in LNCaP cells led to decreased levels of KLF5 mRNA and protein compared to MOCK or untreated controls. Conversely, reducing miR-21 in DU145 and PC-3 cells resulted in increased KLF5 mRNA and protein expression. The direct targeting of KLF5 by miR-21 promotes proliferation, migration, invasion, and resistance to apoptosis in both androgen-dependent and nondependent PCa cells (Guan et al., 2019). FBXO11, a component of the SKP1-CUL1-F-box ubiquitin ligase complex, is involved in targeting proteins for ubiquitination and proteasomal degradation. Through microarray analysis and quantitative PCR, one study identified and validated FBXO11 as a target gene of miR-21, an oncogenic miRNA. The study showed that miR-21 promotes tumorigenesis by inhibiting the expression of FBXO11, which typically functions as a tumor suppressor (Yang C. H. et al., 2015). The cell cycle protein-dependent kinase inhibitor p57(Kip2) is considered a potential oncogene linked to Beckwith-Wiedemann syndrome and sporadic cancers. Recent studies have identified p57(Kip2) as a target of miR-21 in prostate cancer, shedding light on a new oncogenic role of this miR-21. miR-21 has been found to reduce p57(Kip2) expression by targeting the gene's coding region and dampen p57(Kip2)-mediated cellular functions in prostate cancer (Mishra et al., 2014). Research has demonstrated that miR-21 modulates cellular invasiveness by directly regulating the MMP inhibitor RECK, a protein rich in cysteine and featuring a Kazal motif, known for inhibiting multiple MMPs. Notably, the expression levels of RECK can predict the prognosis of various common cancers; lower RECK levels are often linked to increased invasiveness and a poorer prognosis (Takenaka et al., 2004; Kotzsch et al., 2005). Investigations on whether RECK is a target of miR-21 in the DU-145 prostate cancer cell line have revealed that miR-21 directly inhibits RECK and plays a significant role in the progression of prostate cancer by controlling RECK (Reis et al., 2012). Bone morphogenetic proteins (BMPs) belong to the tumor growth factor (TGF)-ß superfamily (Li, 2008). BMP acts by interacting with a diverse receptor complex composed of two types of serine-threonine kinase transmembrane receptors. BMPRII functions as a type 2 receptor for BMP, and mutations in the BMPRII gene are associated with the development of hereditary pulmonary arterial hypertension. Knocking down the BMPRII gene results in early embryonic death (Hassel et al., 2004; Rigelsky et al., 2008). Qin et al. discovered that bone morphogenetic protein receptor II (BMPRII) is directly regulated by miR-21, and they demonstrated a negative correlation between the protein levels of BMPRII and the abundance of miR-21 in PC3 and Lncap cells (Qin et al., 2009). The oncogene PTEN, located on chromosome 10, encodes a protein with lipid phosphatase and protein phosphatase activities. This protein dephosphorylates the PI3K 3-phosphorylation site in cells, inhibiting the phosphorylation of the downstream signal transduction molecule Akt. Research has shown that miR-21 can target and inhibit the expression of PTEN, enhancing the PI3K/Akt signaling pathway in prostate cancer cells. This ultimately promotes the proliferation and invasion of prostate cancer cells (Liu et al., 2011; Yang Y. et al., 2017). Taken together these findings add to the current understanding of the molecular processes involved in the development of prostate cancer and increase the likelihood of using miR-21 as a therapeutic target for prostate cancer.

Mechanism of action associated with miR-21 in bladder cancer

Growth arrest-specific transcript 5 (GAS5) is located at chromosome 1q25 and acts as a tumor suppressor (Pickard et al., 2013). Recent studies have revealed that GAS5 is downregulated in bladder cancer, leading to increased cell proliferation through CDK6 regulation (Liu Z. et al., 2013). Additionally, GAS5 has been found to inhibit metastasis of hepatocellular carcinoma cells by suppressing miR21 (Hu et al., 2016). A study investigating the interplay between GAS5 and miR-21 in bladder cancer cell reversal mechanisms showed that low GAS5 levels and high miR-21 levels were associated with bladder cancer. It was demonstrated that GAS5 directly targets miR-21 through luciferase analysis, while miR-21 targets PTEN. The study further revealed that low GAS5 expression upregulates miR-21, which in turn downregulates PTEN. Dual luciferase reporter gene assays confirmed PTEN as a direct target of miR-21. Overall, GAS5 exerts anti-proliferative and pro-apoptotic effects on bladder cancer cells by modulating the miR-21/PTEN pathway (Chen et al., 2020). RECK is a tumor suppressor gene that plays a role in inhibiting metalloproteinases, such as MMP9. Several studies have shown that inhibiting miR-21 expression through the transfection of a specific miR-21 inhibitor (anti-miR-21) leads to increased RECK expression, decreased MMP9 expression, and impacts the migration and proliferation of bladder cancer cells by modulating this pathway (Dos Santos et al., 2024). Macrophages are a prominent stromal cell type in the tumor microenvironment (TME), with the ability to exhibit either an immunological M1 phenotype that suppresses tumors or an M2 phenotype that promotes tumor inflammation and immunosuppression (Sica and Bronte, 2007). In a study conducted by Lin et al. (2020), it was found that miR-21 downregulated PTEN expression in macrophages, leading to the activation of the PI3K/AKT-mediated STAT3 signaling pathway, thereby promoting bladder cancer progression. A related study also highlighted the significance of the miRNA-21-mediated PTEN/PI3K/AKT pathway in bladder cancer (Yang X. et al., 2015). Marina et al. conducted a study that integrated computational and transcriptomic analyses in 28 bladder cancer cell lines. The study revealed a correlation between the protein phosphatase 2 regulatory subunit Balpha (PPP2R2A) and miR-21 levels. It was demonstrated that PPP2R2A is a direct target of miR-21 and plays a role in regulating the ERK pathway and the growth of bladder cancer cells. The findings suggest that miR-21 promotes tumor growth by inhibiting PPP2R2A expression and activating the ERK pathway (Koutsioumpa et al., 2018). Taken together these findings

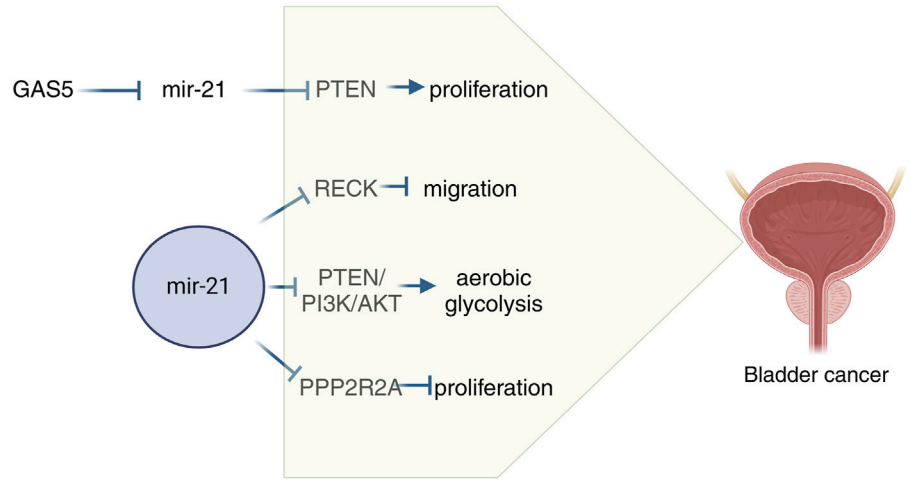


FIGURE 2
Schematic diagram of the mechanism of miR-21 in bladder cancer. GAS5, Growth arrest-specific transcripts 5; PPP2R2A, Protein phosphatase 2 regulatory subunit Balpha.

add to the current understanding of the molecular processes involved in the development of bladder cancer and increase the likelihood of using miR-21 as a therapeutic target for bladder cancer (Figure 2).

Mechanism of action associated with miR-21 in kidney cancer

FDX1, a key regulator of copper metabolism, was initially identified as an electron transfer protein that plays a role in the biosynthesis of steroid hormones, vitamin D, and bile acids in the urinary tissues, kidney, and liver, respectively (Sheftel et al., 2010). Recent research has highlighted the significance of FDX1 in copper-related tumorigenesis and its involvement in the progression of various cancers. A study demonstrated that FDX1 suppressed cell growth and invasion in ccRCC cells, with or without mammary gland formation. Furthermore, a luciferase activity assay indicated a negative correlation between miR-21-5p and FDX1, suggesting that miR-21-5p acts as an upstream regulator of FDX1 in driving ccRCC development (Xie et al., 2022). RhoGAP 24 (ARHGAP24) is a member of the RhoGAP family of proteins known for their oncogenic potential. Yang et al. discovered a close association between ARHGAP24 and hepatocellular carcinoma (Yang et al., 2022). In a study on RCC, Meng et al. observed elevated levels of miR-21-5p in RCC tissues, along with significantly reduced expression of ARHGAP24. They found that miR-21-5p, a known stimulator in RCC, exacerbated the cancer by suppressing the expression of its downstream target gene, ARHGAP24 (Meng et al., 2022). Using real-time fluorescence quantitative polymerase chain reaction, Zhang et al. discovered that miR-21 expression was elevated in human RCC specimens compared to normal renal cell specimens. Upregulation of Pre-miR-21 led to decreased expression of its direct target gene TCF21 and downregulation of KISS1 protein (Zhang et al., 2012). Tumor-associated macrophages (TAM) are classified as M2 and possess various pro-tumorigenic functions (Mohapatra et al., 2021; Munir et al., 2021). TAM are present in all solid tumors, including RCC, and are linked to RCC progression (Pusztai et al., 2019). Exosomes, which are small double-layered membrane vesicles ranging from 30–150 nm in diameter, play a crucial role in intercellular communication by transporting molecules such as mRNAs, miRNAs (miRNAs), long-chain non-coding RNAs, and proteins (Kalluri and LeBleu, 2020). For instance, exosomes derived from M2 macrophages (M2-Exos) were shown to boost the migration and invasion of colorectal cancer cells by transferring miR-21-5p and miR-155-5p (Lan et al., 2019). A study on M2-Exos and miR-21-5p revealed that M2-Exos facilitated the migration, invasion, and EMT of RCC cells, with miR-21-5p in M2-Exos promoting RCC metastasis by inhibiting PTEN/Akt signaling (Zhang et al., 2022). Another study demonstrated that increased miR-21 reduced PTEN protein levels, leading to Akt activation, which then inactivated tuberin to enhance TORC1 activity, resulting in the proliferation, migration, and invasion of renal cancer cells (Dey et al., 2012). Chen et al. discovered that miR-21 directly targets metalloproteinase 3 (TIMP3), which is overexpressed in RCC and acts as a tumor oncogene by promoting cancer cell proliferation and invasion (Chen et al., 2017). Another study revealed that miR-21 may impact the prognosis of hypertensive ccRCC patients. In the ccRCC microenvironment, tissue inhibitor of TIMP3, secreted mainly by tumor endothelial cells (TEC), is downregulated in tumor tissues of hypertensive ccRCC patients. Additionally, overexpression of TIMP3 has been shown to inhibit ccRCC proliferation and metastasis. The study also highlighted that hypertensive ccRCC patients exhibit reduced levels of circulating miR-21-5p, which leads to decreased TIMP3 expression in endothelial cells (EC) via the p38/EGR1 signaling axis (Wang et al., 2023). Programmed cell death 4 (PDCD4) is known to inhibit tumor transformation. Initially identified in a mouse epidermal cell system, the PDCD4 gene encodes a 64 kDa protein that is more abundant in tumor promoter-resistant cells compared to tumor promoter-sensitive cells undergoing transformation (Cmarik et al., 1999). Studies have linked the loss of PDCD4 expression to tumor progression in various cancers such as lung, colon, prostate, and breast (LaRonde-LeBlanc

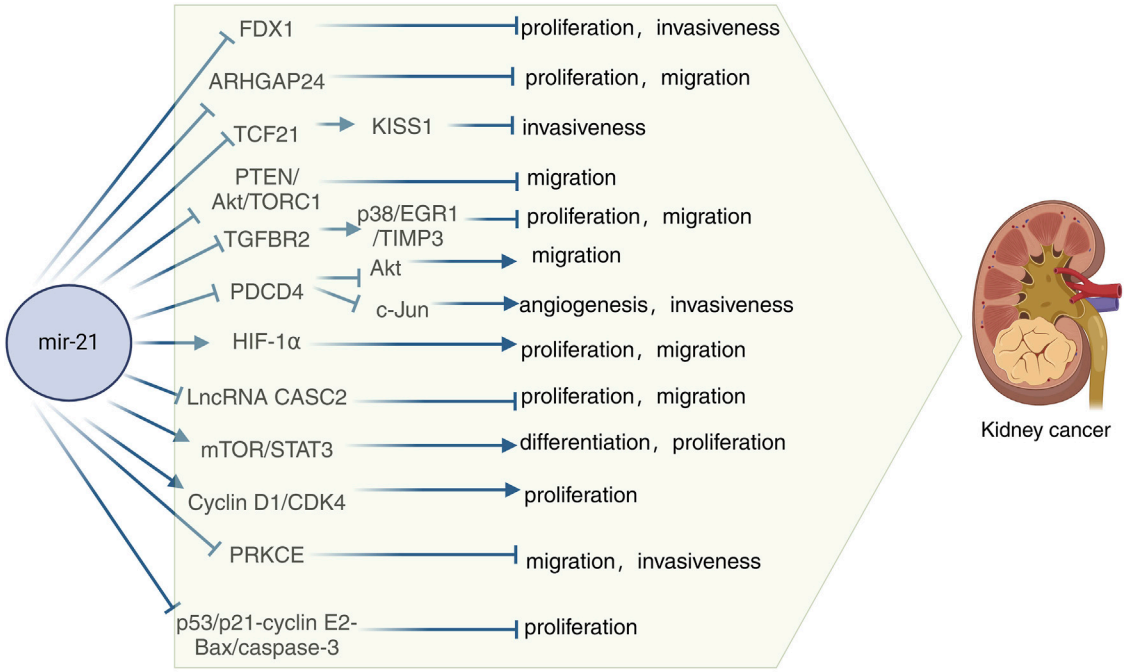


FIGURE 3
Schematic diagram of the mechanism of miR-21 in kidney cancer. TIMP3, Metalloproteinases 3; PTEN, Phosphatase and tensin homolog; PDCD4, Programmed cell death 4; HIF-1α, Hypoxia-inducible factor-1α; lncRNA CASC2, Long-stranded non-coding RNA Cancer susceptibility candidate gene 2; Cyclin D1, Cycle protein D1; PRKCE, Encodes protein kinase C ε.

et al., 2007). Bioinformatics analyses have revealed that PDCD4 acts as a tumor suppressor by regulating processes like cell proliferation, invasion, metastasis, and tumor transformation through its interaction with miR-21 (LaRonde-LeBlanc et al., 2007; Selaru et al., 2009). In RCC, PDCD4 levels were found to be inversely related to miR-21 levels, with a normal renal cell line (HK-2) showing low miR-21 and high PDCD4 protein levels. The tumor suppressor function of PDCD4 is repressed by miR-21 at a post-transcriptional level, leading to increased proliferation, invasion, and metastasis in RCC (Li X. et al., 2014). In a related study, it was demonstrated that miR-21 downregulates PDCD4 at the post-transcriptional level, leading to enhanced cell colony formation and proliferation in a nude mouse model of renal cancer (Yuan et al., 2017). The interplay between PDCD4 and miR-21 in RCC has been further examined in subsequent research. Falguni et al. observed a notable rise in Akt phosphorylation in renal cancer cells, contributing to their proliferation and invasion, and investigated the regulatory role of miR-21 in Akt phosphorylation/activation (Dey et al., 2012). They also elucidated the involvement of PDCD4 in miR-21-mediated Akt phosphorylation, revealing that elevated miR-21 expression in renal cancer cells downregulates PDCD4 levels, resulting in Akt phosphorylation activation and facilitating metastatic adaptation (Bera et al., 2014). Additionally, Fan et al. reported that upregulation of miR-21 and downregulation of PDCD4 led to increased activator protein-1 (AP-1) phosphorylation in renal cancer cells. This alteration activated c-Jun within the AP-1 complex, further promoting the migration, invasion, and angiogenesis of renal cancer cells (Fan et al., 2020) (Figure 3).

Hypoxia-inducible factors-1α and 2-α (HIF-1α and HIF-2α) are transcription factors, with HIF-1α being notably abundant in the majority of ccRCC patients (Stoyanoff et al., 2016). Research has demonstrated that cresyl sulfate (pCS) triggers epithelial-mesenchymal transition (EMT), migration, and proliferation via the HIF-1α pathway. Moreover, pCS has been found to elevate miR-21 levels, promoting cell proliferation and EMT in ccRCC cells. Experimental findings indicate that blocking miR-21 through protein blotting techniques resulted in reduced HIF-1α expression in pCS-treated ccRCC cells (Wu et al., 2019). The long noncoding RNA (lncRNA) cancer susceptibility candidate gene 2 (CASC2), located on chromosome 10q26, was initially identified as downregulated in endometrial cancer, functioning as a tumor suppressor gene (Baldinu et al., 2004). In a study on CASC2 expression and function in RCC, it was discovered that CASC2 is a direct target of miR-21. MiR-21 was shown to decrease CASC2 expression in 786-O and A498 cells. Additionally, the overexpression of miR-21 partially reversed the inhibitory effects of CASC2 on cell proliferation and migration in 786-O and A498 cells (Cao Y. et al., 2016). Several studies have shown that the mTOR-STAT3 signaling pathway plays a role in the proliferation, differentiation, and apoptosis of miR-21 in human RCC cells (Liang et al., 2016). In a xenograft model, silencing miR-21 led to a significant inhibition of tumor growth and a decrease in STAT3 and hTERT expression, indicating that miR-21 regulates STAT3-mediated hTERT expression to modulate glioblastoma cell growth (Wang et al., 2012). This research highlights that miR-21 activates the mTOR-STAT3 signaling pathway to promote the survival and differentiation of human RCC cells while reducing apoptosis (Liang et al., 2016). G1 cell cycle proteins, including cyclin D1, regulate cell progression from G1 to S phase to initiate DNA synthesis. Cyclin D1 activates the cyclin-dependent kinase CDK4 for

TABLE 1 MiR-21 as a diagnostic, prognostic and predictive biomarker for urologic tumors.

Cancer	Expression	Experimental methods	Experimental models	Functions	Targets	Types of biomarkers	Refs.
Prostate cancer	Upward	RT-qPCR,WB Immunohistochemistry protein blot Transwell Testing Luciferase assay	SCID mouse PC3 cells, DU145 cells B16 Mouse melanoma cells, Lncap cells RWPE-1 cells	Apoptosis, proliferation, invasion, metastasis, angiogenesis, epithelial mesenchymal transition	RHOB, KLF5, PTEN	Diagnostic, prognostic markers, predictors of biological recurrence	Liu et al. (2011); Coppola et al. (2013); Leite et al. (2015); Yang et al. (2016); Yang et al. (2017a); Guan et al. (2019); Shukla et al. (2023)
Bladder cancer	Upward	RT-qPCR, WB protein blot flow cytometry immunofluorescence	naked mouse HTB-9 cells T24 cells	Proliferation, apoptosis, migration, invasion	RECK,PI3K/AKT	Prognostic molecular markers, molecular markers of relapse	Zhou et al. (2014); Zhang et al. (2015); Mitash et al. (2017); Lin et al. (2020); Dos Santos et al. (2024)
Kidney cancer	Upward	RT-qPCR,WB IHC, ChIP immunoblotting Immunohistochemistry Transwell Testing, MTT protein blot	Caki-1 cells 786-O cells, A498 cells ACHN cells	Proliferation, apoptosis, differentiation, migration, invasion, angiogenesis, transforming capacity, immune infiltration	PDCD4, mTOR-STAT3, LncRNA-CASC2, PRKCE	Diagnosis, prognostic markers, lymph node metastasis, distant metastasis markers	Faragalla et al. (2012); Li et al. (2014a); Cao et al. (2016b); Liang et al. (2016); Yu et al. (2018); Fan et al. (2020); Wang et al. (2022)

RT-qPCR, Real-time reverse transcription and quantitative PCR; IHC, Immunohistochemistry; WB, Western blot analysis; MTT, Methylthiazolyldiphenyl-tetrazolium bromide; ChIP, Chromatin immunoprecipitation.

cell cycle progression. Studies have shown that miR-21 plays a role in the transcription of cyclin D1, and by inhibiting cyclin D1 mRNA expression, miR-21 Sponge effectively controls renal cancer cell proliferation through the activation of cyclin D1/CDK4 activity. Therefore, this research demonstrates that miR-21 regulates the proliferation of renal cancer cells by modulating the activity of cell cycle protein D1/CDK4 (Bera et al., 2013). Protein kinase C (PKC) is a serine/threonine kinase that regulates various cellular processes, such as proliferation, apoptosis, cell survival, and migration. The gene PRKCE encodes protein kinase C ε (PKC-ε), which plays a role in multiple physiological functions (Gorin and Pan, 2009). In a study by Wang et al., it was observed that reduced expression of PRKCE was linked to a negative prognosis in clear cell renal carcinoma. Through cell invasion assays, they demonstrated that overexpression of PRKCE could inhibit the invasive capabilities of KIRC cells. Furthermore, their analysis suggested that has-miR-21-5p might be a key regulatory miRNA for PRKCE in KIRC, affecting immune infiltration and prognosis by suppressing PRKCE expression (Wang et al., 2022). In a study conducted on RCC A-498 cells, it was discovered that the overexpression of miR-21 resulted in increased cell proliferation, inhibition of apoptosis, and reduced caspase-3 activity. Furthermore, the study revealed that miR-21 overexpression not only promoted cell proliferation and suppressed apoptosis and caspase-3 activity but also downregulated the expression of p53, CDKN1A p21, E2 cell cycle protein, and Bax protein in A-498 cells. Overall, the study demonstrated that the upregulation of miR-21 expression influenced RCC proliferation and apoptosis via the p53/p21-cyclin E2-Bax/caspase-3 signaling pathway (Liu et al., 2017). Taken together these findings add to the current understanding of the molecular processes involved in the development of kidney cancer and raise the possibility of using miR-21 as a therapeutic target for kidney cancer.

Biological pathways, diagnostic and prognostic biomarker role of MiR-21 in urologic cancers

In our investigation of the relationship between miR-21 and urological tumors, we observed that miR-21 influences multiple biological pathways in these tumors. Furthermore, we identified the clinical importance of miR-21 as a valuable diagnostic and prognostic indicator for urological tumors. Our findings regarding these associations are summarized in Table 1.

Potential role of MiR-21 in the treatment of urologic tumors

Using miR-21 as a gene related to urologic cancer treatment

Tumor therapeutic resistance is a prevalent issue in cancer treatment, with the development of chemotherapeutic resistance

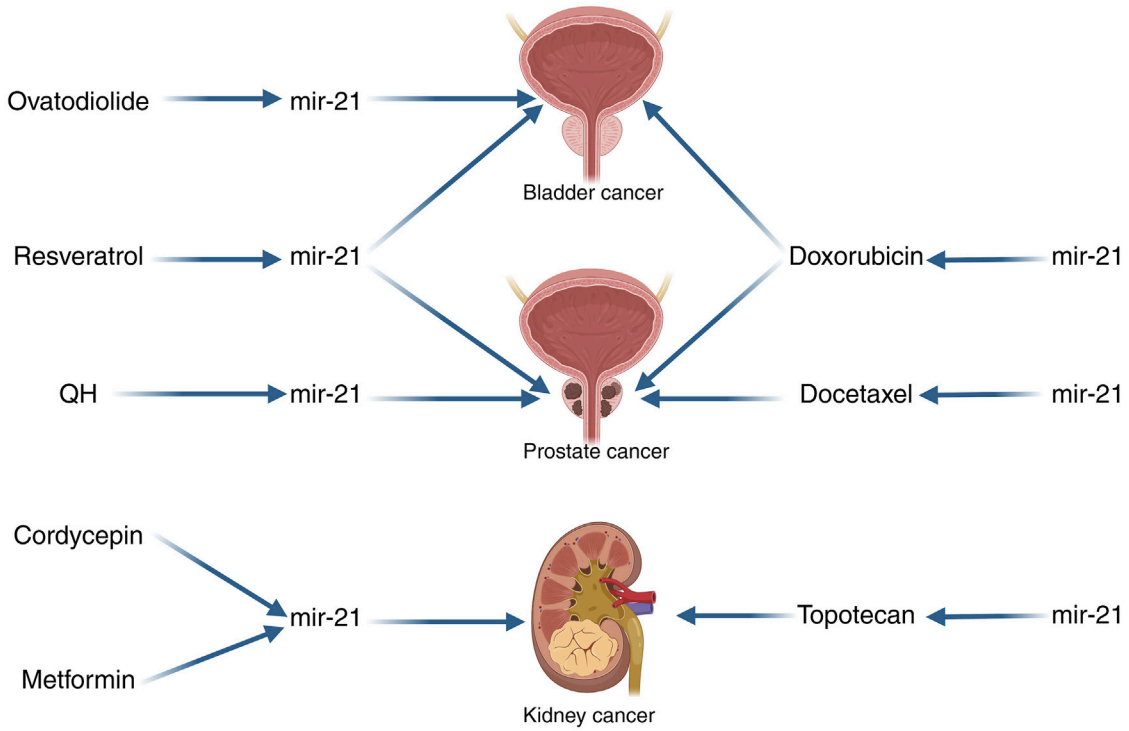


FIGURE 4
miR-21 Schematic diagrams related to the treatment of urologic cancers. QH, The combination of chrysin and quercetin; Note: The arrows in this image only indicate pointing action, not direct targeting. The left side of the image indicates that the drugs can affect the action of miR-21 in the tumor, and the right side of the image indicates that miR-21 can affect the sensitivity of the tumor to the drugs.

posing a significant challenge. Despite advancements in cancer treatment, this remains a major obstacle. Research has indicated that miR-21 can impact the sensitivity of cancer cells to specific drugs. In our integration of the literature, we found that miR-21 was able to influence chemotherapy resistance in urologic tumors. Doxorubicin (DOX) is commonly used as a cytotoxic agent in the treatment of superficial and muscle-invasive bladder cancer, both intravesical and intravenous. This anthracycline antibiotic functions by intercalating into DNA, preventing resealing, halting replication, and ultimately leading to DNA destruction. In a study conducted by Tao et al., it was observed that upregulation of miR-21 resulted in decreased doxorubicin-induced apoptosis in T24 cells, while inhibition of miR-21 enhanced cell death. This suggests that miR-21 overexpression induces resistance to doxorubicin in T24 cell lines, whereas its downregulation sensitizes these cells to the drug (Tao et al., 2011). Furthermore, in a study examining the correlation between doxorubicin and prostate cancer, miR-21 was found to be highly expressed in PC3/DOX cells. Inhibition of miR-21 notably reduced the expression and activity of P-glycoprotein (P-gp) in DOX-resistant cells, thereby eliminating multidrug resistance (MDR) reversal by enhancing intracellular doxorubicin accumulation in PC3/DOX cells (Zhao et al., 2021). Prostate cancer is typically responsive to androgens at the time of initial diagnosis, leading to the administration of anti-androgen therapy for most patients. However, over time, patients may develop androgen-dependent prostate cancer (AIPC), for which docetaxel is the standard treatment (Pazdur et al., 1993). Research has shown that ectopic expression of miR-21 can increase resistance to docetaxel in PC3wt cells, while inhibiting miR-21 expression in PC3R cells can reduce resistance to docetaxel (Shi et al., 2010). Another study has indicated that miR-21 plays a role in the resistance of PC3 cells to docetaxel, suggesting that targeting miR-21 could be a promising therapeutic strategy to enhance the sensitivity of prostate cancer to docetaxel (Zhang et al., 2011). Naro et al. discovered an oxadiazole inhibitor of miR-21 through high-throughput screening. Further studies on structure-activity relationships revealed that the small molecule 37 is a strong inhibitor of miR-21 function. When miR-21 was inhibited in chemotherapy-resistant RCC cell lines using small molecule 37, the expression of tumor suppressor proteins was restored, leading to increased sensitivity to topotecan-induced apoptosis. This resulted in enhanced topotecan activity in cell viability and clone formation assays (Naro et al., 2018) (Figure 4).

Among the numerous treatments for cancer, none are able to completely eliminate cancer cells from a patient's body, with the side effects of current treatment methods being a subject of concern. A more comprehensive comprehension of the molecular mechanisms of cancer has enabled us to target cells at their fundamental source. Given that miRNAs play a crucial role in regulating various key factors in tumorigenesis, the utilization of miRNAs for tumor therapy has garnered significant interest. This review outlines the ongoing research on therapies that impact urological tumors by modulating miR-21 and subsequently influencing them. M2 polarized tumor-associated macrophages (M2 TAM) have been implicated in the progression and drug resistance of bladder cancer (BCa). Wu et al. conducted experiments demonstrating that

M2 TAM can induce malignant properties in BCa cells, partly through the release of oncogenic extracellular vesicles (EVs). Treatment with ovodiolactone (OV) was found to prevent the polarization of M2 TAM, reduce the EV cargo miR-21 derived from M2 TAM, and inhibit the β-catenin/mTOR/CDK6 signaling pathway. Overall, ovodiolactone was shown to inhibit bladder cancer progression by suppressing the mTOR/β-catenin/CDK6 pathway and exosomal miR-21 derived from M2 tumor-associated macrophages (Wu et al., 2020). Resveratrol, a dietary polyphenolic compound found in grapes and red wine, has been shown to inhibit the growth of bladder cancer cells and promote cell death. A recent study utilized real-time fluorescence quantitative PCR to demonstrate that resveratrol downregulated miR-21 expression and modulated the levels of Akt and Bcl-2 proteins, thereby influencing bladder cancer cell apoptosis through the miR-21-mediated Akt/Bcl-2 signaling pathway (Zhou et al., 2014). In another investigation focusing on resveratrol and prostate cancer, researchers observed a decrease in the expression of several prostate cancer-related miRNAs, including miR-21, in PC-3M-MM2 cells, an aggressive form of prostate cancer lacking androgen receptors. This finding suggested that resveratrol could impede the progression and spread of prostate cancer by targeting the Akt/miR-21 pathway. Overall, these studies highlight the potential of resveratrol in slowing down the advancement of prostate cancer through its effects on the Akt/miR-21 pathway (Sheth et al., 2012). The combination of chrysin and quercetin (QH; 1:1) has been shown to inhibit the growth of human leukemia cells (Mertens-Talcott et al., 2003). In a study conducted by Yang et al., QH was found to significantly reduce the invasion and migration of PC3 cells, as well as decrease the expression of various prostate tumor-associated miRNAs, including miR-21, when compared to untreated human prostate cancer cells. These results suggest that QH may act as an anticancer agent against human prostate cancer cells by targeting the miR-21 signaling pathway (Yang F. Q. et al., 2015). Cordycepin, an active ingredient derived from the traditional Chinese herb Cordyceps sinensis, has been found to exhibit antitumor activity across various cancer types. A study revealed that cordycepin downregulated extracellular signal-regulated kinase (ERK) and DUSP5, upregulated phosphorylated JNK (p-JNK), and triggered apoptosis. This research suggests that targeting ERK-JNK signaling with cordycepin-induced apoptosis could serve as a promising therapeutic approach for treating renal cancer (Hwang et al., 2016). Additionally, Zhao et al. demonstrated through quantitative real-time fluorescence quantitative PCR and protein blotting analysis that cordycepin reduced miR-21 expression and Akt phosphorylation levels in a dose-dependent manner, while increasing PTEN phosphatase levels in Caki-1 cells. Their findings indicate that cordycepin induces apoptosis in renal cancer cells by modulating miR-21 and PTEN phosphatase levels (Yang C. et al., 2017). Metformin (MF) is an antidiabetic drug that not only improves glycemic control but also affects various pathways in both normal and cancerous cells. This leads to the inhibition of cell proliferation, cell cycle arrest, and apoptosis (Stumvoll et al., 1995; Li W. et al., 2014; Hadad et al., 2014). Studies have shown that metformin can induce G0/G1 cell cycle arrest and hinder the growth of RCC both in laboratory settings and in living organisms (Liu J. et al., 2013). In a separate investigation on metformin's impact on RCC, it was observed that MF treatment led to a decrease in miR-21 AMPK levels and an increase in PTEN expression in cell lines. The research highlighted that differences in the sensitivity of RCC cells to metformin were linked to the regulation of miR-21/PTEN expression, which subsequently influenced AKT signaling and ultimately affected the growth of RCC (Kalogirou et al., 2016).

Discussion

MiRNAs are stably detected in plasma and serum, making them valuable molecular biomarkers for non-invasive cancer diagnosis and prognosis (Bartel, 2004; Cortez et al., 2011). MiRNAs can be found in biological fluids either within extracellular vesicles or as ribonucleoprotein complexes not associated with vesicles, and can help distinguish between various stages of disease progression (Creemers et al., 2012). Although methods for recognizing, utilizing, and inhibiting miR-21 have advanced in the past decade, there are still many unknown details, such as the factors influencing the formation of typical versus atypical isoomiR forms in specific cancers. Further exploration of these mechanisms and their implications will offer deeper insights into the role of miR-21 in cancer. It is now widely acknowledged that miRNAs operate in both intracellular and extracellular settings. Limited research has investigated the involvement of miR-21 in intercellular communication within the tumor microenvironment, particularly in patient or patient-derived tumor models. Given miR-21's capability to target multiple tumor suppressor and oncogenic pathways, such investigations could uncover additional roles of miR-21 in disease progression. Various miRNA-targeted RNA therapies, including miravirsen, mesoMiR-1, and lademirsen, are currently undergoing clinical trials (Winkle et al., 2021). Different methods of miR-21 inhibition target specific steps in miRNA biogenesis. For instance, small molecule inhibitors like diazobenzene and estradiol have been utilized to target transcription in miRNA biogenesis (Gumireddy et al., 2008; Wickramasinghe et al., 2009; Fu et al., 2021). Additionally, direct targeting of miRNA structures using small molecules that bind to the G-hairpin of the hTERT-G-quadruplex-forming sequence has shown to downregulate expression and exhibit a strong anticancer effect in mice. Despite challenges, RNA therapy holds promise for clinical applications (Song et al., 2019). While advancements have been made in predicting interactions and developing therapeutic strategies to inhibit miR-21, challenges related to cancer heterogeneity and the complex microenvironment network have become more evident. It is now understood that therapy should take into account the intricate miRNA-mRNA, cellular protein regulator, and ncRNA networks to overcome current limitations in cancer treatment. With the growing knowledge of miR-21 and its role in cancer, there is hope for the development of safe and effective RNA-based therapies for clinical use.

Summary and prospect

Recent advances in miRNA research on urologic tumors have revealed significant dysregulation of miRNAs in these cancer types, with implications for key carcinogenic pathways. Interestingly, we

found that miR-21 is playing the role of an oncogene in prostate, bladder and kidney cancers in all the literature we have studied so far. This review focuses on the latest insights into the targeting of signaling pathways by miR-21. While the body of literature on miR-21 in urological tumors is expanding, a comprehensive overview of signal transduction regulation in these tumors is lacking. Therefore, this review consolidates existing findings on the role of miR-21 in regulating signaling pathways in urological tumors. The collective evidence suggests that miR-21 predominantly functions as a tumor suppressor, inhibiting cell proliferation, invasion, metastasis, and tumor growth across various urological cancer types. Many studies have shown that miR-21 is dysregulated in urologic tumors and is believed to target important pathways involved in carcinogenesis. There are various target genes and pathways linked to the function of miR-21, and although therapeutic strategies targeting miR-21 are still in early stages, progress has been made in recent years. This review aims to summarize the current findings on the therapeutic potential of miR-21 in urological cancers. Additionally, the potential interactions of miRNAs with other noncoding RNAs are being explored. Further research is needed to investigate the accuracy and specificity of miR-21 as a diagnostic biomarker, as well as the potential unintended effects of using anti-miR-21 as a therapeutic intervention. A more comprehensive understanding of miR-21, its target genes, and its molecular mechanisms of action will be crucial for successfully translating current research findings into clinical practice.

Author contributions

LG: Conceptualization, Data curation, Methodology, Writing–original draft, Writing–review and editing. LZ: Methodology, Supervision, Writing–review and editing. JZ (3rd author): Formal Analysis, Project administration, Writing–review and editing. PL: Formal Analysis, Project administration, Validation, Writing–review and editing. TC: Data curation, Methodology, Supervision, Writing–review and editing. JZ (6th author): Resources, Visualization, Writing–review and editing. WL: Software, Writing–review and editing. QC: Investigation, Writing–review and editing. LC: Investigation, Writing–review and editing. FZ: Visualization, Writing–review and editing. BQ: Funding acquisition, Resources, Visualization, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Jiangxi Provincial Health Commission Research Project (No. SKJP20203656).

Acknowledgments

All figures are created with BioRender.com.

Conflict of interest

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

Publisher's note

References

Adams, B. K., Cai, J., Armstrong, J., Herold, M., Lu, Y. J., Sun, A., et al. (2005). EF24, a novel synthetic curcumin analog, induces apoptosis in cancer cells via a redox-dependent mechanism. *Anti-cancer drugs* 16, 263–275. doi:10.1097/00001813-200503000-00005

Ambros, V. (2004). The functions of animal microRNAs. *Nature* 431, 350–355. doi:10.1038/nature02871

Angel, C. Z., Stafford, M. Y. C., McNally, C. J., Nesbitt, H., and McKenna, D. J. (2023). MiR-21 is induced by hypoxia and down-regulates RHOB in prostate cancer. *Cancers* 15, 1291. doi:10.3390/cancers15041291

Baldinu, P., Cossu, A., Manca, A., Satta, M. P., Sini, M. C., Rozzo, C., et al. (2004). Identification of a novel candidate gene, CASC2, in a region of common allelic loss at chromosome 10q26 in human endometrial cancer. *Hum. Mutat.* 23, 318–326. doi:10.1002/humu.20015

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–297. doi:10.1016/s0092-8674(04)00045-5

Bera, A., Das, F., Ghosh-Choudhury, N., Kasinath, B. S., Abboud, H. E., and Choudhury, G. G. (2014). microRNA-21-induced dissociation of PDCD4 from rictor contributes to Akt-IKKβ-mTORC1 axis to regulate renal cancer cell invasion. *Exp. Cell Res.* 328, 99–117. doi:10.1016/j.yexcr.2014.06.022

Bera, A., Ghosh-Choudhury, N., Dey, N., Das, F., Kasinath, B. S., Abboud, H. E., et al. (2013). NFκB-mediated cyclin D1 expression by microRNA-21 influences renal cancer cell proliferation. *Cell. Signal.* 25, 2575–2586. doi:10.1016/j.cellsig.2013.08.005

Bommer, G. T., Gerin, I., Feng, Y., Kaczorowski, A. J., Kuick, R., Love, R. E., et al. (2007). p53-mediated activation of miRNA34 candidate tumor-suppressor genes. *Curr. Biol. CB* 17, 1298–1307. doi:10.1016/j.cub.2007.06.068

Bortolin-Cavaillé, M. L., Dance, M., Weber, M., and Cavaillé, J. (2009). C19MC microRNAs are processed from introns of large Pol-II, non-protein-coding transcripts. *Nucleic acids Res.* 37, 3464–3473. doi:10.1093/nar/gkp205

Broughton, J. P., Lovci, M. T., Huang, J. L., Yeo, G. W., and Pasquinelli, A. E. (2016). Pairing beyond the seed supports MicroRNA targeting specificity. *Mol. Cell* 64, 320–333. doi:10.1016/j.molcel.2016.09.004

Bueno, M. J., Pérez de Castro, I., and Malumbres, M. (2008). Control of cell proliferation pathways by microRNAs. *Cell cycle (Georget. Tex.)* 7, 3143–3148. doi:10.4161/cc.7.20.6833

Cai, X., Hagedorn, C. H., and Cullen, B. R. (2004). Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA (New York, N.Y.)* 10, 1957–1966. doi:10.1261/rna.7135204

Calame, K. (2007). MicroRNA-155 function in B cells. *Immunity* 27, 825–827. doi:10.1016/j.immuni.2007.11.010

Calin, G. A., Sevignani, C., Dumitru, C. D., Hyslop, T., Noch, E., Yendamuri, S., et al. (2004). Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc. Natl. Acad. Sci. U. S. A.* 101, 2999–3004. doi:10.1073/pnas.0307323101

Cao, J., Liu, J., Xu, R., Zhu, X., Liu, L., and Zhao, X. (2016a). MicroRNA-21 stimulates epithelial-to-mesenchymal transition and tumorigenesis in clear cell renal cells. *Mol. Med. Rep.* 13, 75–82. doi:10.3892/mmr.2015.4568

Cao, Y., Xu, R., Xu, X., Zhou, Y., Cui, L., and He, X. (2016b). Downregulation of lncRNA CASC2 by microRNA-21 increases the proliferation and migration of renal cell carcinoma cells. *Mol. Med. Rep.* 14, 1019–1025. doi:10.3892/mmr.2016.5337

Chen, D., Guo, Y., Chen, Y., Guo, Q., Chen, J., Li, Y., et al. (2020). LncRNA growth arrest-specific transcript 5 targets miR-21 gene and regulates bladder cancer cell proliferation and apoptosis through PTEN. *Cancer Med.* 9, 2846–2858. doi:10.1002/cam4.2664

Chen, J., Gu, Y., and Shen, W. (2017). MicroRNA-21 functions as an oncogene and promotes cell proliferation and invasion via TIMP3 in renal cancer. *Eur. Rev. Med. Pharmacol. Sci.* 21, 4566–4576.

Cheng, H. Y., Papp, J. W., Varlamova, O., Dziema, H., Russell, B., Curfman, J. P., et al. (2007). microRNA modulation of circadian-clock period and entrainment. *Neuron* 54, 813–829. doi:10.1016/j.neuron.2007.05.017

Cimmino, A., Calin, G. A., Fabbri, M., Iorio, M. V., Ferracin, M., Shimizu, M., et al. (2005). miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc. Natl. Acad. Sci. U. S. A.* 102, 13944–13949. doi:10.1073/pnas.0506654102

Cmarik, J. L., Min, H., Hegamyer, G., Zhan, S., Kulesz-Martin, M., Yoshinaga, H., et al. (1999). Differentially expressed protein Pdcd4 inhibits tumor promoter-induced neoplastic transformation. *Proc. Natl. Acad. Sci. U. S. A.* 96, 14037–14042. doi:10.1073/pnas.96.24.14037

Coppola, V., Musumeci, M., Patrizii, M., Cannistraci, A., Addario, A., Maugeri-Saccà, M., et al. (2013). BTG2 loss and miR-21 upregulation contribute to prostate cell transformation by inducing luminal markers expression and epithelial-mesenchymal transition. *Oncogene* 32, 1843–1853. doi:10.1038/onc.2012.194

Cortez, M. A., Bueso-Ramos, C., Ferdin, J., Lopez-Berestein, G., Sood, A. K., and Calin, G. A. (2011). MicroRNAs in body fluids--the mix of hormones and biomarkers. *Nat. Rev. Clin. Oncol.* 8, 467–477. doi:10.1038/nrclinonc.2011.76

Creemers, E. E., Tijsen, A. J., and Pinto, Y. M. (2012). Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? *Circulation Res.* 110, 483–495. doi:10.1161/circresaha.111.247452

Croce, C. (2012). Introduction to the role of microRNAs in cancer diagnosis, prognosis, and treatment. *Cancer J. (Sudbury, Mass.)* 18, 213–214. doi:10.1097/PPO.0b013e31825efb41

Croce, C. M. (2009). Causes and consequences of microRNA dysregulation in cancer. *Nat. Rev. Genet.* 10, 704–714. doi:10.1038/nrg2634

Dews, M., Homayouni, A., Yu, D., Murphy, D., Sevignani, C., Wentzel, E., et al. (2006). Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat. Genet.* 38, 1060–1065. doi:10.1038/ng1855

Dey, N., Das, F., Ghosh-Choudhury, N., Mandal, C. C., Parekh, D. J., Block, K., et al. (2012). microRNA-21 governs TORC1 activation in renal cancer cell proliferation and invasion. *PloS one* 7, e37366. doi:10.1371/journal.pone.0037366

Dong, B., Shi, Z., Wang, J., Wu, J., Yang, Z., and Fang, K. (2015). IL-6 inhibits the targeted modulation of PDCD4 by miR-21 in prostate cancer. *PloS one* 10, e0134366. doi:10.1371/journal.pone.0134366

Dos Santos, P. R. M., da Silva Gomes, P. R., Romão, P., Maluf, F. C., Guimarães, V. R., Candido, P., et al. (2024). Enhancing RECK expression through miR-21 inhibition: a promising strategy for bladder carcinoma control. *Biochem. Genet.* doi:10.1007/s10528-024-10714-8

Fabian, M. R., Sonenberg, N., and Filipowicz, W. (2010). Regulation of mRNA translation and stability by microRNAs. *Annu. Rev. Biochem.* 79, 351–379. doi:10.1146/annurev-biochem-060308-103103

Fan, B., Jin, Y., Zhang, H., Zhao, R., Sun, M., Sun, M., et al. (2020). MicroRNA-21 contributes to renal cell carcinoma cell invasiveness and angiogenesis via the PDCD4/c-Jun (AP-1) signalling pathway. *Int. J. Oncol.* 56, 178–192. doi:10.3892/ijo.2019.4928

Faragalla, H., Youssef, Y. M., Scorilas, A., Khalil, B., White, N. M. A., Mejia-Guerrero, S., et al. (2012). The clinical utility of miR-21 as a diagnostic and prognostic marker for renal cell carcinoma. *J. Mol. diagnostics JMD* 14, 385–392. doi:10.1016/j.jmoldx.2012.02.003

Feng, Y. H., and Tsao, C. J. (2016). Emerging role of microRNA-21 in cancer. *Biomed. Rep.* 5, 395–402. doi:10.3892/br.2016.747

Fernández-Hernando, C., Ramírez, C. M., Goedeke, L., and Suárez, Y. (2013). MicroRNAs in metabolic disease. *Arteriosclerosis, thrombosis, Vasc. Biol.* 33, 178–185. doi:10.1161/atvbaha.112.300144

Friedman, R. C., Farh, K. K., Burge, C. B., and Bartel, D. P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* 19, 92–105. doi:10.1101/gr.082701.108

Fu, Z., Wang, L., Li, S., Chen, F., Au-Yeung, K. K. W., and Shi, C. (2021). MicroRNA as an important target for anticancer drug development. *Front. Pharmacol.* 12, 736323. doi:10.3389/fphar.2021.736323

Fujita, S., Ito, T., Mizutani, T., Minoguchi, S., Yamamichi, N., Sakurai, K., et al. (2008). miR-21 Gene expression triggered by AP-1 is sustained through a double-negative feedback mechanism. *J. Mol. Biol.* 378, 492–504. doi:10.1016/j.jmb.2008.03.015

Galardi, S., Mercatelli, N., Giorda, E., Massalini, S., Frajese, G. V., Ciafrè, S. A., et al. (2007). miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. *J. Biol. Chem.* 282, 23716–23724. doi:10.1074/jbc.M701805200

Gantier, M. P., Sadler, A. J., and Williams, B. R. (2007). Fine-tuning of the innate immune response by microRNAs. *Immunol. Cell Biol.* 85, 458–462. doi:10.1038/sj.icb.7100091

Gao, Y., Ding, Y., Chen, H., Chen, H., and Zhou, J. (2015). Targeting Krüppel-like factor 5 (KLF5) for cancer therapy. *Curr. Top. Med. Chem.* 15, 699–713. doi:10.2174/1568026615666150302105052

Gillies, J. K., and Lorimer, I. A. (2007). Regulation of p27Kip1 by miRNA 221/222 in glioblastoma. *Cell cycle (Georget. Tex.)* 6, 2005–2009. doi:10.4161/cc.6.16.4526

Gorin, M. A., and Pan, Q. (2009). Protein kinase C epsilon: an oncogene and emerging tumor biomarker. *Mol. cancer* 8, 9. doi:10.1186/1476-4598-8-9

Greco, S. J., and Rameshwar, P. (2007). MicroRNAs regulate synthesis of the neurotransmitter substance P in human mesenchymal stem cell-derived neuronal cells. *Proc. Natl. Acad. Sci. U. S. A.* 104, 15484–15489. doi:10.1073/pnas.0703037104

Griffiths-Jones, S., Grocock, R. J., van Dongen, S., Bateman, A., and Enright, A. J. (2006). miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic acids Res.* 34, D140–D144. doi:10.1093/nar/gkj112

Guan, C., Zhang, L., Wang, S., Long, L., Zhou, H., Qian, S., et al. (2019). Upregulation of MicroRNA-21 promotes tumorigenesis of prostate cancer cells by targeting KLF5. *Cancer Biol. Ther.* 20, 1149–1161. doi:10.1080/15384047.2019.1599659

Guan, Y., Wu, Y., Liu, Y., Ni, J., and Nong, S. (2016). Association of microRNA-21 expression with clinicopathological characteristics and the risk of progression in advanced prostate cancer patients receiving androgen deprivation therapy. *Prostate* 76, 986–993. doi:10.1002/pros.23187

Gumireddy, K., Young, D. D., Xiong, X., Hogenesch, J. B., Huang, Q., and Deiters, A. (2008). Small-molecule inhibitors of microrna miR-21 function. *Angewandte Chemie Int. ed. Engl.* 47, 7482–7484. doi:10.1002/anie.200801555

Hadad, S. M., Hardie, D. G., Appleyard, V., and Thompson, A. M. (2014). Effects of metformin on breast cancer cell proliferation, the AMPK pathway and the cell cycle. *Clin. Transl. Oncol. official Publ. Fed. Span. Oncol. Soc. Natl. Cancer Inst. Mexico* 16, 746–752. doi:10.1007/s12094-013-1144-8

Hammond, S. M., Boettcher, S., Caudy, A. A., Kobayashi, R., and Hannon, G. J. (2001). Argonaute2, a link between genetic and biochemical analyses of RNAi. *Sci. (New York, N.Y.)* 293, 1146–1150. doi:10.1126/science.1064023

Han, J., Lee, Y., Yeom, K. H., Kim, Y. K., Jin, H., and Kim, V. N. (2004). The Drosha-DGCR8 complex in primary microRNA processing. *Genes and Dev.* 18, 3016–3027. doi:10.1101/gad.1262504

Hassel, S., Eichner, A., Yakymovych, M., Hellman, U., Knaus, P., and Souchelnytskyi, S. (2004). Proteins associated with type II bone morphogenetic protein receptor (BMPR-II) and identified by two-dimensional gel electrophoresis and mass spectrometry. *Proteomics* 4, 1346–1358. doi:10.1002/pmic.200300770

He, L., He, X., Lim, L. P., de Stanchina, E., Xuan, Z., Liang, Y., et al. (2007). A microRNA component of the p53 tumour suppressor network. *Nature* 447, 1130–1134. doi:10.1038/nature05939

He, L., Thomson, J. M., Hemann, M. T., Hernando-Monge, E., Mu, D., Goodson, S., et al. (2005). A microRNA polycistron as a potential human oncogene. *Nature* 435, 828–833. doi:10.1038/nature03552

Hilliard, A., Hilliard, B., Zheng, S. J., Sun, H., Miwa, T., Song, W., et al. (2006). Translational regulation of autoimmune inflammation and lymphoma genesis by programmed cell death 4. *J. Immunol. (Baltim. Md. 1950)* 177, 8095–8102. doi:10.4049/jimmunol.177.11.8095

Hu, L., Ye, H., Huang, G., Luo, F., Liu, Y., Liu, Y., et al. (2016). Long noncoding RNA GAS5 suppresses the migration and invasion of hepatocellular carcinoma cells via miR-21. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* 37, 2691–2702. doi:10.1007/s13277-015-4111-x

Hutvágner, G., McLachlan, J., Pasquinelli, A. E., Bálint, E., Tuschl, T., and Zamore, P. D. (2001). A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Sci. (New York, N.Y.)* 293, 834–838. doi:10.1126/science.1062961

Hwang, J. H., Joo, J. C., Kim, D. J., Jo, E., Yoo, H. S., Lee, K. B., et al. (2016). Cordycepin promotes apoptosis by modulating the ERK-JNK signaling pathway via DUSP5 in renal cancer cells. *Am. J. cancer Res.* 6, 1758–1771.

Iorio, M. V., Ferracin, M., Liu, C. G., Veronese, A., Spizzo, R., Sabbioni, S., et al. (2005). MicroRNA gene expression deregulation in human breast cancer. *Cancer Res.* 65, 7065–7070. doi:10.1158/0008-5472.Can-05-1783

Iqbal, M. A., Arora, S., Prakasam, G., Calin, G. A., and Syed, M. A. (2019). MicroRNA in lung cancer: role, mechanisms, pathways and therapeutic relevance. *Mol. aspects Med.* 70, 3–20. doi:10.1016/j.mam.2018.07.003

Jajoo, S., Mukherjea, D., Kaur, T., Sheehan, K. E., Sheth, S., Borse, V., et al. (2013). Essential role of NADPH oxidase-dependent reactive oxygen species generation in regulating microRNA-21 expression and function in prostate cancer. *Antioxidants redox Signal.* 19, 1863–1876. doi:10.1089/ars.2012.4820

Jopling, C. L., Yi, M., Lancaster, A. M., Lemon, S. M., and Sarnow, P. (2005). Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Sci. (New York, N.Y.)* 309, 1577–1581. doi:10.1126/science.1113329

Kalluri, R., and LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Sci. (New York, N.Y.)* 367, eaau6977. doi:10.1126/science.aau6977

Kalogirou, C., Schäfer, D., Krebs, M., Kurz, F., Schneider, A., Riedmiller, H., et al. (2016). Metformin-derived growth inhibition in renal cell carcinoma depends on miR-21-mediated PTEN expression. *Urol. Int.* 96, 106–115. doi:10.1159/000441011

Kanagasabai, T., Li, G., Shen, T. H., Gladoun, N., Castillo-Martin, M., Celada, S. I., et al. (2022). MicroRNA-21 deficiency suppresses prostate cancer progression through downregulation of the IRS1-SREBP-1 signaling pathway. *Cancer Lett.* 525, 46–54. doi:10.1016/j.canlet.2021.09.041

Kim, V. N., Han, J., and Siomi, M. C. (2009). Biogenesis of small RNAs in animals. *Nat. Rev. Mol. Cell Biol.* 10, 126–139. doi:10.1038/nrm2632

Kotzsch, M., Farthmann, J., Meye, A., Fuessel, S., Baretton, G., Tjan-Heijnen, V. C. G., et al. (2005). Prognostic relevance of uPAR-del4/5 and TIMP-3 mRNA expression levels in breast cancer. *Eur. J. cancer (Oxford, Engl. 1990)* 41, 2760–2768. doi:10.1016/j.ejca.2005.09.002

Koutsioumpa, M., Chen, H. W., O'Brien, N., Koinis, F., Mahurkar-Joshi, S., Vorvis, C., et al. (2018). MKAD-21 suppresses the oncogenic activity of the miR-21/PPP2R2A/ERK molecular network in bladder cancer. *Mol. cancer Ther.* 17, 1430–1440. doi:10.1158/1535-7163.Mct-17-1049

Krek, A., Grün, D., Poy, M. N., Wolf, R., Rosenberg, L., Epstein, E. J., et al. (2005). Combinatorial microRNA target predictions. *Nat. Genet.* 37, 495–500. doi:10.1038/ng1536

Kumar, B., Koul, S., Khandrika, L., Meacham, R. B., and Koul, H. K. (2008). Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype. *Cancer Res.* 68, 1777–1785. doi:10.1158/0008-5472.Can-07-5259

Lan, J., Sun, L., Xu, F., Liu, L., Hu, F., Song, D., et al. (2019). M2 macrophage-derived exosomes promote cell migration and invasion in colon cancer. *Cancer Res.* 79, 146–158. doi:10.1158/0008-5472.Can-18-0014

LaRonde-LeBlanc, N., Santhanam, A. N., Baker, A. R., Wlodawer, A., and Colburn, N. H. (2007). Structural basis for inhibition of translation by the tumor suppressor Pdcd4. *Mol. Cell. Biol.* 27, 147–156. doi:10.1128/mcb.00867-06

Lee, R. C., Feinbaum, R. L., and Ambros, V. (1993). The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 75, 843–854. doi:10.1016/0092-8674(93)90529-y

Lee, Y., Kim, M., Han, J., Yeom, K. H., Lee, S., Baek, S. H., et al. (2004). MicroRNA genes are transcribed by RNA polymerase II. *EMBO J.* 23, 4051–4060. doi:10.1038/sj.emboj.7600385

Lehmann, U., Hasemeier, B., Christgen, M., Müller, M., Römermann, D., Länger, F., et al. (2008). Epigenetic inactivation of microRNA gene hsa-mir-9-1 in human breast cancer. *J. pathology* 214, 17–24. doi:10.1002/path.2251

Leite, K. R., Reis, S. T., Viana, N., Morais, D. R., Moura, C. M., Silva, I. A., et al. (2015). Controlling RECK miR21 promotes tumor cell invasion and is related to biochemical recurrence in prostate cancer. *J. Cancer* 6, 292–301. doi:10.7150/jca.11038

le Sage, C., Nagel, R., Egan, D. A., Schrier, M., Mesman, E., Mangiola, A., et al. (2007). Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *EMBO J.* 26, 3699–3708. doi:10.1038/sj.emboj.7601790

Lewis, B. P., Shih, I. H., Jones-Rhoades, M. W., Bartel, D. P., and Burge, C. B. (2003). Prediction of mammalian microRNA targets. *Cell* 115, 787–798. doi:10.1016/s0092-8674(03)01018-3

Li, B. (2008). Bone morphogenetic protein-Smad pathway as drug targets for osteoporosis and cancer therapy. *Endocr. metabolic immune Disord. drug targets* 8, 208–219. doi:10.2174/187153008785700127

Li, C. L., Nie, H., Wang, M., Su, L. P., Li, J. F., Yu, Y. Y., et al. (2012). microRNA-155 is downregulated in gastric cancer cells and involved in cell metastasis. *Oncol. Rep.* 27, 1960–1966. doi:10.3892/or.2012.1719

Li, W., Ma, W., Zhong, H., Liu, W., and Sun, Q. (2014b). Metformin inhibits proliferation of human keratinocytes through a mechanism associated with activation of the MAPK signaling pathway. *Exp. Ther. Med.* 7, 389–392. doi:10.3892/etm.2013.1416

Li, X., Xin, S., He, Z., Che, X., Wang, J., Xiao, X., et al. (2014a). MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor PDCD4 and promotes cell transformation, proliferation, and metastasis in renal cell carcinoma. *Cell. physiology Biochem. Int. J. Exp. Cell. physiology, Biochem. Pharmacol.* 33, 1631–1642. doi:10.1159/000362946

Liang, T., Hu, X. Y., Li, Y. H., Tian, B. Q., Li, Z. W., and Fu, Q. (2016). MicroRNA-21 regulates the proliferation, differentiation, and apoptosis of human renal cell carcinoma cells by the mTOR-STAT3 signaling pathway. *Oncol. Res.* 24, 371–380. doi:10.3727/096504016x14685034103356

Liang, Y., Yin, D., Hou, L., Zheng, T., Wang, J., Meng, X., et al. (2011). Diphenyl difluoroketone: a potent chemotherapy candidate for human hepatocellular carcinoma. *PloS one* 6, e23908. doi:10.1371/journal.pone.0023908

Lin, F., Li, X. Y., Zhu, G. M., He, W. Y., and Gou, X. (2020). Bladder cancer cell-secreted exosomal miR-21 activates the PI3K/AKT pathway in macrophages to promote cancer progression. *Int. J. Oncol.* 56, 151–164. doi:10.3892/ijo.2019.4933

Liu, J., Li, M., Song, B., Jia, C., Zhang, L., Bai, X., et al. (2013b). Metformin inhibits renal cell carcinoma *in vitro* and *in vivo* xenograft. *Urol. Oncol.* 31, 264–270. doi:10.1016/j.urolonc.2011.01.003

Liu, L. Z., Li, C., Chen, Q., Jing, Y., Carpenter, R., Jiang, Y., et al. (2011). MiR-21 induced angiogenesis through AKT and ERK activation and HIF-1α expression. *PloS one* 6, e19139. doi:10.1371/journal.pone.0019139

Liu, Z., Lu, Y., Xiao, Y., and Lu, Y. (2017). Upregulation of miR-21 expression is a valuable predicator of advanced clinicopathological features and poor prognosis in patients with renal cell carcinoma through the p53/p21-cyclin E2-Bax/caspase-3 signaling pathway. *Oncol. Rep.* 37, 1437–1444. doi:10.3892/or.2017.5402

Liu, Z., Wang, W., Jiang, J., Bao, E., Xu, D., Zeng, Y., et al. (2013a). Downregulation of GAS5 promotes bladder cancer cell proliferation, partly by regulating CDK6. *PloS one* 8, e73991. doi:10.1371/journal.pone.0073991

Lujambio, A., and Lowe, S. W. (2012). The microcosmos of cancer. *Nature* 482, 347–355. doi:10.1038/nature10888

Lv, L., Huang, F., Mao, H., Li, M., Li, X., Yang, M., et al. (2013). MicroRNA-21 is overexpressed in renal cell carcinoma. *Int. J. Biol. markers* 28, 201–207. doi:10.5301/jbm.2013.10831

Maatouk, D., and Harfe, B. (2006). MicroRNAs in development. *TheScientificWorldJournal* 6, 1828–1840. doi:10.1100/tsw.2006.313

Meng, B., Wang, P., Zhao, C., Yin, G., Meng, X., Li, L., et al. (2022). miR-21-5p serves as a promoter in renal cell carcinoma progression through ARHGAP24 downregulation. *Environ. Sci. Pollut. Res. Int.* 29, 39985–39993. doi:10.1007/s11356-021-18343-z

Mertens-Talcott, S. U., Talcott, S. T., and Percival, S. S. (2003). Low concentrations of quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells. *J. Nutr.* 133, 2669–2674. doi:10.1093/jn/133.8.2669

Mishra, S., Lin, C. L., Huang, T. H., Bouamar, H., and Sun, L. Z. (2014). MicroRNA-21 inhibits p57Kip2 expression in prostate cancer. *Mol. cancer* 13, 212. doi:10.1186/1476-4598-13-212

Mitash, N., Agnihotri, S., Tiwari, S., Agrawal, V., and Mandhani, A. (2017). MicroRNA-21 could be a molecular marker to predict the recurrence of nonmuscle invasive bladder cancer. *Indian J. urology IJU J. Urological Soc. India* 33, 283–290. doi:10.4103/iju.IJU_4_17

Mohapatra, S., Pioppini, C., Ozpolat, B., and Calin, G. A. (2021). Non-coding RNAs regulation of macrophage polarization in cancer. *Mol. cancer* 20, 24. doi:10.1186/s12943-021-01313-x

Mott, J. L., Kobayashi, S., Bronk, S. F., and Gores, G. J. (2007). mir-29 regulates Mcl-1 protein expression and apoptosis. *Oncogene* 26, 6133–6140. doi:10.1038/sj.onc.1210436

Munir, M. T., Kay, M. K., Kang, M. H., Rahman, M. M., Al-Harrasi, A., Choudhury, M., et al. (2021). Tumor-associated macrophages as multifaceted regulators of breast tumor growth. *Int. J. Mol. Sci.* 22, 6526. doi:10.3390/ijms22126526

Naro, Y., Ankenbruck, N., Thomas, M., Tivon, Y., Connelly, C. M., Gardner, L., et al. (2018). Small molecule inhibition of MicroRNA miR-21 rescues chemosensitivity of renal-cell carcinoma to topotecan. *J. Med. Chem.* 61, 5900–5909. doi:10.1021/acs.jmedchem.7b01891

Pagotto, S., Colorito, M. L., Nicotra, A., Apuzzo, T., Tinari, N., Protasi, F., et al. (2022). A perspective analysis: microRNAs, glucose metabolism, and drug resistance in colon cancer stem cells. *Cancer Gene Ther.* 29, 4–9. doi:10.1038/s41417-021-00298-5

Palma, C. A., Al Sheikha, D., Lim, T. K., Bryant, A., Vu, T. T., Jayaswal, V., et al. (2014). MicroRNA-155 as an inducer of apoptosis and cell differentiation in Acute Myeloid Leukaemia. *Mol. cancer* 13, 79. doi:10.1186/1476-4598-13-79

Pazdur, R., Kudelka, A. P., Kavanagh, J. J., Cohen, P. R., and Raber, M. N. (1993). The taxoids: paclitaxel (Taxol) and docetaxel (Taxotere). *Cancer Treat. Rev.* 19, 351–386. doi:10.1016/0305-7372(93)90010-o

Pickard, M. R., Mourtada-Maarabouni, M., and Williams, G. T. (2013). Long non-coding RNA GAS5 regulates apoptosis in prostate cancer cell lines. *Biochimica biophysica acta* 1832, 1613–1623. doi:10.1016/j.bbadis.2013.05.005

Poy, M. N., Eliasson, L., Krutzfeldt, J., Kuwajima, S., Ma, X., Macdonald, P. E., et al. (2004). A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* 432, 226–230. doi:10.1038/nature03076

Pusztai, C., Yusenko, M. V., Banyai, D., Szanto, A., and Kovacs, G. (2019). M2 macrophage marker chitinase 3-like 2 (CHI3L2) associates with progression of conventional renal cell carcinoma. *Anticancer Res.* 39, 6939–6943. doi:10.21873/anticanres.13915

Qin, W., Zhao, B., Shi, Y., Yao, C., Jin, L., and Jin, Y. (2009). BMPRII is a direct target of miR-21. *Acta biochimica biophysica Sinica* 41, 618–623. doi:10.1093/abbs/gmp049

Reis, S. T., Pontes-Junior, J., Antunes, A. A., Dall'Oglio, M. F., Dip, N., Passerotti, C. C., et al. (2012). miR-21 may acts as an oncomir by targeting RECK, a matrix metalloproteinase regulator, in prostate cancer. *BMC Urol.* 12, 14. doi:10.1186/1471-2490-12-14

Ribas, J., Ni, X., Haffner, M., Wentzel, E. A., Salmasi, A. H., Chowdhury, W. H., et al. (2009). miR-21: an androgen receptor-regulated microRNA that promotes hormone-dependent and hormone-independent prostate cancer growth. *Cancer Res.* 69, 7165–7169. doi:10.1158/0008-5472.Can-09-1448

Rigelsky, C. M., Jennings, C., Lehtonen, R., Minai, O. A., Eng, C., and Aldred, M. A. (2008). BMPR2 mutation in a patient with pulmonary arterial hypertension and suspected hereditary hemorrhagic telangiectasia. *Am. J. Med. Genet. Part A* 146a, 2551–2556. doi:10.1002/ajmg.a.32468

Rodriguez, A., Griffiths-Jones, S., Ashurst, J. L., and Bradley, A. (2004). Identification of mammalian microRNA host genes and transcription units. *Genome Res.* 14, 1902–1910. doi:10.1101/gr.2722704

Rupaimoole, R., and Slack, F. J. (2017). MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat. Rev. Drug Discov.* 16, 203–222. doi:10.1038/nrd.2016.246

Scott, G. K., Goga, A., Bhaumik, D., Berger, C. E., Sullivan, C. S., and Benz, C. C. (2007). Coordinate suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR-125a or miR-125b. *J. Biol. Chem.* 282, 1479–1486. doi:10.1074/jbc.M609383200

Selaru, F. M., Olaru, A. V., Kan, T., David, S., Cheng, Y., Mori, Y., et al. (2009). MicroRNA-21 is overexpressed in human cholangiocarcinoma and regulates programmed cell death 4 and tissue inhibitor of metalloproteinase 3. *Hepatol. (Baltim. Md.)* 49, 1595–1601. doi:10.1002/hep.22838

Sevignani, C., Calin, G. A., Nnadi, S. C., Shimizu, M., Davuluri, R. V., Hyslop, T., et al. (2007). MicroRNA genes are frequently located near mouse cancer susceptibility loci. *Proc. Natl. Acad. Sci. U. S. A.* 104, 8017–8022. doi:10.1073/pnas.0702177104

Sheftel, A. D., Stehling, O., Pierik, A. J., Elsässer, H. P., Mühlenhoff, U., Webert, H., et al. (2010). Humans possess two mitochondrial ferredoxins, Fdx1 and Fdx2, with distinct roles in steroidogenesis, heme, and Fe/S cluster biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* 107, 11775–11780. doi:10.1073/pnas.1004250107

Sheth, S., Jajoo, S., Kaur, T., Mukherjea, D., Sheehan, K., Rybak, L. P., et al. (2012). Resveratrol reduces prostate cancer growth and metastasis by inhibiting the Akt/MicroRNA-21 pathway. *PloS one* 7, e51655. doi:10.1371/journal.pone.0051655

Shi, G. H., Ye, D. w., Yao, X. d., Zhang, S. l., Dai, B., Zhang, H. l., et al. (2010). Involvement of microRNA-21 in mediating chemo-resistance to docetaxel in androgen-independent prostate cancer PC3 cells. *Acta Pharmacol. Sin.* 31, 867–873. doi:10.1038/aps.2010.48

Shukla, K. K., Choudhary, G. R., Sankanagoudar, S., Misra, S., Vishnoi, J. R., Pareek, P., et al. (2023). Deregulation of miR-10b and miR-21 correlate with cancer stem cells expansion through the apoptotic pathway in prostate cancer. *Asian Pac. J. cancer Prev. APJCP* 24, 2105–2119. doi:10.31557/apjcp.2023.24.6.2105

Sica, A., and Bronte, V. (2007). Altered macrophage differentiation and immune dysfunction in tumor development. *J. Clin. investigation* 117, 1155–1166. doi:10.1172/jci31422

Song, J. H., Kang, H. J., Luevano, L. A., Gokhale, V., Wu, K., Pandey, R., et al. (2019). Small-molecule-targeting hairpin loop of hTERT promoter G-quadruplex induces cancer cell death. *Cell Chem. Biol.* 26, 1110–1121. doi:10.1016/j.chembiol.2019.04.009

Stoyanoff, T. R., Rodríguez, J. P., Todaro, J. S., Espada, J. D., Colavita, J. P. M., Brandan, N. C., et al. (2016). Tumor biology of non-metastatic stages of clear cell renal cell carcinoma; overexpression of stearoyl desaturase-1, EPO/EPO-R system and hypoxia-related proteins. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* 37, 13581–13593. doi:10.1007/s13277-016-5279-4

Stumvoll, M., Nurjhan, N., Perriello, G., Dailey, G., and Gerich, J. E. (1995). Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 333, 550–554. doi:10.1056/nejm199508313330903

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA a cancer J. Clin.* 71, 209–249. doi:10.3322/caac.21660

Sylvestre, Y., De Guire, V., Querido, E., Mukhopadhyay, U. K., Bourdeau, V., Major, F., et al. (2007). An E2F/miR-20a autoregulatory feedback loop. *J. Biol. Chem.* 282, 2135–2143. doi:10.1074/jbc.M608939200

Tabara, H., Sarkissian, M., Kelly, W. G., Fleenor, J., Grishok, A., Timmons, L., et al. (1999). The rde-1 gene, RNA interference, and transposon silencing in *C. elegans*. *Cell* 99, 123–132. doi:10.1016/s0092-8674(00)81644-x

Takamizawa, J., Konishi, H., Yanagisawa, K., Tomida, S., Osada, H., Endoh, H., et al. (2004). Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res.* 64, 3753–3756. doi:10.1158/0008-5472.Can-04-0637

Takenaka, K., Ishikawa, S., Kawano, Y., Yanagihara, K., Miyahara, R., Otake, Y., et al. (2004). Expression of a novel matrix metalloproteinase regulator, RECK, and its clinical significance in resected non-small cell lung cancer. *Eur. J. cancer (Oxford, Engl. 1990)* 40, 1617–1623. doi:10.1016/j.ejca.2004.02.028

Tao, J., Lu, Q., Wu, D., Li, P., Xu, B., Qing, W., et al. (2011). microRNA-21 modulates cell proliferation and sensitivity to doxorubicin in bladder cancer cells. *Oncol. Rep.* 25, 1721–1729. doi:10.3892/or.2011.1245

Thomson, J. M., Newman, M., Parker, J. S., Morin-Kensicki, E. M., Wright, T., and Hammond, S. M. (2006). Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes and Dev.* 20, 2202–2207. doi:10.1101/gad.1444406

Volinia, S., Calin, G. A., Liu, C. G., Ambs, S., Cimmino, A., Petrocca, F., et al. (2006). A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc. Natl. Acad. Sci. U. S. A.* 103, 2257–2261. doi:10.1073/pnas.0510565103

Wang, C., Xu, H., Liao, X., Wang, W., Wu, W., Li, W., et al. (2023). Hypertension promotes the proliferation and migration of ccRCC cells by downregulation of TIMP3 in tumor endothelial cells through the miR-21-5p/TGFBR2/P38/EGR1 Axis. *Mol. cancer Res. MCR* 21, 62–75. doi:10.1158/1541-7786.Mcr-22-0089

Wang, D., Yan, S., Wang, L., Li, Y., and Qiao, B. (2021b). circSLC8A1 acts as a tumor suppressor in prostate cancer via sponging miR-21. *BioMed Res. Int.* 2021, 6614591. doi:10.1155/2021/6614591

Wang, J., Jin, J., Liang, Y., Zhang, Y., Wu, N., Fan, M., et al. (2022). miR-21-5p/PRKCE axis implicated in immune infiltration and poor prognosis of kidney renal clear cell carcinoma. *Front. Genet.* 13, 978840. doi:10.3389/fgene.2022.978840

Wang, X., Liu, J., Wang, D., Feng, M., and Wu, X. (2021a). Epigenetically regulated gene expression profiles reveal four molecular subtypes with prognostic and therapeutic implications in colorectal cancer. *Briefings Bioinforma.* 22, bbaa309. doi:10.1093/bib/bbaa309

Wang, Y., and Lee, C. G. (2009). MicroRNA and cancer--focus on apoptosis. *J. Cell. Mol. Med.* 13, 12–23. doi:10.1111/j.1582-4934.2008.00510.x

Wang, Y. Y., Sun, G., Luo, H., Wang, X. F., Lan, F. M., Yue, X., et al. (2012). MiR-21 modulates hTERT through a STAT3-dependent manner on glioblastoma cell growth. *CNS Neurosci. Ther.* 18, 722–728. doi:10.1111/j.1755-5949.2012.00349.x

Wickramasinghe, N. S., Manavalan, T. T., Dougherty, S. M., Riggs, K. A., Li, Y., and Klinge, C. M. (2009). Estradiol downregulates miR-21 expression and increases miR-21 target gene expression in MCF-7 breast cancer cells. *Nucleic acids Res.* 37, 2584–2595. doi:10.1093/nar/gkp117

Wightman, B., Ha, I., and Ruvkun, G. (1993). Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. *Cell* 75, 855–862. doi:10.1016/0092-8674(93)90530-4

Winkle, M., El-Daly, S. M., Fabbri, M., and Calin, G. A. (2021). Noncoding RNA therapeutics - challenges and potential solutions. *Nat. Rev. Drug Discov.* 20, 629–651. doi:10.1038/s41573-021-00219-z

Wu, A. T. H., Srivastava, P., Yadav, V. K., Tzeng, D. T. W., Iamsaard, S., Su, E. C. Y., et al. (2020). Ovatodiolide, isolated from Anisomeles indica, suppresses bladder carcinogenesis through suppression of mTOR/β-catenin/CDK6 and exosomal miR-21 derived from M2 tumor-associated macrophages. *Toxicol. Appl. Pharmacol.* 401, 115109. doi:10.1016/j.taap.2020.115109

Wu, K., Li, L., and Li, S. (2015). Circulating microRNA-21 as a biomarker for the detection of various carcinomas: an updated meta-analysis based on 36 studies. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* 36, 1973–1981. doi:10.1007/s13277-014-2803-2

Wu, T. K., Wei, C. W., Pan, Y. R., Hsu, R. J., Wu, C. Y., and Yu, Y. L. (2019). The uremic toxin p-cresyl sulfate induces proliferation and migration of clear cell renal cell carcinoma via microRNA-21/HIF-1α axis signals. *Sci. Rep.* 9, 3207. doi:10.1038/s41598-019-39646-9

Xie, M., Cheng, B., Yu, S., He, Y., Cao, Y., Zhou, T., et al. (2022). Cuproptosis-related MiR-21-5p/FDX1 Axis in clear cell renal cell carcinoma and its potential impact on tumor microenvironment. *Cells* 12, 173. doi:10.3390/cells12010173

Yang, B., Liu, Z., Ning, H., Zhang, K., Pan, D., Ding, K., et al. (2016). MicroRNA-21 in peripheral blood mononuclear cells as a novel biomarker in the diagnosis and prognosis of prostate cancer. *Cancer biomarkers Sect. A Dis. markers* 17, 223–230. doi:10.3233/cbm-160634

Yang, C., Zhao, L., Yuan, W., and Wen, J. (2017b). Cordycepin induces apoptotic cell death and inhibits cell migration in renal cell carcinoma via regulation of microRNA-21 and PTEN phosphatase. *Biomed. Res. (Tokyo, Jpn.)* 38, 313–320. doi:10.2220/biomedres.38.313

Yang, C. H., Pfeffer, S. R., Sims, M., Yue, J., Wang, Y., Linga, V. G., et al. (2015a). The oncogenic microRNA-21 inhibits the tumor suppressive activity of FBXO11 to promote tumorigenesis. *J. Biol. Chem.* 290, 6037–6046. doi:10.1074/jbc.M114.632125

Yang, C. H., Yue, J., Fan, M., and Pfeffer, L. M. (2010). IFN induces miR-21 through a signal transducer and activator of transcription 3-dependent pathway as a suppressive negative feedback on IFN-induced apoptosis. *Cancer Res.* 70, 8108–8116. doi:10.1158/0008-5472.Can-10-2579

Yang, C. H., Yue, J., Sims, M., and Pfeffer, L. M. (2013). The curcumin analog EF24 targets NF-κB and miRNA-21, and has potent anticancer activity *in vitro* and *in vivo*. *PloS one* 8, e71130. doi:10.1371/journal.pone.0071130

Yang, F. Q., Liu, M., Li, W., Che, J. P., Wang, G. C., and Zheng, J. H. (2015c). Combination of quercetin and hyperoside inhibits prostate cancer cell growth and metastasis via regulation of microRNA-21. *Mol. Med. Rep.* 11, 1085–1092. doi:10.3892/mmr.2014.2813

Yang, W., Wang, B., Yu, Q., Liu, T., Li, T., Tian, T., et al. (2022). ARHGAP24 represses β-catenin transactivation-induced invasiveness in hepatocellular carcinoma mainly by

acting as a GTPase-independent scaffold. *Theranostics* 12, 6189–6206. doi:10.7150/thno.72134

Yang, X., Cheng, Y., Li, P., Tao, J., Deng, X., Zhang, X., et al. (2015b). A lentiviral sponge for miRNA-21 diminishes aerobic glycolysis in bladder cancer T24 cells via the PTEN/PI3K/AKT/mTOR axis. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* 36, 383–391. doi:10.1007/s13277-014-2617-2

Yang, Y., Guo, J. X., and Shao, Z. Q. (2017a). miR-21 targets and inhibits tumor suppressor gene PTEN to promote prostate cancer cell proliferation and invasion: an experimental study. *Asian Pac. J. Trop. Med.* 10, 87–91. doi:10.1016/j.apjtm.2016.09.011

Yu, L., Xiang, L., Feng, J., Li, B., Zhou, Z., Li, J., et al. (2018). miRNA-21 and miRNA-223 expression signature as a predictor for lymph node metastasis, distant metastasis and survival in kidney renal clear cell carcinoma. *J. Cancer* 9, 3651–3659. doi:10.7150/jca.27117

Yuan, H., Xin, S., Huang, Y., Bao, Y., Jiang, H., Zhou, L., et al. (2017). Downregulation of PDCD4 by miR-21 suppresses tumor transformation and proliferation in a nude mouse renal cancer model. *Oncol. Lett.* 14, 3371–3378. doi:10.3892/ol.2017.6605

Zaman, M. S., Shahryari, V., Deng, G., Thamminana, S., Saini, S., Majid, S., et al. (2012). Up-regulation of microRNA-21 correlates with lower kidney cancer survival. *PloS one* 7, e31060. doi:10.1371/journal.pone.0031060

Zhang, B., Pan, X., Cobb, G. P., and Anderson, T. A. (2007). microRNAs as oncogenes and tumor suppressors. *Dev. Biol.* 302, 1–12. doi:10.1016/j.ydbio.2006.08.028

Zhang, H., Guo, Y., Shang, C., Song, Y., and Wu, B. (2012). miR-21 downregulated TCF21 to inhibit KISS1 in renal cancer. *Urology* 80, 1298–1302. doi:10.1016/j.urology.2012.08.013

Zhang, H. H., Qi, F., Cao, Y. H., Zu, X. B., and Chen, M. F. (2015). Expression and clinical significance of microRNA-21, maspin and vascular endothelial growth factor-C in bladder cancer. *Oncol. Lett.* 10, 2610–2616. doi:10.3892/ol.2015.3540

Zhang, H. L., Yang, L. F., Zhu, Y., Yao, X. D., Zhang, S. L., Dai, B., et al. (2011). Serum miRNA-21: elevated levels in patients with metastatic hormone-refractory prostate cancer and potential predictive factor for the efficacy of docetaxel-based chemotherapy. *Prostate* 71, 326–331. doi:10.1002/pros.21246

Zhang, Z., Hu, J., Ishihara, M., Sharrow, A. C., Flora, K., He, Y., et al. (2022). The miRNA-21-5p payload in exosomes from M2 macrophages drives tumor cell aggression via PTEN/Akt signaling in renal cell carcinoma. *Int. J. Mol. Sci.* 23, 3005. doi:10.3390/ijms23063005

Zhao, W., Ning, L., Wang, L., Ouyang, T., Qi, L., Yang, R., et al. (2021). miR-21 inhibition reverses doxorubicin-resistance and inhibits PC3 human prostate cancer cells proliferation. *Andrologia* 53, e14016. doi:10.1111/and.14016

Zhou, B., Wang, J., Zheng, G., and Qiu, Z. (2016). Methylated urolithin A, the modified ellagitannin-derived metabolite, suppresses cell viability of DU145 human prostate cancer cells via targeting miR-21. *Food Chem. Toxicol. Int. J. Publ. Br. Industrial Biol. Res. Assoc.* 97, 375–384. doi:10.1016/j.fct.2016.10.005

Zhou, C., Ding, J., and Wu, Y. (2014). Resveratrol induces apoptosis of bladder cancer cells via miR-21 regulation of the Akt/Bcl-2 signaling pathway. *Mol. Med. Rep.* 9, 1467–1473. doi:10.3892/mmr.2014.1950

Zhu, S., Moore, T. W., Lin, X., Morii, N., Mancini, A., Howard, R. B., et al. (2012). Synthetic curcumin analog EF31 inhibits the growth of head and neck squamous cell carcinoma xenografts. *Integr. Biol. quantitative Biosci. Nano macro* 4, 633–640. doi:10.1039/c2ib20007d

frontiers | Frontiers in Cell and Developmental Biology

TYPE Review
PUBLISHED 23 July 2024
DOI 10.3389/fcell.2024.1430486

OPEN ACCESS

EDITED BY
Sambhavi Animesh,
Harvard Medical School, United States

REVIEWED BY
Volker Blank,
McGill University, Canada
Jinghui Liang,
Wistar Institute, United States

*CORRESPONDENCE
Fang Yang,
fangyang@swmu.edu.cn
Yuancai Xiang,
yuancaix@swmu.edu.cn

†These authors have contributed equally to this work

RECEIVED 10 May 2024
ACCEPTED 08 July 2024
PUBLISHED 23 July 2024

CITATION
Xiong G, Li J, Yao F, Yang F and Xiang Y (2024), New insight into the CNC-bZIP member, NFE2L3, in human diseases.
Front. Cell Dev. Biol. 12:1430486.
doi: 10.3389/fcell.2024.1430486

New insight into the CNC-bZIP member, NFE2L3, in human diseases

Guanghui Xiong[1,2†], Jie Li[3†], Fuli Yao[1], Fang Yang[4,5*] and Yuancai Xiang[1*]

[1]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Southwest Medical University, Luzhou, Sichuan, China, [2]Department of Children Rehabilitation, Maternal and Child Health Hospital of Jintang County, Chendu, Sichuan, China, [3]Department of Anaesthesia, The Affiliated Hospital, Southwest Medical University, Luzhou, Sichuan, China, [4]Department of Physiology, School of Basic Medical Sciences, Southwest Medical University, Luzhou, Sichuan, China, [5]Department of Pathophysiology, College of High Altitude Military Medicine, Third Military Medical University (Army Medical University), Chongqing, China

Nuclear factor erythroid 2 (NF-E2)-related factor 3 (NFE2L3), a member of the CNC-bZIP subfamily and widely found in a variety of tissues, is an endoplasmic reticulum (ER) membrane-anchored transcription factor that can be released from the ER and moved into the nucleus to bind the promoter region to regulate a series of target genes involved in antioxidant, inflammatory responses, and cell cycle regulation in response to extracellular or intracellular stress. Recent research, particularly in the past 5 years, has shed light on NFE2L3's participation in diverse biological processes, including cell differentiation, inflammatory responses, lipid homeostasis, immune responses, and tumor growth. Notably, NFE2L3 has been identified as a key player in the development and prognosis of multiple cancers including colorectal cancer, thyroid cancer, breast cancer, hepatocellular carcinoma, gastric cancer, renal cancer, bladder cancer, esophageal squamous cell carcinoma, T cell lymphoblastic lymphoma, pancreatic cancer, and squamous cell carcinoma. Furthermore, research has linked NFE2L3 to other cancers such as lung adenocarcinoma, malignant pleural mesothelioma, ovarian cancer, glioblastoma multiforme, and laryngeal carcinoma, indicating its potential as a target for innovative cancer treatment approaches. Therefore, to gain a better understanding of the role of NFE2L3 in disease, this review offers insights into the discovery, structure, function, and recent advancements in the study of NFE2L3 to lay the groundwork for the development of NFE2L3-targeted cancer therapies.

KEYWORDS

cancer, homoeostasis, stress, CNC-bZIP, NFE2L3/NRF3, post-transcriptional modification, transcriptional regulation

1 Introduction

Cells respond to changes in their internal and external environments by initiating a series of biological reactions to preserve their stability and normal physiological functions, known as cellular stress. This form of stress can manifest in different ways, including oxidative stress, heat, hypoxia, endoplasmic reticulum (ER) stress, and genotoxic stress. Oxidative stress is a cellular stress reaction caused by an increase in cellular oxidative damage due to various factors, such as environmental changes, drug exposure, and metabolic abnormalities (Sies, 2015). This stress is primarily induced by oxygen free radicals such as superoxide anions, hydroxyl free radicals, and hydrogen peroxide.

Prolonged oxidative stress can lead to the destruction of biological macromolecules, resulting in cellular dysfunction and death. Numerous studies have demonstrated that genes from the cap'n'collar (CNC) -basic region leucine zipper (bZIP) subfamily act as a critical molecular switch for cells to counter intracellular oxidative stress (Bathish et al., 2022; Liu et al., 2023; Hu et al., 2024). These transcription factors are able to bind to the antioxidant or electrophile response element (ARE or EpRE) site in the promoter region of genes that code for anti-oxidative enzymes (Zhang and Xiang, 2016). The CNC-bZIP subfamily comprises six members in vertebrates, including nuclear factor-erythroid 2 (NF-E2) p45, NF-E2-related factor 1 (NFE2L1), NFE2L2, NFE2L3, BTB domain and CNC homolog 1 (BACH1), and BACH2, each of which plays key roles in various cellular functions, including proliferation, apoptosis, inflammatory response, embryonic development, and metabolic regulation (Yang et al., 2020; Cirone and D'Orazi, 2022; Waku and Kobayashi, 2021; Zhou et al., 2016).

Compared with the well-known CNC-bZIP members NFE2L1 and NFE2L2, less attention has been paid to NFE2L3. However, recent research, particularly in the past 5 years, has shed light on NFE2L3's participation in diverse biological processes including cell differentiation, inflammatory responses, oxidative stress, lipid homeostasis, transcription activation, immune response, and tumor growth (Figure 1). Notably, NFE2L3 has been identified as a key player in the development and prognosis of multiple cancer types, including colorectal (Waku and Kobayashi, 2021), liver (Ren Y. et al., 2020), thyroid (Wang et al., 2017), pancreatic (Wang et al., 2018), and renal cancers (Wang et al., 2019; Zhang et al., 2022). Furthermore, studies have linked *NFE2L3* to other cancers such as lung adenocarcinoma (Ren J. et al., 2020), malignant pleural mesothelioma (Wang et al., 2022), and ovarian cancer (Dou et al., 2022), indicating its potential as a target for innovative cancer treatment approaches. Therefore, to gain a better understanding of the role of NFE2L3, this review offers insights into the discovery, structure, function, and recent advancements in the study of NFE2L3 to lay the groundwork for the development of NFE2L3-targeted therapies for cancer.

2 Discovery, distribution, and sequence structure of NFE2L3

2.1 Discovery of NFE2L3

In the 1980s, the discovery of homeobox (Hox) gene clusters, HoxA, HoxB, HoxC, and HoxD, confirmed their critical role in early embryonic development in *Drosophila* (McGinnis and Krumlauf, 1992). These gene clusters encode a series of transcription factors essential for biological processes, such as embryonic development, body axis formation, and cell differentiation (Du and Taylor, 2015). Thereafter, the genes within these clusters have been extensively studied. Notably, cDNA encoding NF-E2 p45 (Chan and Kan, 1993a), NFE2L1 (Chan and Kan, 1993b), and NFE2L2 (Moi et al., 1994) have been found near the HoxC, HoxB and HoxD clusters, respectively. In 1999, Kobayashi et al. (1999) found an expressed sequence tag (EST) clone (668 bp) located near HoxA in The Institute for Genomic Research Human database (GenBank accession number THC181377); the encoded protein was highly homologous to the C-terminal amino acid sequence of NFE2L1. They then obtained a fragment that matched the EST clone in HeLa cell genomic DNA. To obtain the full-length cDNA, the human placental cDNA library was screened using PCR, and four positive overlapping phage clones were isolated. Furthermore, domain structure analysis showed that these newly cloned cDNAs contained CNC and bZIP domain, and had high homology with NF-E2 p45, NFE2L1, and NFE2L2. Therefore, this new member of the CNC subfamily is called nuclear factor-erythroid two related factor 3 (NRF3 or NFE2L3) (Kobayashi et al., 1999). Since then, the structure and function of NFE2L3 have gradually been unveiled with advancements in NFE2L3 research (Figure 1).

2.2 Distribution and cell localization of NFE2L3

Fluorescence *in situ* hybridization experiments have identified the human *NFE2L3* gene's location on chromosome seven p15-p14, with widespread expression across multiple tissues (Kobayashi et al., 1999). Notably, *NFE2L3* displays low expression levels in most normal tissues but exhibits increased expression in specific organs such as the placenta (Kobayashi et al., 1999). Additionally, elevated *NFE2L3* expression has been detected in B cells, monocytes, placental trophoblasts, and tumor cells (Kobayashi et al., 1999; Chenais et al., 2005). Within cells, the full-length glycosylated isoform NFE2L3A is localized in the ER, while the full-length non-glycosylated isoform NFE2L3B is found in the cytosol. In contrast, the truncated isoform NFE2L3C is released from the ER and translocated to the nucleus to exert its transcriptional regulatory functions (Nouhi et al., 2007).

2.3 Domain distribution and their role for NFE2L3

Human NFE2L3 comprises of 694 amino acids, whereas mouse NFE2L3 is composed of 660 amino acids (Kobayashi et al., 1999). As a member of the CNC-bZIP subfamily, the structural domains of NFE2L3 exhibit high similarities to those of NFE2L1 and possess typical domains, including an N-terminal domain (NTD), a transactivation domain (TAD), and a DNA-binding domain (Figures 2A, B). Sequence alignment result revealed that human NFE2L3 consists of seven major domains: NTD (1–146 aa), Pro/Glu/Ser/Thr-rich sequence (PEST, 161–173 aa), TAD (233–431 aa), Nrf2-ECH homology 6-like (Neh6L, 432–535 aa), CNC (536–579 aa), bZIP (580–631 aa), and Neh3L (632–683 aa) (Zhang et al., 2009). These domains play crucial roles in the regulation of NFE2L3 activation, transcription activity, and degradation, with detailed information provided in Table 1.

3 Expression and regulation of NFE2L3

The discovery of NFE2L3 has led researchers to uncover its structure and function, generating interest in its associated

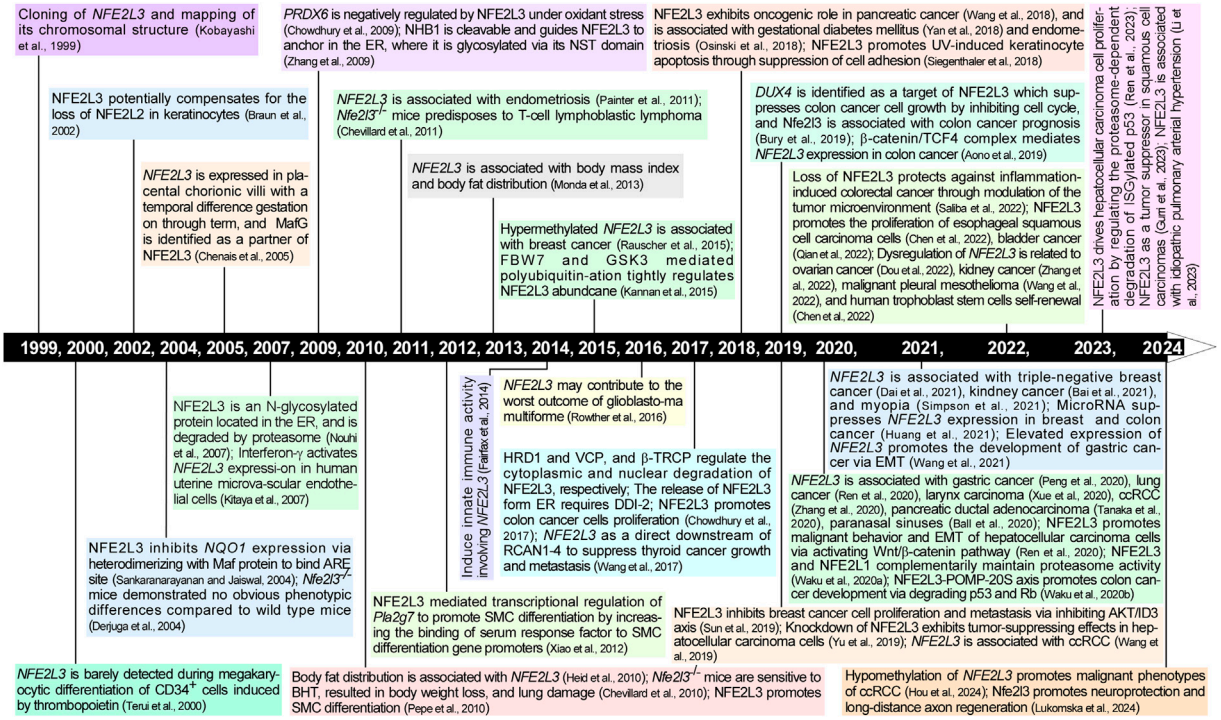


FIGURE 1
Timeline of research progression in NFE2L3 from 1999 to 2024. The timeline represents a major history of scientific research on NFE2L3 in the last two and half decades. Further details are provided in the text.

regulatory mechanisms. Recent studies have suggested that the regulation of NFE2L3 mainly occurs at the transcriptional, post-transcriptional, and post-translational levels.

3.1 Transcriptional and post-transcriptional regulation of *NFE2L3*

A search of the Ensembl database revealed that human *NFE2L3* contains four exons that generating three transcripts (two protein-coding sequences and one retained intron sequence; Figure 2A), with the highest mRNA levels observed in embryos in the GenBank database (Figure 2C). Notably, in a study by Chenais et al. (2005), the mRNA and protein levels of NFE2L3 in choriocarcinoma JAR cells could be significantly increased in the treatment of TNF-α. Further, they found that TNF-α can promote *NFE2L3* transcription in colon adenocarcinoma through activating transcription factor p65 (RELA) which can bind to the first intron of *NFE2L3* (Bury et al., 2019). In addition, a similar work showed that the β-catenin/transcription factor 4 (TCF4) complex can bind to the TCF recognition element (CTTTGAA, Wnt responsive element, WRE site) within the first intron region of *NFE2L3* to promote its transcription (Aono et al., 2019). In addition to these direct regulations of the transcriptional process of *NFE2L3*, miRNAs are also important factors in regulating *NFE2L3* expression at the post-transcriptional level. For example, a luciferase reporter activity assay revealed that miR-1246 (Dai et al., 2021) and miR-23b-3p inhibit the expression of *NFE2L3* (Huang et al., 2021). Additionally, factors such as keratinocyte growth factor (KGF) (Braun et al., 2002), interferon-γ (Kitaya et al., 2007), and calcineurin 1-subtype 4 (RCAN1-4) (Wang et al., 2017) have been shown to influence *NFE2L3* expression, although the underlying mechanisms remain unknown.

3.2 Post-translational modification of NFE2L3

Post-translational modifications (PTMs) increase the functional diversity of the proteome by covalently attaching functional groups or proteins to protein molecules. The modifications included phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, and lipidation. These modifications play a critical role in various aspects of both normal cell biology and pathogenesis (Ramazi and Zahiri, 2021; Peng et al., 2023). However, current studies have only revealed glycosylation, ubiquitination, and phosphorylation as the PTMs of NFE2L3, which was shown as follows.

3.2.1 Glycosylation

Glycosylation is a common post-translational modification of proteins that involves the transfer of sugars to proteins or specific amino acid residues via glycosyltransferases to form glycosidic bonds. In a study by Nouhi et al. (2007), NFE2L3 has three main isoforms (NFE2L3A, NFE2L3B, and NFE2L3C). When treated with deglycosylase, isoform A was disappeared, leading to an increase in the abundance of isoform B, whereas that of isoform C remained unchanged. These findings suggest that the ER located isoform A is glycosylated, isoform B is non-glycosylated, and isoform C may be a processed non-glycosylated protein. This

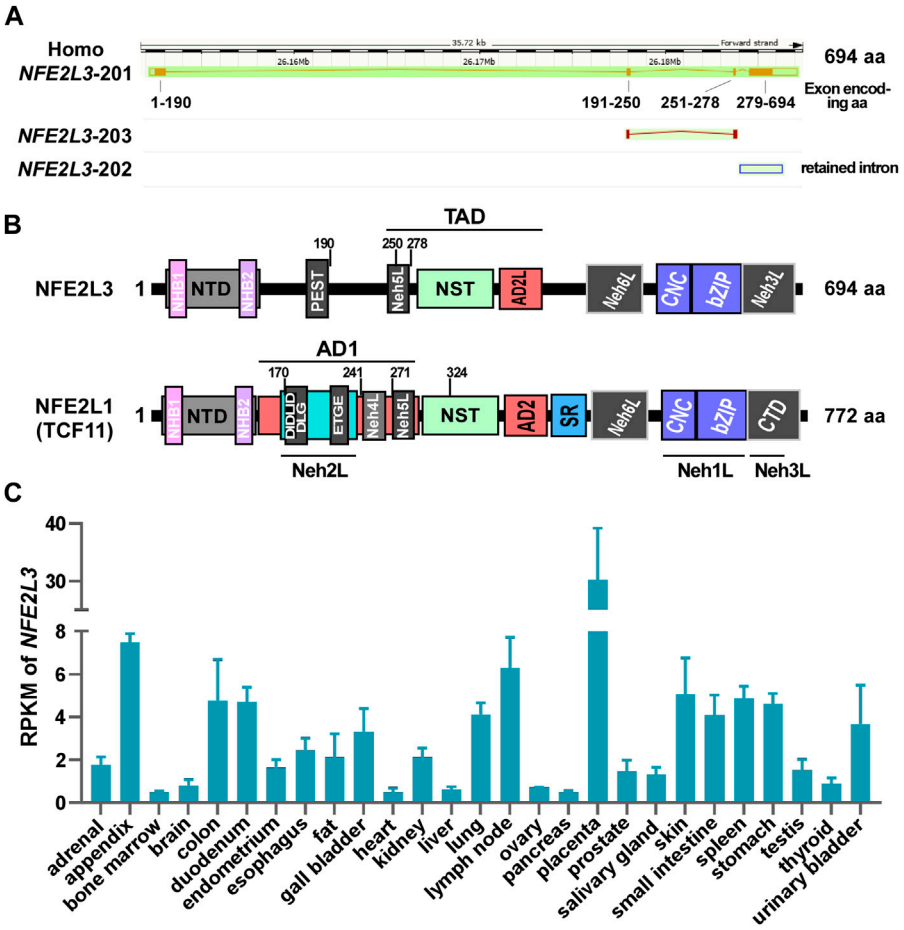


FIGURE 2
The detailed information of NFE2L3 and NFE2L1. **(A)** The genomic and transcriptional information of human *NFE2L3* were obtained from Ensembl genome browser. **(B)** The domain distribution of human NFE2L3 and NFE2L1 (TCF11). The detailed amino acid information of Nfe2L3 domains is provided in Table 1 **(C)** The reads per kilobase per million mapped reads (RPKM) of *NFE2L3* in different human tissues, which was obtained from GenBank database. AD1/2: acidic domain 1 or 2; bZIP: basic-leucine zipper; CNC: cap 'n' collar; CTD: C-terminal domain; Neh L: Neh-like; NHB1/2: N-terminal homology box 1 or 2; NST: Asn/Ser/Thr-rich; NTD: N-terminal domain; SR: serine-repeat.

conclusion is supported by Zhang et al. (2009), who further demonstrated that glycosylation inhibits the transcriptional activity of NFE2L3, and speculated that isoform B may represent a glycosylated cleaved protein or deglycosylated protein. Overall, among NFE2L3 PTMs, glycosylation appears to be a crucial factor to modulate the generation of multiple isoforms with varying activities.

3.2.2 Ubiquitination

Ubiquitination, an essential post-translational modification, plays a dual role in maintaining intracellular protein homeostasis and mediating intracellular signaling cascades. In their study of NFE2L3 function, Nouhi et al. (2007) discovered that NFE2L3 can undergo degradation via the ubiquitin-proteasome pathway. They identified two types of ubiquitination on NFE2L3 (Lys48 and Lys63) and highlighted K77 as a major ubiquitination site for NFE2L3 turnover facilitated by the E3 ligase F-box/WD repeat protein 7 (FBW 7) (Kannan et al., 2015). Chowdhury et al. (2017) revealed multiple ubiquitin-dependent degradation mechanisms for NFE2L3 at different cellular locations. For example, NFE2L3 can be rapidly degraded in the cytoplasm by the ER-associated ubiquitin ligase synoviolin (HRD1) and valosin containing protein (VCP), whereas in the nucleus, β-transducin repeat-containing protein (β-TRCP)-based E3 ubiquitin ligase mediates NFE2L3 degradation. Collectively, NFE2L3 ubiquitination occurs diverse forms within the cell and is targeted for degradation by various molecules, contributing to the regulation of NFE2L3 protein levels and signaling.

3.2.3 Phosphorylation

In addition to glycosylation and ubiquitination modification, Kannan et al. (2015) discovered through an immunoprecipitation experiment that glycogen synthase kinase 3 (GSK3) can interact with NFE2L3. Furthermore, *in vitro* kinase assay revealed that the phosphorylation of NFE2L3 significantly increases when GSK3B and NFE2L3 are combined, compared to the control group. Notably, GSK3-mediated phosphorylation is a prerequisite for FBW7 to degrade NFE2L3 through ubiquitination.

TABLE 1 Protein functional domains of NFE2L3.

Domain	Abbreviation	Residues (aa)		Functional role	References
		Human	Mouse		
N-terminal domain	NTD	1–146	1–126	Negative regulates NFE2L3 activity	Chenais et al. (2005), Zhang et al. (2009)
N-terminal homology box 1	NHB1	13–32	12–31	This domain is part of a tripartite signal peptide sequence transmembrane region	Zhang et al. (2009)
N-terminal homology box 2	NHB2	90–114	76–100	Controlled the proteolytic processing of NFE2L3 into cleavage products	Zhang et al. (2009), Chowdhury et al. (2017)
Pro/Glu/Ser/Thr-rich	PEST	161–173	137–152	Contributes to protein rapid turnover, and negatively regulates NFE2L3 activity	Nouhi et al. (2007), Zhang et al. (2009)
Transactivation domain	TAD	233–431	211–400	Activate transcription	Zhang et al. (2009)
Neh5L	Neh5L	233–279	211–256	Highly conserved domains; a part of the TAD.	Zhang et al. (2009)
Asn/Ser/Thr-rich	NST	281–377	258–350	A potential glycosylation domain; a part of the TAD; this domain possesses potential a transcriptional activation function	Zhang et al. (2009)
Acidic Domain 2 like	AD2L	378–431	351–400	Highly conserved domains; a part of the TAD.	Chenais et al. (2005)
Neh6-like	Neh6L	432–535	401–498	Highly conserved domains; this domain exhibits a transcriptional activation role	Chenais et al. (2005)
Cap 'n' Collar	CNC	536–579	499–542	Highly conserved domain; deletion both CNC and bZIP increases transactivation	Chenais et al. (2005), Zhang et al. (2009)
Basic-leucine zipper	bZIP	580–631	543–604	DNA binding domain; deletion both CNC and bZIP increases transactivation	Chenais et al. (2005), Zhang et al. (2009)
Neh3-like	Neh3L	632–683	605–646	Highly conserved domains	Zhang et al. (2009)
Basic c-tail	BCT	684–694	647–660	Conserved domains between NFE2L3 and NFE2L1	Zhang et al. (2009)

Taken together, NFE2L3 is structurally similar to NFE2L1 and undergoes similar post-translational modification processing (Yang et al., 2020) (Figures 2B, 3): when anchored to the ER via signal peptides related to the N-terminal homology box 1 (NHB1) subdomain, the amino terminal of NFE2L3 orients to the cytoplasmic side while the carboxyl terminal locates in the ER lumen (Zhang et al., 2009); multiple modifications such as glycosylation occur in the ER to produce the precursor of the mature protein; upon specific stimulation, NFE2L3 is released from the ER with the aid of signal peptidase or DNA-damage inducible one homolog 2 (DDI2), then translocates to the nucleus for downstream gene regulation. Excess NFE2L3 is degraded through the ubiquitination-proteasome system in the cytoplasm and nucleus, involving in ubiquitination-related proteins such as FBW7, HRD1/VCP, and β-TRCP (Chowdhury et al., 2017).

4 Biological function of NFE2L3

4.1 Role of NFE2L3 in regulating intracellular biological processes

4.1.1 Regulation of NFE2L3 in oxidative stress

Wild-type mice treated with the antioxidant butylated hydroxytoluene (BTH) exhibited abnormal symptoms such as respiratory distress and weight loss, along with significant downregulation of *Nfe2l3* expression in the lungs. When *Nfe2l3* was knocked out in the entire body, mice became more sensitive to antioxidants, leading to acute lung injury and substantial weight loss (Chevillard et al., 2010). These findings indicate a crucial role for NFE2L3 in maintaining intracellular redox homeostasis. This notion has been further confirmed by several groups, such as silencing *Nfe2l3* in *Nfe2l2*-deficient keratinocytes, which inhibits the induction of antioxidant enzymes (heme oxygenase one and glutathione S-transferase Ya) in response to reactive oxygen species (ROS) inducers (Braun et al., 2002). However, Pepe et al. (2010) showed that overexpression of *Nfe2l3* could enhance intracellular ROS production during smooth muscle cell (SMC) differentiation. Chowdhury et al. (2009) revealed that the overexpression of *NFE2L3* significantly inhibited antioxidant oxidase *PRDX6* expression under oxidative stress. Notably, a dual-luciferase reporter assay showed that NFE2L3 can directly control the expression of NAD(P)H dehydrogenase, quinone 1 (*NQO1*) (Sankaranarayanan and Jaiswal, 2004). Interestingly, a contradictory outcome was observed in mouse *Nfe2l3*-overexpressed setting (Zhang et al., 2009), which may be attributed to differences in the length or species of the *NQO1* ARE site, or to disparities in the specific model cells utilized in their studies. These findings indicate that NFE2L3 plays a crucial role in preserving the intracellular redox balance. Nevertheless, the exact mechanism through which NFE2L3 regulates oxidative stress remains unclear.

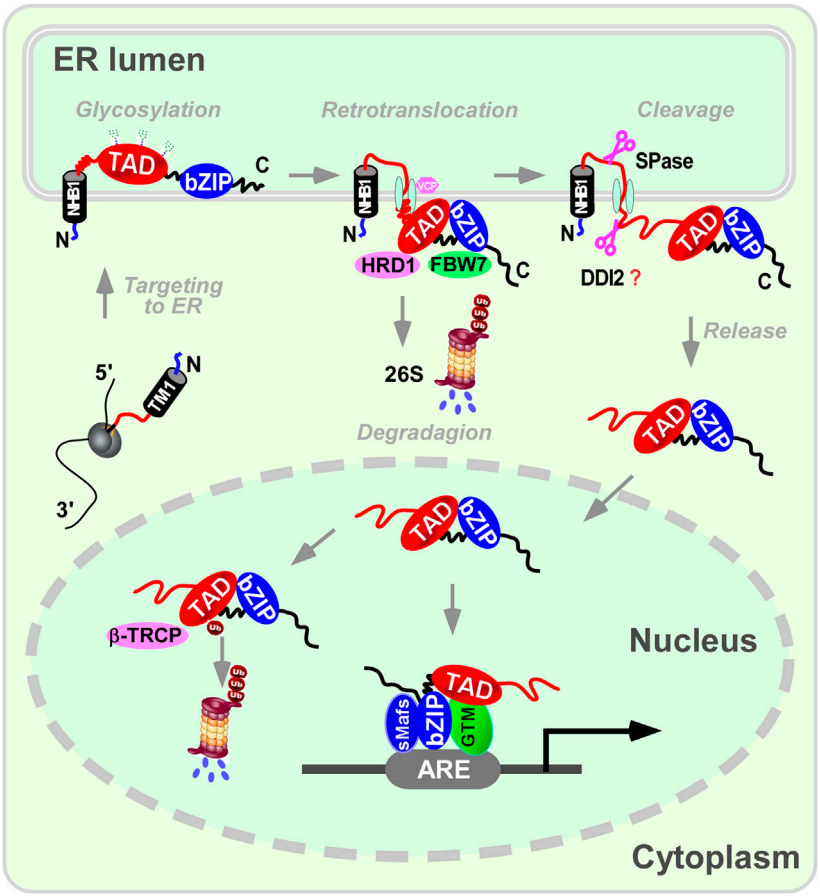


FIGURE 3
A proposed mechanism for NFE2L3 dynamic cleavage processing in endoplasmic reticulum. When anchored to the endoplasmic reticulum (ER) via signal peptides related to the N-terminal homology box 1 (NHB1) subdomain, the amino terminal of NFE2L3 orients to the cytoplasmic side while the carboxyl terminal locates in the ER lumen. Multiple modifications like glycosylation occur in the ER to produce the precursor of the mature protein. Upon specific stimulation, NFE2L3 is released from the ER with the aid of signal peptidase or DNA-damage inducible one homolog 2 (DDI2), then translocates to the nucleus for downstream gene regulation. Excess NFE2L3 is degraded through the ubiquitination-proteasome system in the cytoplasm and nucleus, involving ubiquitination-related proteins such as F-box/WD repeat protein 7 (FBW7), synoviolin/valosin containing protein (HRD1/VCP), and β-transducin repeat-containing protein (β-TRCP). It is important to note that there is no direct evidence showing DDI2 can cleave NFE2L3. ARE: antioxidant response element; bZIP: basic leucine-zipper; GTM: general transcriptional machineries; sMafs: small Maf; TAD: transactivation domain.

4.1.2 Regulation of NFE2L3 in proteostasis

Proteasomes are, large protein complexes found in eukaryotes and archaea that play crucial roles in the degradation of unfolded, damaged, or redundant proteins to maintain cellular homeostasis (Cockram et al., 2021). It is well documented that NFE2L1 has been identified as a key transcription factor that activates the expression of genes related to proteasomes, mitigating the effects of proteasome activity inhibition, known as the proteasome 'bounce-back effect' (Yang et al., 2020). Owing to its similarities to NFE2L1, NFE2L3 is speculated to be involved in the regulation of proteasome activity. In fact, a ChIP-seq experiment indeed found that NFE2L3 can directly bind to the ARE site in the promoter region of proteasome subunit genes (*PSMB3*, *PSMB7*, *PSMC2*, *PSMD3*, *PSMG3*, and *POMP*) (Waku et al., 2020a), and knockdown *NFE2L1* and *NFE2L3* simultaneously resulted in significantly downregulated proteasome activity, although no noticeable changes were observed in cells with individual knockdowns. Of note, knockdown *NFE2L3* showed a significant increase in the protein levels of NFE2L1 in colon cancer cells, implying a complex regulatory relationship between NFE2L3 and proteasome activity (Waku et al., 2020a). Further analysis revealed that when cells demonstrated a low level of NFE2L3, it could directly regulate the expression of proteasome subunit genes through elevating NFE2L1; when intracellular NFE2L3 was at a high level, it could not only directly regulate the expression of proteasome subunits, but also prevent *NFE2L1* translation through regulating its downstream gene cytoplasmic polyadenylation element binding protein 3 (CPEB3) to target the 3' untranslated region of *NFE2L1* mRNA (Waku et al., 2020a). Meanwhile, another study from the same group revealed that NFE2L3 promotes the assembly of the 20S proteasome by directly inducing the expression of *POMP*, leading to accelerated degradation of tumor suppressor genes p53 and Rb (Waku et al., 2020b). These findings suggest that NFE2L3 directly regulates the expression of proteasome subunits and indirectly affects the translation of *NFE2L1*. The coordination between NFE2L3 and NFE2L1 plays an essential role in regulating proteasome function, ultimately contributing to the stable growth of tumor cells.

4.1.3 Role of NFE2L3 in ER stress

Besides NFE2L3 regulates protein degradation via the proteasome, abnormal protein processing also affects the function of NFE2L3. This was evidenced by the time-dependent downregulation of all three NFE2L3 isoforms in tunicamycin (TU) or thapsigargin (TG)-induced ER stress in JAR cells (Nouhi et al., 2007). However, in contrast to these findings, Zhang et al. (2009) demonstrated that only the full glycosylation of NFE2L3 was decreased in response to the ER stressors TU and brefeldin A in *Nfe2l3*-overexpressed COS-1 cells, with the activation of its transcriptional activity, rather than TG. They proposed that these effects were not directly influenced by ER stress, but by glycosylation. Surprisingly, Gurri et al. (2023) recently reported that TU treatment increased the abundance of NFE2L3 in skin cancer SCC13 cells, and the knockdown of *NFE2L3* protected cancer cells from TU-induced apoptosis, possibly because of the stabilization of its partner, heat shock protein family A (Hsp70) member 5 (HSPA5). These results suggest that the role of NFE2L3 in ER stress is extremely complex and that we should carefully consider the different effects of experimental conditions, such as cell lines, treatment times, concentrations, and species.

4.1.4 Regulation of NFE2L3 in lipid metabolism

Lipids, such as fatty acids, triglycerides, cholesterol, and phospholipids, play a significant role in maintaining the normal functioning of cellular processes (Xiang and Miao, 2021). As a transcription factor in the ER, NFE2L3 may play a role in lipid metabolism. This hypothesis is supported by the fact that the homologous protein NFE2L1 is essential for maintaining cholesterol homeostasis (Widenmaier et al., 2017). Actually, some findings from *Nfe2l3* knockout mice have revealed a connection between NFE2L3 and lipid metabolism, for example, there was a significant increase in the expression of the adipocyte differentiation gene peroxisome proliferator activated receptor γ in the white adipose tissue of *Nfe2l3*$^{-/-}$ mice, suggesting that NFE2L3 may be involved in the transcriptional regulation of this gene (Chevillard et al., 2010). Recently, Waku et al. (2021) discovered that NFE2L3 directly regulates genes associated with cholesterol synthesis. Their findings showed that NFE2L3 can either directly enhance the transcription of sterol regulatory element binding transcription factor 2 (SREBP2) or form a transcriptional complex with SREBP2 to boost the expression of mevalonate pathway genes such as 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), and isopentenyl-diphosphate delta isomerase 1 (IDI1). Despite the overexpression of *NFE2L3*, intracellular cholesterol synthesis did not increase and the levels of its precursor lanosterol decreased. Further investigation revealed that NFE2L3 can convert the lanosterol precursor into a substantial amount of geranylgeranyl pyrophosphate to inhibit adipogenesis by upregulating geranylgeranyl diphosphate synthase 1 (GGPS1). Moreover, NFE2L3 directly enhances the transcription of Ras-related protein 5 (RAB5) to facilitate extracellular cholesterol uptake, thus ensuring intracellular cholesterol stability. In summary, these results demonstrated that NFE2L3 is crucial for intracellular lipid metabolism, especially for maintaining cholesterol homeostasis.

4.1.5 Regulation of NFE2L3 in inflammatory response

KGF plays a vital role in the inflammatory process by reducing inflammation, exerting immunosuppressive effects, inhibiting the release of inflammatory mediators, and promoting skin healing. In a study on the role of NFE2L2 in wound healing, Braun et al. (2002) discovered that KGF triggered the expression of *Nfe2l3* in keratinocytes, and this increase was also noted in wounded skin, indicating the potential involvement of NFE2L3 in inflammation regulation. This was corroborated by the fact that colon inflammation was significantly reduced in *Nfe2l3*$^{-/-}$ mice (Saliba et al., 2022). Moreover, the transcription levels of the inflammatory factor interleukin 33 (Il33) were decreased in *Nfe2l3* knockout mice, and the mRNA and protein levels of ras-related protein Rab-27A (RAB27A), an important regulator of mast cells, were increased in mast cells obtained from azoxymethane (AOM) and dextran sodium sulfate (DSS) -induced *Nfe2l3*$^{-/-}$ mice, which resulted from the direct binding of NFE2L3 at the loci of *Il33* and *Rab27a*. Notably, the number of Tregs were increased in this model. Furthermore, analysis of RNA-seq data from the Human Microbiome Project (HMP2) showed that *NFE2L3* transcript levels were higher in the rectum of patients with ulcerative colitis (Saliba et al., 2022). Similarly, Chevillard et al. (2010) found that BTH can promote the expression of prostaglandinendoperoxide synthase 2 (*Ptgs2*), which was blocked by *Nfe2l3* knockout, thereby inducing an inflammatory response caused by lung injury in vitro animal experiments. It is worth mentioning that the inflammatory factor TNF-α can promote RELA to bind to the first intron of *NFE2L3* to activate its transcription, thereby affecting tumor progression (Bury et al., 2019). These results indicate that NFE2L3 participates in the regulation of inflammation. However, the precise regulatory mechanisms may vary temporally and spatially, necessitating further comprehensive investigation.

4.1.6 Role of NFE2L3 in embryonic development and cell differentiation

Studies have found that *NFE2L3* is highly expressed in placental trophoblast cells (Chenais et al., 2005) and chicken mesoderm derivatives with spatio-temporal specificity (Etchevers, 2005). Interestingly, through the analysis of single-cell gene expression profiles from zygote to mid-gestation combined with siRNA library screening, *NFE2L3* was identified as one of the 15 hub genes involved in the self-renewal of human trophoblast stem cells (Chen Y. et al., 2022). These results strongly indicate that *NFE2L3* participates in mbryonic development, however, no significant differences were observed in growth, development, and fertility between *Nfe2l3* knockout mice and wild-type mice (Derjuga et al., 2004) and the expression of *NFE2L3* was also not changed during the process of thrombopoietin-induced megakaryocytic differentiation of CD34^{+} cells (Terui et al., 2000). Nevertheless, two *in vitro* experiments showed that NFE2L3 promotes the differentiation of embryonic stem cells into SMC by directly regulating the expression of the SMC

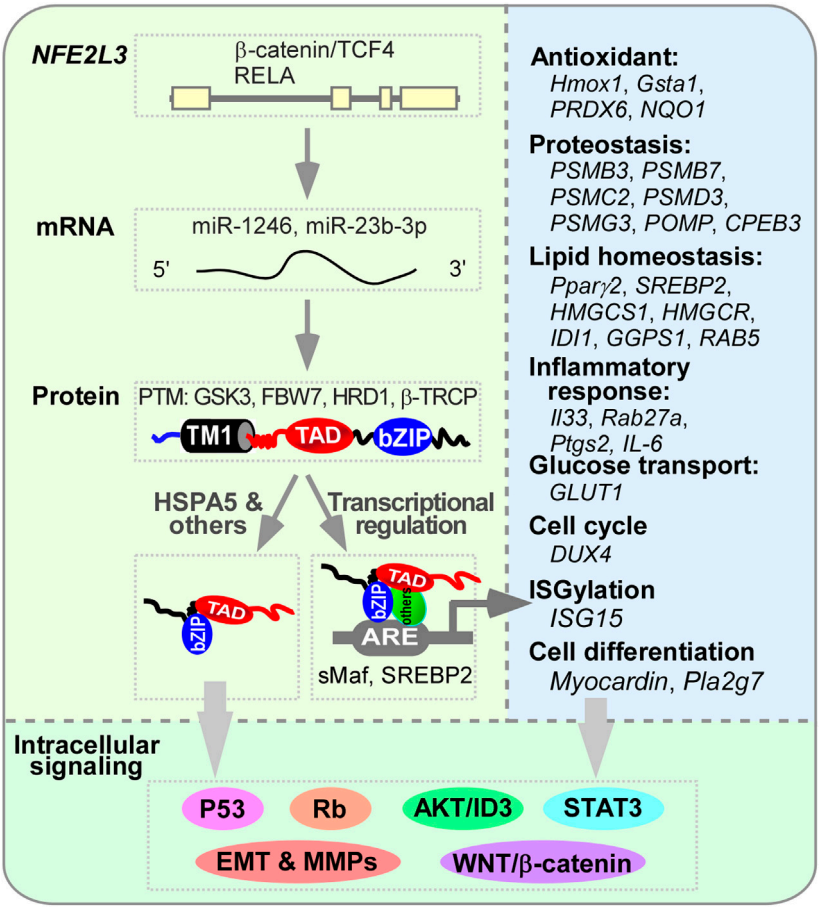


FIGURE 4
The regulation of NFE2L3 and its downstream genes. The transcription of *NFE2L3*, which consists of four exons, is activated by the β-catenin/TCF4 complex or RELA (RELA proto-oncogene, NF-kB subunit) through binding to its first intron. This activation is further regulated by microRNAs such as miR-1246 and miR-23b-3p. Following translation, NFE2L3 located in the endoplasmic reticulum (ER) undergoes post-translational modifications like phosphorylation and ubiquitination by GSK3, FBW7, HRD1/VCP, and β-TRCP before maturing into a protein. Once released from the ER, NFE2L3 can translocate into the nucleus and bind to sMaf or SREBP2 to activate the expression of various genes involved in processes of antioxidant, proteostasis, lipid homeostasis, inflammatory responses, glucose transport, cell cycle regulation, ISGylation, and cell differentiation. Through direct regulation of these gene expressions or binding to HSPA5 or other unidentified factors, NFE2L3 can modulate multiple intracellular signaling pathways such as p53, Rb, STAT3, EMT, MMPs, AKT/ID3, and Wnt/β-catenin, thereby influencing cellular activities.

transcription factors *Myocardin* (Pepe et al., 2010) and phospholipase A2 group VII (Pla2g7) (Xiao et al., 2012). It is remarkable that to investigate the potential functional redundancy between NFE2L3 and other CNC subfamily members in growth and development, Derjuga et al. (2004) generated *Nfe2l3*$^{-/-}$/*Nfe2l2*$^{-/-}$ and *Nfe2l3*$^{-/-}$/*p45*$^{-/-}$ knockout mice, and found both of which exhibited normal growth. This phenomenon implies that the deficiency in NFE2L3 function may be functionally compensated for by its homologous protein or other unknown proteins.

Besides its involvement in the processes of oxidative stress, proteostasis, ER stress, lipid metabolism, inflammatory response, and cell differentiation (Figure 4), NFE2L3 was also found to participate in the regulation of cell adhesion (Siegenthaler et al., 2018), neuroprotection and long-distance axon regeneration (Lukomska et al., 2024), and other undetermined processes, such as stress granule assembly, extracellular exosomes, cellular iron ion homeostasis, and autolysosomes (Liu et al., 2019).

4.2 Role of NFE2L3 in human disease

Given its importance in regulating intracellular redox balance, protein and lipid homeostasis, and cell differentiation, abnormal expression of NFE2L3 is strongly associated with both normal bodily functions and the development of various tumors (Table 2), which was elucidated as follows.

4.2.1 Role of NFE2L3 in tumor progression

4.2.1.1 Thyroid cancer

When analyzing The Cancer Genome Atlas (TCGA) database, Wang et al. (2017) discovered a significant increase in the expression level of *NFE2L3* in human thyroid cancer tissues compared to that in normal tissues. This finding was further validated by qPCR experiments in another study (Khanal et al., 2023). Subsequent gene chip screening and experimental analysis indicated that the downregulation of RCAN1-4 could enhance the growth and metastasis of thyroid cancer cells by

TABLE 2 The role of NFE2L3 in human cancers.

Tumor type	Object of study	Intervention	Biological function	Clinical relevance	References
Thyroid cancer	Cell lines (FTC236, and HTh74); The Cancer Genome Atlas (TCGA) dataset	siRNA, Overexpression	Knockdown of *NFE2L3* decreases the spheroid formation, growth, and invasiveness of thyroid cancer cells	*NFE2L3* is elevated in thyroid cancer samples and in distant metastasis samples	Wang et al. (2017)
	Papillary thyroid tumor samples	—	—	*NFE2L3* expression is higher in papillary thyroid cancer samples	Khanal et al. (2023)
Colorectal cancer	Cell lines (DLD-1, and HCT116)	siRNA	Silencing *NFE2L3* triggers cell cycle arrest (G0/G1), and declines cell proliferation	—	Chowdhury et al. (2017)
	Gene Expression Omnibus (GEO) datasets (GSE32323, GSE74602, and GSE113513) and TCGA	—	—	*NFE2L3* is identified as one of nine prognostic gene for diagnosis and prognosis prediction of colorectal cancer patients	Chen et al. (2019)
	GEO (GSE31279, GSE35602, and GSE46824), TCGA, SurvExpress (colon metabase)	—	—	*NFE2L3* is identified as a colon tumor stroma-specific transcriptional gene	Uddin et al. (2019)
	Cell lines (HCT116 and HT29); Female athymic nu/nu mice; TCGA, BioGPS and Oncomine datasets; Clinical samples	shRNA	*NFE2L3* transcripts were upregulated in colon adenocarcinoma; Silencing *NFE2L3* inhibits colon cancer cell proliferation and tumor growth in mouse xenograft model	Upregulation of *NFE2L3* correlates with poor prognosis for colon cancer	Bury et al. (2019)
	Cell line (HCT116) *Apc* $^{flox/flox}$ and *Apc*$^{\Delta716}$ mice	siRNA	Silencing *NFE2L3* significantly reduced cell proliferation; *Apc* gene deletion induces *Nfe2l3* expression in mouse intestine and organoids	*NFE2L3* expression is induced in colon and rectal carcinoma	Aono et al. (2019)
	Cell lines (HCT116 and SW480) UCSC Cancer Browser and TCGA; Clinical samples	siRNA	*NFE2L3* knockdown arrests cell cycle at the G0/G1 phase through downregulation of CCND1 and pRb1-ser$^{807/811}$	*NFE2L3* is markedly upregulated in colorectal cancer	Zhang et al. (2019)
	Cell lines (HCT116 and H1299); BALB/cA-nu female mouse	siRNA, Overexpression	*NFE2L3* knockdown significantly inhibited the growth of cancer cells; *NFE2L3* overexpression increased 20S proteasome activity via increase *POMP*; *NFE2L3* overexpression decreases Rb and p53 protein through ubiquitin-dependent degradation; Overexpression of *NFE2L3* induced tumor growth and hepatic metastasis	Higher *NFE2L3* expression is correlated with poor prognoses in colorectal or rectal adenocarcinoma patients	Waku et al. (2020b)
	Cell lines (NCM460, SW620, SW1116, CW-2, and LoVo) TCGA	Overexpression	miR-23b-3p inhibited the proliferation, migration and invasion of colon adenocarcinoma cells by directly downregulating *NFE2L3*	*NFE2L3* expression is elevated in colon adenocarcinoma at different stages	Huang et al. (2021)
	Cell line (HCT116); *Nfe2l3*$^{-/-}$ mice; HMP2 datasets	AOM (7 mg/kg)/DSS (2.5%) treatment	*Nfe2l3*$^{-/-}$ mice exhibit significantly less inflammation in the colon, reduced tumor size and numbers; NFE2L3 deficiency disrupts mast cell homeostasis via downregulation of *Il33* and *Rab27a*	*NFE2L3* transcript levels are higher in the rectum of ulcerative colitis patients, compared to these in normal groups	Saliba et al. (2022)

(Continued on following page)

TABLE 2 (*Continued*) The role of NFE2L3 in human cancers.

Tumor type	Object of study	Intervention	Biological function	Clinical relevance	References
Breast cancer	TCGA; Clinical samples	—	—	Hypermethylated *NFE2L3* is associated with invasive breast cancer; *NFE2L3* displays hypermethylation for estrogen receptor positive tumors and hypomethylation for estrogen receptor negative tumors	Rauscher et al. (2015)
	Cell lines (MCF-7, SKBR3, and MDA-MB-231)	siRNA, Overexpression	NFE2L3 inhibited breast cancer cell proliferation and migration by inhibiting AKT/ID3 axis; Silencing NFE2L3 increased the percentage of cell number in S and G2/M phase, and decreased these in G0/G1 phase	*NFE2L3* is positively related to the survival of breast cancer patients	Sun et al. (2019)
	Cell lines (MDA-MB-231 and SKBR3)	siRNA	Inhibition of miR-1246 increases *NFE2L3* expression, which may contribute to increase the sensitivity of cells to docetaxel and decrease the ability of cell migration	—	Dai et al. (2021)
	Cell lines (BT-549, MDA-MB-231, and HCC-70); Sequence Read Archive database	siRNA	Knockdown of *NFE2L3* inhibits colony formation of triple-negative breast cancer (TNBC) models, and enhances the sensitivity of paclitaxel	*NFE2L3* is upregulated in **TNBC** cancer but not enriched in any TNBC subsets	Elango et al. (2021)
Hepatocellular carcinoma	Cell lines (SMCC-7721 and BEL7404); TCGA	shRNA	Knockdown of *NFE2L3* inhibits cell proliferation, induces cell apoptosis, and suppresses the migration, invasion, and EMT of hepatocellular carcinoma cell	*NFE2L3* positively correlated with tumor grade, T stage, and pathologic stage	Yu et al. (2019)
	Cell line (HepG2); GEO (GSE25097, and GSE76427) and TCGA	shRNA	Knockdown of *NFE2L3* inhibits cell proliferation and migration, arrests cell cycle at G0/G1 phase and induces cell apoptosis, thereby inhibiting the malignant growth of subcutaneous carcinoma xenograft. Deficiency of *NFE2L3* decrease the process of EMT.	*NFE2L3* expression is upregulated and associated with hepatocellular carcinoma	Ren et al. (2020a)
	Cell lines (HepG2, MHCC97H); Nude mice; TCGA; Tissue microarrays and clinical samples	siRNA, shRNA	Overexpression of *NFE2L3* promotes hepatocellular carcinoma cell proliferation; NFE2L3 enhances p53 degradation	*NFE2L3* is associated with overall survival in hepatocellular carcinoma	Ren et al. (2023)
Gastric cancer	TCGA	—	—	The methylation of *NFE2L3* is associated with gastric cancer	Peng et al. (2020)
	Cell lines (SGC-7901 and MGC803); GEO (GSE103236) and TCGA	shRNA	Inhibiting *NFE2L3* expression blocks the cell cycle at G0/G1 phage, induces cell apoptosis, and decreases biomarkers in EMT.	*NFE2L3* is upregulated in gastric cancer patients, with a shorter survival time	Wang et al. (2021)
	TCGA, UCSC Xena, Human Protein Atlas; Clinical samples	siRNA, overexpression	Knockdown of *NFE2L3* inhibits the proliferation and migration of cancer cells; *vice versa*	*NFE2L3* is elevated in cancer, and high levels of *NFE2L3* are associated with poor overall survival, progress-free interval, and disease-specific survival	Li and Wen (2024)
Renal cancer	GEO (GSE70303, GSE113501, GSE6344 and GSE53757) and TCGA	—	—	The methylation of *NFE2L3* is decreased in tumor tissue; DNA methylation-driven *NFE2L3* may be a prognostic marker in human clear cell renal cell carcinoma	Wang et al. (2019)

(Continued on following page)

TABLE 2 (*Continued*) The role of NFE2L3 in human cancers.

Tumor type	Object of study	Intervention	Biological function	Clinical relevance	References
	GEO (GSE29609, GSE40435, GSE53757, and GSE70303), and TCGA	—	—	DNA methylation-driven *NFE2L3* may be a prognostic marker in human clear cell renal cell carcinoma	Zhang et al. (2020)
	Cell lines (LoMet-ccRCC, RPTEC, and Caki-1); TCGA	5-Aza-CdR (0–10 μM), shRNA, Overexpression	*NFE2L3* overexpression increases cancer cell proliferation, migration, and invasion	The methylation levels of *NFE2L3* are decreased in tumor tissues, with an increase in *NFE2L3* mRNA levels, which is negatively correlated to survival time of patients	Hou et al. (2024)
Pancreatic cancer	Cell lines (PANC-1 and SW 1990); GEO (GSE16515, GSE15471, GSE55643, and GSE28735); TCGA; Clinical smaples	siRNA	Silencing *NFE2L3* inhibits cell invasion ability, whereas cell proliferation demonstrates no obvious changes	High levels of *NFE2L3* are associated with the poor prognosis of pancreatic cancer patients	Wang et al. (2018)
	GEO (GSE15471), TCGA, and OncoLnc; Clinical samples	—	—	*NFE2L3* is an independent prognostic factors for short patient survival times in pancreatic ductal adenocarcinoma	Tanaka et al. (2020)
Bladder cancer	Cell lines (RT4, BIU-87, J82, EJ, UM-UC-3, 5637, T24, and SW780); GEO (GSE40355, GSE12507, GSE37815, GSE32548, and GSE19915), and UCSC Xena	shRNA, Overexpression	Knockdown of *NFE2L3* inhibits cells proliferation, arrests cell cycle, and induces cell apoptosis; Overexpression of *NFE2L3* promotes cell migration and invasion, *in vitro* and *in vivo*, and EMT.	*NFE2L3* is increased in bladder cancer samples, which is associated with poor clinical outcomes	Qian et al. (2022)
Squamous cell carcinomas	Cell lines (KYSE-150 and ECA-109R); GEO (GSE53625) and TCGA	shRNA, Overexpression	Cells proliferation and migration is promoted by *NFE2L3* overexpression, and is inhibited by silencing *NFE2L3*; NFE2L3 increased radioresistance in esophageal squamous cell carcinoma cells	A significant upregulation of *NFE2L3* expression was documented in esophageal cancer	Chen et al. (2022b)
	Cell lines (SCC13 and HaCaT); *Nfe2l3*$^{-/-}$ mice and NOD-SCID mice; Clinical samples	7,12-dimethylbenz(a)anthracene (100 μg in 300 μL acetone), 12-O-tetradecanoylphorbol-13-acetate (15 lg in 200 ll acetone), siRNA, CRISPR/Cas9-ko, Lentivirus (Overexpression)	Knockdown of *Nfe2l3* promotes growth and malignant conversion of chemically induced skin tumors; Loss of NFE2L3 promotes clonogenicity and migration of the cancer cells, enhances tumorigenesis and invasiveness	NFE2L3 is downregulated at the protein level in invasively growing skin cancer cells	Gurri et al. (2023)
T-cell lymphoblastic lymphoma	*Nfe2l3*$^{-/-}$ mice	benzo-[a]pyrene (B[a]P) (100 mg/kg)	*Nfe2l3*$^{-/-}$ mice exhibits highly susceptible to B[a]P, with a high incidence of T-cell lymphoblastic lymphoma, and demonstrates significantly increased mortality	—	Chevillard et al. (2011)
Lung cancer	GEO (GSE72094), and TCGA	—	—	Methylation driven *NFE2L3* is correlated with lung adenocarcinoma prognosis	Ren et al. (2020b)
Larynx carcinoma	Cell line (TU686)	—	APOM overexpression increases the mRNA level of *NFE2L3*, with no changes in protein level	—	Xue et al. (2020)

upregulating NFE2L3 expression (Wang et al., 2017). In general, although both *in vivo* and *in vitro* experimental and clinical evidence suggest that inhibiting NFE2L3 expression may impede the progression of thyroid cancer, the precise mechanism by which NFE2L3 exerts its pro-tumor effects remains unclear.

4.2.1.2 Colorectal cancer

Recent studies have shown that *NFE2L3* is increased in colorectal cancer and has a positive correlation between *NFE2L3* expression in tumor grade and stage (Bury et al., 2019; Chen et al., 2019; Uddin et al., 2019; Liu et al., 2022). Both *in vitro* and *in vivo* experiments have demonstrated that knocking down *NFE2L3* leads

to a reduction in the proliferation of colon cancer cells, thereby inhibiting tumor growth (Chowdhury et al., 2017; Bury et al., 2019). These findings are further supported by studies showing that modulation *NFE2L3* with miR23b-3p can reproduce similar results (Huang et al., 2021). Notably, one of the key mechanisms by which NFE2L3 exerts its tumor-promoting effects is the regulation of cell cycle progression. Bury et al. (2019) demonstrated that NFE2L3 promotes the proliferation of colon cancer cells by transcriptionally inhibiting the expression of DUX4, a molecule that inhibits cyclin CDK1. Moreover, following *NFE2L3* knockdown, the cell cycle regulatory factors UHMK1, CCND1, and pRb1-Ser$^{807/811}$ are downregulated in colon cancer cells, leading to cell cycle arrest at the G0/G1 phase (Chowdhury et al., 2017; Zhang et al., 2019). In addition to regulating cell cycle-related proteins, NFE2L3 can also affect cell activity by enhancing the degradation of tumor suppressor genes such as p53 and Rb by increasing the function of the 20S proteasome (Waku et al., 2020b). In addition, Saliba et al. (2022) found a significant reduction in both the size and number of colon tumors in *Nfe2l3*$^{-/-}$ mice, along with weakened colon inflammation, compared to wild-type mice in an inflammation-induced colon cancer model (AOM/DSS). Subsequent RNA-seq analysis revealed that only mast cells showing significant changes in tumor tissues, that is, activated mast cells were predominant in wild-type mouse tumors, whereas resting mast cells were more prevalent in *Nfe2l3*$^{-/-}$ mice. Moreover, digital spatial profiling and immunohistochemistry demonstrated that *Nfe2l3*$^{-/-}$ mice promote the infiltration of tumor-suppressive Tregs, ultimately leading to an immunosuppressive tumor microenvironment (Saliba et al., 2022). Despite demonstrating the impact of *Nfe2l3*$^{-/-}$ on mast cell function through *Il33* and *Rab27a*, this study utilized systemic gene knockout mice, leaving out the specific cellular and regulatory mechanisms underlying the creation of an immunosuppressive tumor microenvironment by NFE2L3 deletion.

Additionally, Aono et al. (2019) discovered that the β-catenin/TCF4 complex directly regulates *NFE2L3* expression, and both *β-catenin/TCF4* and *Nfe2l3* are significantly activated in a spontaneous colon cancer model resulting from *APC* gene mutation. Of note, one reason for the carcinogenesis of this mutation was the global cellular metabolic reprogramming mediated by the abnormal expression of protooncogenes such as β-catenin. These findings, together with the fact that abnormal expression of NFE2L3 can also lead to metabolic reprogramming by affecting the expression of glucose transporter *GLUT1* in colorectal cancer cells (Aono et al., 2019) and *Nfe2l3* was identified as one of the five most variable genes in the AOM/DSS model (Suzuki et al., 2007), suggest that NFE2L3 may play a crucial role in the early stages of colon cancer development, although distinct phenotypes were not observed in *Nfe2l3* knockout mice. In conclusion, NFE2L3 could serve as a valuable biomarker or therapeutic target in the context of colorectal cancer.

4.2.1.3 Breast cancer

Breast cancer is the second most common cause of cancer-related death in women (Sung et al., 2021). Sun et al. (2019) revealed that the expression of *NFE2L3* in breast cancer tissues was significantly reduced and was negatively correlated with lymph node metastasis and tumor stage. Furthermore, silencing *NFE2L3* increased MCF-7 cell cycle progression and enhanced cell proliferation, whereas overexpression of *NFE2L3* effectively restricted the growth and metastasis of cancer cells and inhibited the epithelial-mesenchymal transformation (EMT) and MMPs expression. Additionally, activated NFE2L3 can prevent the malignant progression of breast cancer by inhibiting the AKT/ID3 axis (Sun et al., 2019). This mechanism was further confirmed by Dai et al. (2021), who found that miR-1246 inhibits the activation of AKT/ID3 axis by targeting *NFE2L3* mRNA, thus promoting drug resistance and metastasis of breast cancer. However, Elango et al. (2021) showed that the knockdown of NFE2L3 significantly inhibits colony formation in triple-negative breast cancer cells. These contradictory results may be related to the discrepancy in the methylation level of *NFE2L3* in different types of breast cancer cells (Rauscher et al., 2015), which requires further verification.

4.2.1.4 Hepatocellular carcinoma

In contrast to breast cancer, Liu et al. (2022) analyzed an RNA-sequencing database and found that the expression of *NFE2L3* in liver cancer was positively correlated with tumor grade and stage, and analysis of clinical patient tissue samples also revealed a significant association between high *NFE2L3* levels and poor prognosis in patients with liver cancer. *In vitro* experiments have demonstrated that suppression of *NFE2L3* inhibits cell proliferation, migration, invasion, and EMT, ultimately leading to apoptosis in liver cancer cells (Yu et al., 2019; Ren Y. et al., 2020). Ren Y. et al. (2020) also noted a positive correlation between *NFE2L3* expression and the aggressive behavior of liver cancer cells and EMT. This oncogenic function of NFE2L3 is likely attributed to its downregulation of cyclin D1 and TCF4 by inhibiting the Wnt/β-catenin signaling pathway. Recently, another study by this group uncovered a new role for NFE2L3 in promoting liver cancer (Ren et al., 2023). NFE2L3 upregulates the expression of interferon-stimulated gene 15 (ISG15), leading to the ISG modification of the p53. The modified p53 protein is rapidly degraded by NFE2L3-regulated proteasomes, ultimately enhancing the malignant capabilities of liver cancer cells.

In summary, these results indicate that NFE2L3 plays an important role in regulating the occurrence, development, and prognosis of hepatocellular carcinoma. Therefore, targeting NFE2L3 may be an important strategy for the treatment of hepatocellular carcinoma.

4.2.1.5 Gastric cancer

Gastric cancer is a prevalent gastrointestinal malignancy with an incidence of 5.6% and a mortality rate of 7.7%, ranking fifth and fourth among all cancer types, respectively (Sung et al., 2021). Owing to the limitations of the current TNM staging system in gastric cancer, Peng et al. (2020) identified new biomarkers for the diagnosis, prognosis, and prediction of gastric cancer. They analyzed the DNA methylation characteristics of gastric cancer using public databases and identified 10 candidate genes, including *NFE2L3*, that were associated with gastric cancer recurrence. Subsequent research by Wang et al. (2021) confirmed these findings by analyzing clinical samples and common tumor cell lines. They observed

that *NFE2L3* expression in gastric cancer tissues was significantly higher than that in the adjacent tissues. Moreover, the knockdown of *NFE2L3* led to inhibited cellular biological behaviors such as proliferation, migration, and invasion of gastric cancer cells, along with cell cycle arrest and increased apoptosis (Wang et al., 2021; Li and Wen, 2024). Further studies revealed that E-cadherin expression was upregulated, whereas vimentin and N-cadherin expression were downregulated, suggesting a possible connection between NFE2L3 and EMT in gastric cancer. However, the underlying regulatory mechanisms remain unclear.

4.2.1.6 Renal cancer

Current research on NFE2L3 in renal cancer has primarily focused on analyzing sequencing data (Wang et al., 2019; Zhang et al., 2020; Bai et al., 2021; Zhang et al., 2022). Findings showed that *NFE2L3* expression was higher in clear cell carcinoma of the kidney (KIRC) than in normal tissue. This trend has also been observed in renal papillary cell carcinoma (Zhang et al., 2022), suggesting a potential role for *NFE2L3* in renal cancer progression. Additionally, these data suggest that NFE2L3 may affect the tumor immune microenvironment by influencing antigen processing and presentation, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, lymphocyte-mediated immune regulation, and adaptive immune response, thereby contributing to the development of renal clear cell carcinoma (Wang et al., 2019; Zhang et al., 2022). Furthermore, Bai et al. (2021) identified NFE2L3 as one of the six genes significantly associated with survival differences in KIRC in a hypoxia-immune-related prognostic risk model. Importantly, through the analysis of multiple databases, *NFE2L3* was found to be strongly correlated with the prognosis of DNA methylation-driven KIRC (Wang et al., 2019; Zhang et al., 2020), indicating its potential as a prognostic biomarker for renal cancer. Notably, the tumor-promoting effect of NFE2L3 in renal cancer was recently confirmed *in vitro* experiments (Hou et al., 2024) and our ongoing work. However, the molecular mechanisms underlying the action of NFE2L3 in the pathogenesis and progression of renal cancer remain largely unknown.

4.2.1.7 Bladder cancer

Qian et al. (2022) demonstrated a significant increase in *NFE2L3* expression in bladder cancer (BLCA) samples compared to normal tissues and found a strong correlation between high levels of *NFE2L3* and advanced clinicopathological features, as well as poor prognosis. Importantly, intervention in *NFE2L3* expression suppressed the progression of BLCA, potentially through the regulation of NFE2L3 in the cell cycle, apoptosis, and EMT (Qian et al., 2022), indicating that NFE2L3 is involved in the advancement of BLCA. These findings imply that NFE2L3 could serve as a crucial biomarker and potential therapeutic target for predicting clinical outcomes in BLCA. However, *Nfe2l3* knockout mice-based BLCA models must be used to validate these results.

4.2.1.8 Pancreatic cancer

By analyzing various datasets, cell lines, and clinical samples, Wang et al. (2018) discovered that *NFE2L3* levels in pancreatic cancer tissues were elevated compared to those in normal tissues at both the mRNA and protein levels. Additionally, clinical data indicated a strong correlation between high *Nfe2l3* expression and lymph node metastasis, advanced TNM stage, and poor prognosis, which may be attributed to alterations in VEGFA. In addition, NFE2L3 also was identified as an independent prognostic factor for the survival of patients with pancreatic ductal adenocarcinoma (Tanaka et al., 2020).

4.2.1.9 Squamous cell carcinoma

It is reported that *Nfe2l3* participates in the normal healing of skin cells and potentially compensates for the loss of NFE2L2 (Braun et al., 2002), highlighting the importance of NFE2L3 in the regulation of skin function. However, no healing abnormalities were observed in *Nfe2l3*$^{-/-}$ mice with full-thickness excisional wounds compared with wild-type mice (Siegenthaler et al., 2018). Surprisingly, the deficiency of *Nfe2l3* protects keratinocytes from UVB-, oxidative-, and hyperosmotic stress-induced apoptosis by activating cell adhesion signals. Recently, Gurri et al. (2023) demonstrated that the NFE2L3 protein level was decreased in human non-melanoma skin cancer. Moreover, knockout of *NFE2L3* promotes the growth and malignant conversion of squamous cell carcinomas induced by 7,12-dimethylbenzo(a) anthracene and 12-O-tetradecanoylphorbol-13-acetate. This conclusion was further confirmed in multiple models, such as ear tumorigenicity assay, UVB irradiation, and organotypic skin cultures. Taken together, these results strongly imply that NFE2L3 plays a protective role against skin cells, especially during tumor growth. Notably, a contrasting role of NFE2L3 was observed in multiple esophageal squamous cell carcinoma cell lines, and *in vitro* and *in vivo* experiments showed that the knockdown *NFE2L3* enhanced the radiosensitivity of esophageal squamous cell cancer through the transcriptional regulation of IL-6-mediated STAT3 signaling (Chen T. et al., 2022). These findings suggest that the role of NFE2L3 in squamous cell carcinoma may be tissue specific, and requires further investigation.

4.2.1.10 T cell lymphoblastic lymphoma

In a study investigating NFE2L3 function, Chevillard et al. (2011) exposed *Nfe2l3*$^{-/-}$ mice to the carcinogen B[a]P (benzo[a] pyrene), and observed that only one out of 16 wild-type mice died before the 30th week, whereas six out of 19 (32%) *Nfe2l3*$^{-/-}$ mice died starting at week 15 after B[a]P treatment. This indicated that *Nfe2l3*$^{-/-}$ mice exhibited increased sensitivity to carcinogen exposure. Furthermore, following B[a]P treatment, 6% of the wild-type mice developed lymphoma, in contrast to 32% of *Nfe2l3*$^{-/-}$ mice. These findings suggest a potential protective role for NFE2L3 in the development of hematopoietic malignancies, warranting further identification of the underlying mechanisms.

In addition to the aforementioned studies, which have been verified through *in vitro* experiments, there are reports based on RNA sequencing data analysis indicating a potential correlation between abnormal *NFE2L3* expression and the prognosis of various cancers, such as lung adenocarcinoma (Ren J. et al., 2020), malignant pleural mesothelioma (Wang et al., 2022), ovarian cancer (Dou et al., 2022), glioblastoma multiforme (Rowther et al., 2016), and laryngeal carcinoma (Xue et al., 2020). Nevertheless, it is essential to validate these findings by modulating the expression of NFE2L3.

4.2.2 Role of NFE2L3 in other diseases

Recent studies utilizing GWAS, microarray, transcriptome sequencing, and other technologies have identified a strong association between *NFE2L3* and other diseases, such as endometriosis (Painter et al., 2011; Osinski et al., 2018; Cardoso et al., 2020), gestational diabetes mellitus (Yan et al., 2018), chronic sinusitis (Ball et al., 2020), idiopathic pulmonary hypertension (Li et al., 2023), myopia (Simpson et al., 2021), diabetic foot ulcers (Jin et al., 2024), and obesity-related body fat distribution (Heid et al., 2010; Monda et al., 2013). However, further animal studies are required to elucidate the role of NFE2L3 in the development of these diseases.

5 Conclusion and future perspective

The structure and biological function of NFE2L3 suggest that it plays a role in various cellular processes, such as oxidative stress, the inflammatory response, lipid homeostasis, proteostasis, and cell differentiation, as a member of the CNC-bZIP subfamily. Dysregulation of NFE2L3 is closely linked to the development of various diseases, particularly tumors. Thus, targeted modulation of NFE2L3 is crucial for preserving normal cellular function. Current research on NFE2L3 is in its early stages, with many aspects yet to be explored. One key question is why NFE2L3 exhibits different functions in various tissues and organs, particularly during tumor progression. For instance, although highly expressed NFE2L3 promotes cancer in most tumors, it inhibits tumor progression in T cell lymphoblastic lymphoma, skin squamous cell carcinoma, and some breast cancers. Moreover, given the similarity between NFE2L3 and NFE2L1, whether NFE2L3 is a receptor of ER stress like NFE2L1 remains to be clarified. Notably, NFE2L3 not only regulates proteasome-mediated protein degradation, but also participates in the regulation of signals related to glucose metabolism (Aono et al., 2019) and cholesterol synthesis (Waku et al., 2021). These findings suggest that NFE2L3, similar to NFE2L1, is a crucial protein involved in intracellular glucose, lipid, and protein metabolism. However, the mechanism by which these two proteins collaborate to maintain the balance between these three major nutrients within cells remains unknown. Furthermore, bioinformatics analysis revealed a close connection between abnormal NFE2L3 function and changes in the tumor microenvironment. However, the impact of abnormal NFE2L3 expression in tumor cells on the tumor immune microenvironment and the specific role of NFE2L3 in immune cells remain unclear, although it was identified as a crucial gene involving in the stimulation of innate immune activity (Fairfax et al., 2014). It is noteworthy that previous research on NFE2L3 in tumor-related studies has shown a significant correlation with poor prognosis in various diseases, and modulating NFE2L3 expression can potentially impede tumor progression, highlighting NFE2L3 as a promising target for tumor treatment. Therefore, the identification of drugs targeting NFE2L3 is crucial for advancing tumor therapy. Importantly, present studies have made the targeting of transcription factors in cancer a reality (Bushweller, 2019), and clinical trials have shown that transcription factors are prospective therapeutic targets and reliable biomarkers for cancer diagnosis and prognosis (Silva et al., 2024), offering promising avenues for the development of novel transcription factor-based strategies in cancer treatment. Moreover, the development of chemicals targeting or regulating the activity of NFE2L1 and NFE2L2, two closely-related transcription factors of NFE2L3, shows significant potential to overcome chemotherapy drug resistance (Yuan et al., 2018; Jia et al., 2022) and inhibit tumor cell growth (Robledinos-Anton et al., 2019). Therefore, given the pivotal role of NFE2L3 in cancer, we firmly believe that advancing NFE2L3-targeted therapies will greatly improve cancer treatment.

Author contributions

GX: Writing–original draft. JL: Funding acquisition, Writing–review and editing. FuY: Supervision, Validation, Writing–review and editing. FaY: Conceptualization, Supervision, Validation, Writing–original draft, Writing–review and editing. YX: Conceptualization, Funding acquisition, Software, Supervision, Validation, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported in part by The Project of Southwest Medical University (2023QN002) and Sichuan Science and Technology Program (2022YFS0632-B2).

Acknowledgments

We are very thankful to the reviewers for providing their thoughtful suggestions to improve the quality of this work.

Conflict of interest

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

Publisher's note

References

Aono, S., Hatanaka, A., Hatanaka, A., Gao, Y., Hippo, Y., Taketo, M. M., et al. (2019). β-Catenin/TCF4 complex-mediated induction of the NRF3 (NFE2L3) gene in cancer cells. *Int. J. Mol. Sci.* 20, 3344. doi:10.3390/ijms20133344

Bai, S., Chen, L., Yan, Y., Wang, X., Jiang, A., Li, R., et al. (2021). Identification of hypoxia-immune-related gene signatures and construction of a prognostic model in kidney renal clear cell carcinoma. *Front. Cell. Dev. Biol.* 9, 796156. doi:10.3389/fcell.2021.796156

Ball, S. L., Cockell, S. J., Wilson, J. A., Mann, D. A., and Fisher, A. J. (2020). Microarray analysis of primary epithelial and fibroblast cells in chronic rhinosinusitis without nasal polyps. *Rhinology* 58, 581–587. doi:10.4193/Rhin19.319

Bathish, B., Robertson, H., Dillon, J. F., Dinkova-Kostova, A. T., and Hayes, J. D. (2022). Nonalcoholic steatohepatitis and mechanisms by which it is ameliorated by activation of the CNC-bZIP transcription factor Nrf2. *Free Radic. Biol. Med.* 188, 221–261. doi:10.1016/j.freeradbiomed.2022.06.226

Braun, S., Hanselmann, C., Gassmann, M. G., auf dem Keller, U., Born-Berclaz, C., Chan, K., et al. (2002). Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound. *Mol. Cell. Biol.* 22, 5492–5505. doi:10.1128/mcb.22.15.5492-5505.2002

Bury, M., Le Calve, B., Lessard, F., Dal Maso, T., Saliba, J., Michiels, C., et al. (2019). NFE2L3 controls colon cancer cell growth through regulation of DUX4, a CDK1 inhibitor. *Cell. Rep.* 29, 1469–1481. doi:10.1016/j.celrep.2019.09.087

Bushweller, J. H. (2019). Targeting transcription factors in cancer - from undruggable to reality. *Nat. Rev. Cancer* 19, 611–624. doi:10.1038/s41568-019-0196-7

Cardoso, J. V., Perini, J. A., Machado, D. E., Pinto, R., and Medeiros, R. (2020). Systematic review of genome-wide association studies on susceptibility to endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 255, 74–82. doi:10.1016/j.ejogrb.2020.10.017

Chan, J. Y. H. X., and Kan, Y. W. (1993a). Isolation of cDNA encoding the human NF-E2 protein. *Proc. Natl. Acad. Sci. U. S. A.* 90, 11366–11370. doi:10.1073/pnas.90.23.11366

Chan, J. Y. H. X., and Kan, Y. W. (1993b). Cloning of Nrf1, an NF-E2-related transcription factor, by genetic selection in yeast. *Proc. Natl. Acad. Sci. U. S. A.* 90, 11371–11375. doi:10.1073/pnas.90.23.11371

Chen, L., Lu, D., Sun, K., Xu, Y., Hu, P., Li, X., et al. (2019). Identification of biomarkers associated with diagnosis and prognosis of colorectal cancer patients based on integrated bioinformatics analysis. *Gene* 692, 119–125. doi:10.1016/j.gene.2019.01.001

Chen, T., Xu, B., Chen, H., Sun, Y., Song, J., Sun, X., et al. (2022b). Transcription factor NFE2L3 promotes the proliferation of esophageal squamous cell carcinoma cells and causes radiotherapy resistance by regulating IL-6. *Comput. Methods Programs Biomed.* 226, 107102. doi:10.1016/j.cmpb.2022.107102

Chen, Y., Siriwardena, D., Penfold, C., Pavlinek, A., and Boroviak, T. E. (2022a). An integrated atlas of human placental development delineates essential regulators of trophoblast stem cells. *Development* 149, dev200171. doi:10.1242/dev.200171

Chenais, B., Derjuga, A., Massrieh, W., Red-Horse, K., Bellingard, V., Fisher, S. J., et al. (2005). Functional and placental expression analysis of the human NRF3 transcription factor. *Mol. Endocrinol.* 19, 125–137. doi:10.1210/me.2003-0379

Chevillard, G., Nouhi, Z., Anna, D., Paquet, M., and Blank, V. (2010). Nrf3-deficient mice are not protected against acute lung and adipose tissue damages induced by butylated hydroxytoluene. *FEBS Lett.* 584, 923–928. doi:10.1016/j.febslet.2010.01.028

Chevillard, G., Paquet, M., and Blank, V. (2011). Nfe2l3 (Nrf3) deficiency predisposes mice to T-cell lymphoblastic lymphoma. *Blood* 117, 2005–2008. doi:10.1182/blood-2010-02-271460

Chowdhury, A., Katoh, H., Hatanaka, A., Iwanari, H., Nakamura, N., Hamakubo, T., et al. (2017). Multiple regulatory mechanisms of the biological function of NRF3 (NFE2L3) control cancer cell proliferation. *Sci. Rep.* 7, 12494. doi:10.1038/s41598-017-12675-y

Chowdhury, I., Mo, Y., Gao, L., Kazi, A., Fisher, A. B., and Feinstein, S. I. (2009). Oxidant stress stimulates expression of the human peroxiredoxin 6 gene by a transcriptional mechanism involving an antioxidant response element. *Free Radic. Biol. Med.* 46, 146–153. doi:10.1016/j.freeradbiomed.2008.09.027

Cirone, M., and D'Orazi, G. (2022). NRF2 in cancer: cross-talk with oncogenic pathways and involvement in gammaherpesvirus-driven carcinogenesis. *Int. J. Mol. Sci.* 24, 595. doi:10.3390/ijms24010595

Cockram, P. E., Kist, M., Prakash, S., Chen, S. H., Wertz, I. E., and Vucic, D. (2021). Ubiquitination in the regulation of inflammatory cell death and cancer. *Cell. Death Differ.* 28, 591–605. doi:10.1038/s41418-020-00708-5

Dai, Y. C., Pan, Y., Quan, M. M., Chen, Q., Pan, Y., Ruan, Y. Y., et al. (2021). MicroRNA-1246 mediates drug resistance and metastasis in breast cancer by targeting NFE2L3. *Front. Oncol.* 11, 677168. doi:10.3389/fonc.2021.677168

Derjuga, A., Gourley, T. S., Holm, T. M., Heng, H. H., Shivdasani, R. A., Ahmed, R., et al. (2004). Complexity of CNC transcription factors as revealed by gene targeting of the Nrf3 locus. *Mol. Cell. Biol.* 24, 3286–3294. doi:10.1128/mcb.24.8.3286-3294.2004

Dou, R., Wang, X., and Zhang, J. (2022). Prognostic value and immune infiltration analysis of nuclear factor erythroid-2 family members in ovarian cancer. *Biomed. Res. Int.* 2022, 8672258. doi:10.1155/2022/8672258

Du, H., and Taylor, H. S. (2015). The role of hox genes in female reproductive tract development, adult function, and fertility. *Cold Spring Harb. Perspect. Med.* 6, a023002. doi:10.1101/cshperspect.a023002

Elango, R., Vishnubalaji, R., Shaath, H., and Alajez, N. M. (2021). Molecular subtyping and functional validation of TTK, TPX2, UBE2C, and LRP8 in sensitivity of TNBC to paclitaxel. *Mol. Ther. Methods Clin. Dev.* 20, 601–614. doi:10.1016/j.omtm.2021.01.013

Etchevers, H. C. (2005). The cap 'n' collar family member NF-E2-related factor 3 (Nrf3) is expressed in mesodermal derivatives of the avian embryo. *Int. J. Dev. Biol.* 49, 363–367. doi:10.1387/ijdb.041942he

Fairfax, B. P., Humburg, P., Makino, S., Naranbhai, V., Wong, D., Lau, E., et al. (2014). Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. *Science* 343, 1246949. doi:10.1126/science.1246949

Gurri, S., Siegenthaler, B., Cangkrama, M., Restivo, G., Huber, M., Saliba, J., et al. (2023). NRF3 suppresses squamous carcinogenesis, involving the unfolded protein response regulator HSPA5. *EMBO Mol. Med.* 15, e17761. doi:10.15252/emmm.202317761

Heid, I. M., Jackson, A. U., Randall, J. C., Winkler, T. W., Qi, L., Steinthorsdottir, V., et al. (2010). Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat. Genet.* 42, 949–960. doi:10.1038/ng.685

Hou, X., Liao, Q., Wu, Y., Wang, L., Zhao, J., and Liao, X. (2024). Hypomethylation-mediated upregulation of NFE2L3 promotes malignant phenotypes of clear cell renal cell carcinoma cells. *Mol. Biotechnol.* 66, 198–207. doi:10.1007/s12033-023-00727-w

Hu, D., Zhang, Z., Luo, X., Li, S., Jiang, J., Zhang, J., et al. (2024). Transcription factor BACH1 in cancer: roles, mechanisms, and prospects for targeted therapy. *Biomark. Res.* 12, 21. doi:10.1186/s40364-024-00570-4

Huang, G., Yang, Y., Lv, M., Huang, T., Zhan, X., Yao, Y., et al. (2021). miR-23b-3p inhibits the oncogenicity of colon adenocarcinoma by directly targeting NFE2L3. *J. Oncol.* 2021, 8493225. doi:10.1155/2021/8493225

Jia, M., Lv, J. J., and Xiang, Y. C. (2022). The mechanisms of the production of different isoforms of nuclear factor E2-related factor 1 and their role in diseases. *Chin. J. Biochem. Mol. Biol.* 38, 595–602. doi:10.13865/j.cnki.cjbmb.2021.09.1204

Jin, Y., Huang, Y., Zeng, G., Hu, J., Li, M., Tian, M., et al. (2024). Advanced glycation end products regulate macrophage apoptosis and influence the healing of diabetic foot wound through miR-361-3p/CSF1R and PI3K/AKT pathway. *Heliyon* 10, e24598. doi:10.1016/j.heliyon.2024.e24598

Kannan, M. B., Dodard-Friedman, I., and Blank, V. (2015). Stringent control of NFE2L3 (nuclear factor, erythroid 2-like 3; NRF3) protein degradation by FBW7 (F-box/WD repeat-containing protein 7) and glycogen synthase kinase 3 (GSK3). *J. Biol. Chem.* 290, 26292–26302. doi:10.1074/jbc.M115.666446

Khanal, T., Rajan, N., Li, W., Liyanarachchi, S., and Ringel, M. D. (2023). The RCAN1.4 metastasis suppressor is hypermethylated at intron 1 in thyroid cancer. *Thyroid* 33, 965–973. doi:10.1089/thy.2022.0687

Kitaya, K., Yasuo, T., Yamaguchi, T., Fushiki, S., and Honjo, H. (2007). Genes regulated by interferon-gamma in human uterine microvascular endothelial cells. *Int. J. Mol. Med.* 20, 689–697. doi:10.3892/ijmm.20.5.689

Kobayashi, A., Ito, E., Toki, T., Kogame, K., Takahashi, S., Igarashi, K., et al. (1999). Molecular cloning and functional characterization of a new Cap'n' collar family transcription factor Nrf3. *J. Biol. Chem.* 274, 6443–6452. doi:10.1074/jbc.274.10.6443

Li, F., and Wen, Z. (2024). Identification roles of NFE2L3 in digestive system cancers. *J. Cancer Res. Clin. Oncol.* 150, 150. doi:10.1007/s00432-024-05656-y

Li, Q., Hujiaaihemaiti, M., Wang, J., Uddin, M. N., Li, M. Y., Aierken, A., et al. (2023). Identifying key transcription factors and miRNAs coregulatory networks associated with immune infiltrations and drug interactions in idiopathic pulmonary arterial hypertension. *Math. Biosci. Eng.* 20, 4153–4177. doi:10.3934/mbe.2023194

Liu, M., Wei, H., Yang, J., Chen, X., Wang, H., Zheng, Y., et al. (2022). Multi-omics analysis of molecular characteristics and carcinogenic effect of NFE2L3 in pan-cancer. *Front. Genet.* 13, 916973. doi:10.3389/fgene.2022.916973

Liu, P., Kerins, M. J., Tian, W., Neupane, D., Zhang, D. D., and Ooi, A. (2019). Differential and overlapping targets of the transcriptional regulators NRF1, NRF2, and NRF3 in human cells. *J. Biol. Chem.* 294, 18131–18149. doi:10.1074/jbc.RA119.009591

Liu, X., Xu, C., Xiao, W., and Yan, N. (2023). Unravelling the role of NFE2L1 in stress responses and related diseases. *Redox Biol.* 65, 102819. doi:10.1016/j.redox.2023.102819

Lukomska, A., Frost, M. P., Theune, W. C., Xing, J., Gupta, M., and Trakhtenberg, E. F. (2024). Nfe2l3 promotes neuroprotection and long-distance axon regeneration after injury *in vivo*. *Exp. Neurol.* 375, 114741. doi:10.1016/j.expneurol.2024.114741

McGinnis, W., and Krumlauf, R. (1992). Homeobox genes and axial patterning. *Cell.* 68, 283–302. doi:10.1016/0092-8674(92)90471-n

Moi, P., Chan, K., Asunis, I., Cao, A., and Kan, Y. W. (1994). Isolation of nf-E2-related factor-2 (Nrf2), a nf-E2-like basic leucine-zipper transcriptional activator that binds to

the tandem nf-E2/ap1 repeat of the beta-globin locus-control region. *Proc. Natl. Acad. Sci. U. S. A.* 91, 9926–9930. doi:10.1073/pnas.91.21.9926

Monda, K. L., Chen, G. K., Taylor, K. C., Palmer, C., Edwards, T. L., Lange, L. A., et al. (2013). A meta-analysis identifies new loci associated with body mass index in individuals of African ancestry. *Nat. Genet.* 45, 690–696. doi:10.1038/ng.2608

Nouhi, Z., Chevillard, G., Derjuga, A., and Blank, V. (2007). Endoplasmic reticulum association and N-linked glycosylation of the human Nrf3 transcription factor. *FEBS Lett.* 581, 5401–5406. doi:10.1016/j.febslet.2007.10.041

Osinski, M., Mostowska, A., Wirstlein, P., Wender-Ozegowska, E., Jagodzinski, P. P., and Szczepanska, M. (2018). The assessment of GWAS - identified polymorphisms associated with infertility risk in Polish women with endometriosis. *Ginekol. Pol.* 89, 304–310. doi:10.5603/GP.a2018.0052

Painter, J. N., Anderson, C. A., Nyholt, D. R., Macgregor, S., Lin, J., Lee, S. H., et al. (2011). Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. *Nat. Genet.* 43, 51–54. doi:10.1038/ng.731

Peng, Y., Liu, J., Inuzuka, H., and Wei, W. (2023). Targeted protein posttranslational modifications by chemically induced proximity for cancer therapy. *J. Biol. Chem.* 299, 104572. doi:10.1016/j.jbc.2023.104572

Peng, Y., Wu, Q., Wang, L., Wang, H., and Yin, F. (2020). A DNA methylation signature to improve survival prediction of gastric cancer. *Clin. Epigenetics* 12, 15. doi:10.1186/s13148-020-0807-x

Pepe, A. E., Xiao, Q., Zampetaki, A., Zhang, Z., Kobayashi, A., Hu, Y., et al. (2010). Crucial role of nrf3 in smooth muscle cell differentiation from stem cells. *Circ. Res.* 106, 870–879. doi:10.1161/CIRCRESAHA.109.211417

Qian, J., Huang, C., Zhu, Z., He, Y., Wang, Y., Feng, N., et al. (2022). NFE2L3 promotes tumor progression and predicts a poor prognosis of bladder cancer. *Carcinogenesis* 43, 457–468. doi:10.1093/carcin/bgac006

Ramazi, S., and Zahiri, J. (2021). Posttranslational modifications in proteins: resources, tools and prediction methods. *Database (Oxford)* 2021, baab012. doi:10.1093/database/baab012

Rauscher, G. H., Kresovich, J. K., Poulin, M., Yan, L., Macias, V., Mahmoud, A. M., et al. (2015). Exploring DNA methylation changes in promoter, intragenic, and intergenic regions as early and late events in breast cancer formation. *BMC Cancer* 15, 816. doi:10.1186/s12885-015-1777-9

Ren, J., Yang, Y., Li, C., Xie, L., Hu, R., Qin, X., et al. (2020). A novel prognostic model of early-stage lung adenocarcinoma integrating methylation and immune biomarkers. *Front. Genet.* 11, 634634. doi:10.3389/fgene.2020.634634

Ren, Y., Wang, Y., Hao, S., Yang, Y., Xiong, W., Qiu, L., et al. (2020). NFE2L3 promotes malignant behavior and EMT of human hepatocellular carcinoma (HepG2) cells via Wnt/β-catenin pathway. *J. Cancer* 11, 6939–6949. doi:10.7150/jca.48100

Ren, Y., Yang, J., Ding, Z., Zheng, M., Qiu, L., Tang, A., et al. (2023). NFE2L3 drives hepatocellular carcinoma cell proliferation by regulating the proteasome-dependent degradation of ISGylated p53. *Cancer Sci.* 114, 3523–3536. doi:10.1111/cas.15887

Robledinos-Anton, N., Fernandez-Gines, R., Manda, G., and Cuadrado, A. (2019). Activators and inhibitors of NRF2: a review of their potential for clinical development. *Oxid. Med. Cell. Longev.* 2019, 9372182. doi:10.1155/2019/9372182

Rowther, F. B., Wei, W., Dawson, T. P., Ashton, K., Singh, A., Madiesse-Timchou, M. P., et al. (2016). Cyclic nucleotide phosphodiesterase-1C (PDE1C) drives cell proliferation, migration and invasion in glioblastoma multiforme cells *in vitro*. *Mol. Carcinog.* 55, 268–279. doi:10.1002/mc.22276

Saliba, J., Coutaud, B., Makhani, K., Roth, N. E., Jackson, J., Park, J. Y., et al. (2022). Loss of NFE2L3 protects against inflammation-induced colorectal cancer through modulation of the tumor microenvironment. *Oncogene* 41, 1563–1575. doi:10.1038/s41388-022-02192-2

Sankaranarayanan, K., and Jaiswal, A. K. (2004). Nrf3 negatively regulates antioxidant-response element-mediated expression and antioxidant induction of NAD(P)H:quinone oxidoreductase1 gene. *J. Biol. Chem.* 279, 50810–50817. doi:10.1074/jbc.M404984200

Siegenthaler, B., Defila, C., Muzumdar, S., Beer, H. D., Meyer, M., Tanner, S., et al. (2018). Nrf3 promotes UV-induced keratinocyte apoptosis through suppression of cell adhesion. *Cell. Death Differ.* 25, 1749–1765. doi:10.1038/s41418-018-0074-y

Sies, H. (2015). Oxidative stress: a concept in redox biology and medicine. *Redox Biol.* 4, 180–183. doi:10.1016/j.redox.2015.01.002

Silva, K. C. S., Tambwe, N., Mahfouz, D. H., Wium, M., Cacciatore, S., Paccez, J. D., et al. (2024). Transcription factors in prostate cancer: insights for disease development and diagnostic and therapeutic approaches. *Genes. (Basel)* 15, 450. doi:10.3390/genes15040450

Simpson, C. L., Musolf, A. M., Cordero, R. Y., Cordero, J. B., Portas, L., Murgia, F., et al. (2021). Myopia in african Americans is significantly linked to chromosome 7p15.2-14.2. *Investig. Ophthalmol. Vis. Sci.* 62, 16. doi:10.1167/iovs.62.9.16

Sun, J., Zheng, Z., Chen, Q., Pan, Y., Lu, H., Zhang, H., et al. (2019). NRF3 suppresses breast cancer cell metastasis and cell proliferation and is a favorable predictor of survival in breast cancer. *Onco Targets Ther.* 12, 3019–3030. doi:10.2147/OTT.S197409

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 71, 209–249. doi:10.3322/caac.21660

Suzuki, R., Miyamoto, S., Yasui, Y., Sugie, S., and Tanaka, T. (2007). Global gene expression analysis of the mouse colonic mucosa treated with azoxymethane and dextran sodium sulfate. *BMC Cancer* 7, 84. doi:10.1186/1471-2407-7-84

Tanaka, T., Okada, R., Hozaka, Y., Wada, M., Moriya, S., Satake, S., et al. (2020). Molecular pathogenesis of pancreatic ductal adenocarcinoma: impact of miR-30c-5p and miR-30c-2-3p regulation on oncogenic genes. *Cancers* 12, 2731. doi:10.3390/cancers12102731

Terui, K., Takahashi, Y., Kitazawa, J., Toki, T., Yokoyama, M., and Ito, E. (2000). Expression of transcription factors during megakaryocytic differentiation of CD34+ cells from human cord blood induced by thrombopoietin. *Tohoku J. Exp. Med.* 192, 259–273. doi:10.1620/tjem.192.259

Uddin, M. N., Li, M., and Wang, X. (2019). Identification of transcriptional signatures of colon tumor stroma by a meta-analysis. *J. Oncol.* 2019, 8752862. doi:10.1155/2019/8752862

Waku, T., Hagiwara, T., Tamura, N., Atsumi, Y., Urano, Y., Suzuki, M., et al. (2021). NRF3 upregulates gene expression in SREBP2-dependent mevalonate pathway with cholesterol uptake and lipogenesis inhibition. *iScience* 24, 103180. doi:10.1016/j.isci.2021.103180

Waku, T., Katayama, H., Hiraoka, M., Hatanaka, A., Nakamura, N., Tanaka, Y., et al. (2020a). NFE2L1 and NFE2L3 complementarily maintain basal proteasome activity in cancer cells through CPEB3-mediated translational repression. *Mol. Cell. Biol.* 40, e00010-20–e00020. doi:10.1128/MCB.00010-20

Waku, T., and Kobayashi, A. (2021). Pathophysiological potentials of NRF3-regulated transcriptional axes in protein and lipid homeostasis. *Int. J. Mol. Sci.* 22, 12686. doi:10.3390/ijms222312686

Waku, T., Nakamura, N., Koji, M., Watanabe, H., Katoh, H., Tatsumi, C., et al. (2020b). NRF3-POMP-20S proteasome assembly Axis promotes cancer development via ubiquitin-independent proteolysis of p53 and retinoblastoma protein. *Mol. Cell. Biol.* 40, e00597. doi:10.1128/MCB.00597-19

Wang, C., Saji, M., Justiniano, S. E., Yusof, A. M., Zhang, X., Yu, L., et al. (2017). RCAN1-4 is a thyroid cancer growth and metastasis suppressor. *JCI Insight* 2, e90651. doi:10.1172/jci.insight.90651

Wang, H., Zhan, M., Yang, R., Shi, Y., Liu, Q., and Wang, J. (2018). Elevated expression of NFE2L3 predicts the poor prognosis of pancreatic cancer patients. *Cell. Cycle* 17, 2164–2174. doi:10.1080/15384101.2018.1520558

Wang, J., Zhao, H., Dong, H., Zhu, L., Wang, S., Wang, P., et al. (2019). LAT, HOXD3 and NFE2L3 identified as novel DNA methylation-driven genes and prognostic markers in human clear cell renal cell carcinoma by integrative bioinformatics approaches. *J. Cancer* 10, 6726–6737. doi:10.7150/jca.35641

Wang, X., Li, Y., Fang, Z., and Li, Y. (2021). Elevated expression of NFE2L3 promotes the development of gastric cancer through epithelial-mesenchymal transformation. *Bioengineered* 12, 12204–12214. doi:10.1080/21655979.2021.2005915

Wang, Z., Yang, H., Luo, B., Duan, P., and Lin, P. (2022). NFE2L3 as a novel biomarker associated with IL-2/STAT5/NLRP3 signaling pathway in malignant pleural mesothelioma and other cancers. *Front. Genet.* 13, 805256. doi:10.3389/fgene.2022.805256

Widenmaier, S. B., Snyder, N. A., Nguyen, T. B., Arduini, A., Lee, G. Y., Arruda, A. P., et al. (2017). NRF1 is an ER membrane sensor that is central to cholesterol homeostasis. *Cell.* 171, 1094–1109. doi:10.1016/j.cell.2017.10.003

Xiang, Y., and Miao, H. (2021). Lipid metabolism in tumor-associated macrophages. *Adv. Exp. Med. Biol.* 1316, 87–101. doi:10.1007/978-981-33-6785-2_6

Xiao, Q., Pepe, A. E., Wang, G., Luo, Z., Zhang, L., Zeng, L., et al. (2012). Nrf3-Pla2g7 interaction plays an essential role in smooth muscle differentiation from stem cells. *Arterioscler. Thromb. Vasc. Biol.* 32, 730–744. doi:10.1161/ATVBAHA.111.243188

Xue, H., Yu, M., Zhou, Y., Zhang, J., Mu, Q., Chen, T., et al. (2020). Apolipoprotein M inhibits proliferation and migration of larynx carcinoma cells. *Sci. Rep.* 10, 19424. doi:10.1038/s41598-020-76480-w

Yan, J., Su, R., Ao, D., Wang, Y., Wang, H., and Yang, H. (2018). Genetic variants and clinical relevance associated with gestational diabetes mellitus in Chinese women: a case-control study. *J. Matern. Fetal Neonatal Med.* 31, 2115–2121. doi:10.1080/14767058.2017.1336225

Yang, F., Jia, M., Dai, R. Y., and Xiang, Y. C. (2020). Progress on the biological functions of transmembrane factor Nrf1. *Prog. Biochem. Biophys.* 47, 582–594. doi:10.16476/j.pibb.2020.0057

Yu, M. M., Feng, Y. H., Zheng, L., Zhang, J., and Luo, G. H. (2019). Short hairpin RNA-mediated knockdown of nuclear factor erythroid 2-like 3 exhibits tumor-suppressing effects in hepatocellular carcinoma cells. *World J. Gastroenterol.* 25, 1210–1223. doi:10.3748/wjg.v25.i10.1210

Yuan, J., Zhang, S., and Zhang, Y. (2018). Nrf1 is paved as a new strategic avenue to prevent and treat cancer, neurodegenerative and other diseases. *Toxicol. Appl. Pharmacol.* 360, 273–283. doi:10.1016/j.taap.2018.09.037

Zhang, D., Wang, Y., and Hu, X. (2020). Identification and comprehensive validation of a DNA methylation-driven gene-based prognostic model for clear cell renal cell carcinoma. *DNA Cell. Biol.* 39, 1799–1812. doi:10.1089/dna.2020.5601

Zhang, L., Hu, D. L., Tang, B., Cheng, Y., Jiao, C., Cheng, L., et al. (2019). NFE2L3 inhibition induces cell cycle arrest at the G0/G1 phase in colorectal cancer cells through downregulating CCND1 and pRb1-ser807/811. *Dis. Markers* 2019, 2829798. doi:10.1155/2019/2829798

Zhang, Q., Tang, D., Zha, A., He, J., Li, D., Chen, Y., et al. (2022). NFE2L3 as a potential functional gene regulating immune microenvironment in human kidney cancer. *Biomed. Res. Int.* 2022, 9085186. doi:10.1155/2022/9085186

Zhang, Y., Kobayashi, A., Yamamoto, M., and Hayes, J. D. (2009). The Nrf3 transcription factor is a membrane-bound glycoprotein targeted to the endoplasmic reticulum through its N-terminal homology box 1 sequence. *J. Biol. Chem.* 284, 3195–3210. doi:10.1074/jbc.M805337200

Zhang, Y., and Xiang, Y. (2016). Molecular and cellular basis for the unique functioning of Nrf1, an indispensable transcription factor for maintaining cell homoeostasis and organ integrity. *Biochem. J.* 473, 961–1000. doi:10.1042/BJ20151182

Zhou, Y., Wu, H., Zhao, M., Chang, C., and Lu, Q. (2016). The bach family of transcription factors: a comprehensive review. *Clin. Rev. Allergy Immunol.* 50, 345–356. doi:10.1007/s12016-016-8538-7

frontiers | Frontiers in Cell and Developmental Biology

TYPE Review
PUBLISHED 02 August 2024
DOI 10.3389/fcell.2024.1408852

OPEN ACCESS

EDITED BY
Lucio Miele,
Louisiana State University, United States

REVIEWED BY
Massimiliano Cadamuro,
University of Milano Bicocca, Italy
Abhishek Bhardwaj,
New York University, United States
Karan Singh,
New York University, United States

*CORRESPONDENCE
Yong Chen,
doctorcy007@163.com

RECEIVED 28 March 2024
ACCEPTED 26 June 2024
PUBLISHED 02 August 2024

CITATION
Wang J, Liu S, Cao Y and Chen Y (2024), Overcoming treatment resistance in cholangiocarcinoma: current strategies, challenges, and prospects.
Front. Cell Dev. Biol. 12:1408852.
doi: 10.3389/fcell.2024.1408852

Overcoming treatment resistance in cholangiocarcinoma: current strategies, challenges, and prospects

Jiayi Wang[1], Siyan Liu[1], Yi Cao[2] and Yong Chen[3]*

[1]International Medical College, Chongqing Medical University, Chongqing, China, [2]Second Clinical College, Chongqing Medical University, Chongqing, China, [3]Department of Hepatobiliary Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

Significant advancements in our understanding and clinical treatment of cholangiocarcinoma (CCA) have been achieved over the past 5 years. Groundbreaking studies have illuminated the immune landscape and pathological characteristics of the tumor microenvironment in CCA. The development of immune- and metabolism-based classification systems has enabled a nuanced exploration of the tumor microenvironment and the origins of CCA, facilitating a detailed understanding of tumor progression modulation. Despite these insights, targeted therapies have not yet yielded satisfactory clinical results, highlighting the urgent need for innovative therapeutic strategies. This review delineates the complexity and heterogeneity of CCA, examines the current landscape of therapeutic strategies and clinical trials, and delves into the resistance mechanisms underlying targeted therapies. Finally, from a single-cell and spatial transcriptomic perspective, we address the challenge of therapy resistance, discussing emerging mechanisms and potential strategies to overcome this barrier and enhance treatment efficacy.

KEYWORDS

resistance in cholangiocarcinoma, targeted therapies, treatment of cholangiocarcinoma, single-cell and spatial transcriptomic perspective, cholangiocarcinoma (CCA)

Introduction

Cholangiocarcinoma (CCA) represents a highly lethal epithelial carcinoma within the hepatobiliary system, usually classified as intrahepatic, perihilar, and distal based on various anatomical locations (Brindley et al., 2021). CCA is the second most common liver cancer accounting for 15%–20% of all primary liver cancers (Gingold et al., 2018). In contrast to hepatocellular carcinoma (Xue et al.), the rare incidence of it has in fact increased the complexity and challenge of treatment (Balogh et al., 2016). The late diagnoses and poor prognoses are an obstacle to further improvement of therapeutic effectiveness: patients have an overall 5-year overall survival (Bridgewater et al.) ranges from 7% to 20% (Balogh et al., 2016; Banales et al., 2019; Zhu and Kwong, 2020).

Considering the continuing high recurrence and rapid progression after treatment worldwide, understanding the risk factors for CCA is essential to improve therapy efficacy. In Thailand with the highest incidence (30–40 out of 100,000), the top one pathogenic factor is the infection of liver fluke (Bridgewater et al., 2014). In contrast, in western countries with

relatively low incidences (Xue et al., 2019), risk factors are diverse and usually include hepatitis B/C virus, fatty liver, alcohol, and biliary inflammation (Palmer and Patel, 2012; Bridgewater et al., 2014). Recently, a few studies pointed out wider risk factors for CCA in a perspective of liver diseases such as fibroinflammatory biliary duct diseases and primary sclerosing hepatitis (Razumilava and Gores, 2014; Bertuccio et al., 2019; Kelley et al., 2020).

In our recent summary and review, we have consolidated significant advancements, including studies utilizing next-generation sequencing, single-cell sequencing, spatial transcriptomic sequencing, and other multi-omics analyses. These studies have provided insights into the mechanisms of CCA resistance, addressing aspects such as the identification of driver genes, challenges related to specific target resistance, cell-cell interactions within the tumor microenvironment, and the spatial heterogeneity of tumors. This review underscores the critical issue of therapeutic resistance and the development of novel combination treatment strategies. The objective is to innovate therapeutic approaches and improve the adverse clinical outcomes associated with CCA.

Advances in the genomic landscape and laboratory technology of CCA

Our understanding of the genomic landscape of cholangiocarcinoma (CCA) has significantly deepened (Figure 1). Since 2013, extensive next-generation sequencing efforts have identified diverse subgroups of intrahepatic cholangiocarcinoma (ICC) for clinical consideration. Notably, Sia, Moeini, and Montal, along with their teams, have made significant contributions to elucidating the molecular signatures and actionable targets in CCA at various localizations. In 2013, Sia et al. identified two types of ICC—proliferation and inflammation—by analyzing signaling pathways activated in tumors of specific molecular classes and copy number variation (Sia et al., 2013).

In 2014, Gao and colleagues reported a whole-exome sequencing (Schneider et al.) study linking PTPN3 mutations to CCA proliferation, migration, and recurrence potential (Gao et al., 2014). The Lawrence Kwong lab at MD Anderson Cancer Center contributed to The Cancer Genome Atlas (Farshidfar et al.), performing comprehensive analyses of somatic mutations, DNA methylation, whole-genome expression, and copy number variation. Their work highlighted the isocitrate dehydrogenase (IDH) mutation as a stratification marker for the CCA population (Farshidfar et al., 2017). Kwong's work is regarded as a cornerstone in ICC bulk sequencing studies.

In 2019, researchers from China, Japan, and Singapore conducted a joint study sequencing the genome and transcriptome of 133 East Asian patients, elucidating CCA heterogeneity and providing molecular subtypes for pathological reference (Xue et al., 2019). In 2022, Gao, Q.'s lab performed in-depth sequencing on different spots for each patient, delineating the heterogeneity of immune infiltration in CCA (Lin et al., 2022). Additionally, Sia's team provided comprehensive molecular characterization and identified multiple subtypes of mixed hepatocellular-cholangiocarcinoma (HCC-ICC) and extrahepatic cholangiocarcinoma (eCCA) in 2017 and 2020, respectively (Moeini et al., 2017; Montal et al., 2020). These studies have facilitated an in-depth exploration of the molecular mechanisms of CCA across different locations and types.

Advancements in laboratory technology have also been noteworthy. The study by Calvisi DF and Chen X in 2014 achieved stable reproduction of mouse liver cancer models through hydrodynamic tail vein injection (HTVI) technology. They demonstrated that liver tumors could be induced by transfecting oncogenes and tumor suppressor genes into hepatocytes, replicating specific pathological environments and tumor progression periods (Chen and Calvisi, 2014). This method allows for the efficient delivery of oncogenes, eliminating the need for breeding transgenic mice to study liver tumors. Consequently, new insights into the pathology and origin of hepatocellular carcinoma and intrahepatic CCA have been revealed.

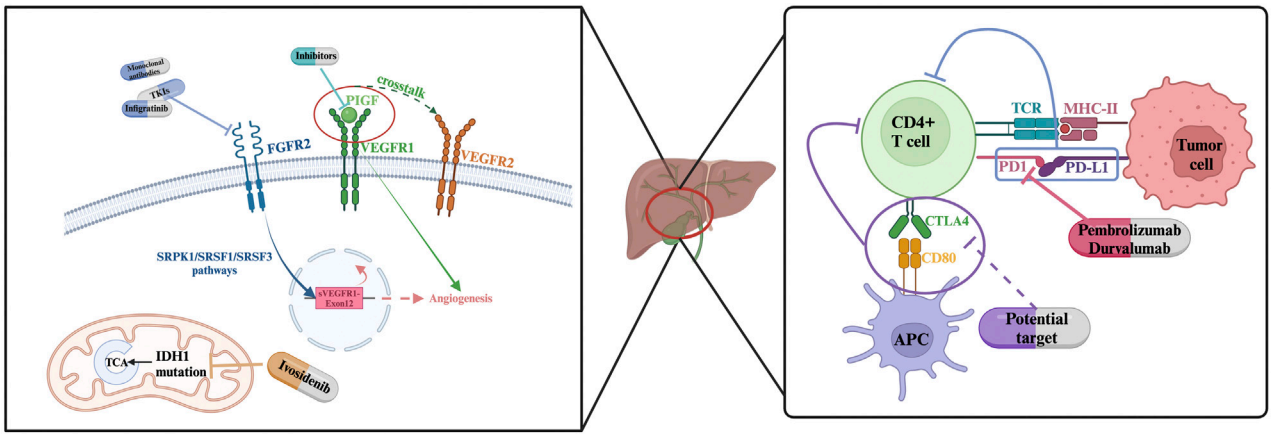


FIGURE 1
General condition of targeted therapy for cholangiocarcinoma.

TABLE 1 Cholangiocarcinoma clinical trials.

Trial code	Study arm	Study phase	Inhibitor	Primary endPoint(s)	ORR (advances)
TOPAZ-1 (Oh et al., 2022)	durvalumab	Phase III	PD-L1	OS	26.7%
FIGHT-202 (Abou-Alfa et al., 2020b)	Pemigatinib	Phase II	FGFR2	ORR	35.5%
FIGHT-302 (Bekaii-Saab et al., 2020)	Pemigatinib	Phase III	FGFR2	OS, ORR	-ongoing
BGJ398 (Javle et al., 2021a)	Infigratinib	Phase II	FGFR2	ORR	23.1%
ClarIDHy (Abou-Alfa et al., 2020a)	Ivosidenib	Phase III	IDH1	PFS	2–7 months
KEYNOTE-158 (Marabelle et al., 2020)	Pembrolizumab	Phase II	MSI-H/ dMMR	ORR	34.3%
LEAP-005 (Perez-Fidalgo and Martinelli, 2023)	Lenvatinib + pembrolizumab	Phase II	ICIs	ORR	22%
FIDES-01 (Park et al., 2019)	Derazantinib	Phase II	FGFR2	ORR	-ongoing
FOENIX-CCA2 (Goyal et al., 2021b)	Futibatinib	Phase II	FGFR2	ORR	41.7%
ECOG-ACRIN EA6134 (Atkins et al., 2023)	Dabrafenib + trametinib	Phase III	BRAF	ORR, OS	47.8%–29.6%

Standard treatment

Understanding the current clinical treatment landscape for cholangiocarcinoma is crucial for recognizing the significance of immunotherapy. Standard treatment strategies for CCA include surgical resection, systemic chemotherapy, and combinations of chemotherapy with targeted therapy. For intrahepatic cholangiocarcinoma (iCCA), only 30%–40% of cases are suitable for surgical resection. Even among those who undergo early surgical resection, the recurrence rate remains higher than 50% (Bridgewater et al., 2014). Monotherapy chemotherapy regimens have also shown limited success. Since 2019, two phase III randomized studies assessing adjuvant therapy with gemcitabine alone after surgical resection for CCA and gallbladder cancer (GBC) reported no significant improvement in survival rates (Primrose et al., 2019; Lamarca et al., 2020b; Lamarca et al., 2022).

In the quest to improve survival rates and immune response in CCA, resistance to targeted therapy inhibitors remains a significant challenge. This review aims to elucidate the mechanisms behind inhibitor resistance and the pathways leading to immune escape in CCA, with the goal of identifying new targets and perspectives for future treatments. A substantial barrier to the development of targeted therapies for CCA is the general lack of a predominant oncogenic driver in many cases, limiting the subset of patients who could benefit from these therapies. However, targeted treatment can still be relevant for patients harboring specific mutations, with potential targets including Isocitrate Dehydrogenase 1/2 (IDH1/2), Fibroblast Growth Factor Receptor 2 (FGFR2), Neurotrophic Receptor Tyrosine Kinase (NTRK), HER2, BRAF, ROS, and RET (Lamarca et al., 2020a; Harding et al., 2023).

Enhanced by the availability of open-source bulk sequencing and single-cell RNA sequencing data, combination therapies that include targeted treatments are showing promise. In the realm of first-line treatments, there is growing evidence that combining the immune checkpoint inhibitor durvalumab, a Programmed Death-Ligand 1 (PD-L1) inhibitor, with gemcitabine and cisplatin can significantly improve survival rates, establishing a new standard of care for patients with advanced-stage CCA (O'Rourke et al.). In the subsequent sections, we explore a range of prominent therapeutic agents, from inhibitors developed for traditional targetable molecules to immune checkpoint inhibitors (ICIs). This discussion encompasses drugs currently in clinical trial phases as well as those that have received regulatory approval. We delve into the phenomena of resistance to inhibitors and ICIs, highlighting several studies investigating the underlying mechanisms of resistance. Furthermore, we propose potential pathways to overcome this resistance, offering insights into future strategies for enhancing the efficacy of CCA treatments.

Molecular targeted therapy

In recent years, clinical trials targeting specific molecules have provided good support and information for the introduction of new treatment options into clinical practice. Table 1 summarizes some of the clinical trials that have recently ended and are ongoing. In the following sections, we further discuss some of these and other CCA clinical results that have attracted attention in recent years.

FGFR2 Inhibitors: clinical trial, toxicity, resistance mechanisms, and revisit of clinicogenomic analysis

Fibroblast growth factor receptor (FGFR) encompasses a family of tyrosine kinase receptors instrumental in the regulation of cellular proliferation and growth (Turner and Grose, 2010). Genetic alterations such as fusions and rearrangements of FGFR2 occur in 10%–15% of intrahepatic cholangiocarcinoma (iCCA) cases but are rarely observed in extrahepatic cholangiocarcinoma (Cadamuro et al., 2019; Goyal et al., 2021a). Pemigatinib, the first therapy approved by the U.S. FDA for the treatment of advanced CCA patients with FGFR2 fusion and rearrangement positivity, demonstrated objective responses in 38 out of 146 enrolled

patients (Liu et al., 2020). Furthermore, other targeted drugs developed for the FGFR pathway are in clinical trial phases, including selective and non-selective FGFR2 tyrosine kinase inhibitors (TKIs), and anti-FGF/FGFR monoclonal antibodies (Ghedini et al., 2018). Numerous studies reporting on the clinical efficacy of targeting FGFR2 fusion positive CCA have been published. Promising agents such as Debio 1,347, Derazantinib, Erdafitinib, and Infigratinib are currently undergoing phase II-III studies, with some of the results reported as of the writing of this article (Park et al., 2019; Cleary et al., 2020; Goyal et al., 2021a; Javle et al., 2021b). For patients with refractory advanced CCA harboring FGFR2 fusions or rearrangements, these therapies have demonstrated objective response rate (ORR) ranging from 20.7% to 47%. A multi-national, single-group, phase II study published in 2023 indicated that the covalent FGFR inhibitor futibatinib provided objective clinical benefits to patients with iCCA who had FGFR2 fusions or rearrangements and who had previously undergone treatment (Goyal et al., 2023).

Despite FDA has approved multiple FGFR2 inhibitors in clinical treatment, unsatisfied ORRs (<45%) were seen upon this type of treatment so far. Progression-free periods are maintained at 6–12 months and there is evidence that this may be associated with acquired alterations in the FGFR2 kinase domain and FGFR inhibitors disfunction of binding (Byron et al., 2013; Goyal et al., 2017; Goyal et al., 2019; Krook et al., 2019; Silverman et al., 2021; Varghese et al., 2021). FGFR1-3 inhibitor, Futibatinib (TAS-120) remains efficacy against a series of secondary FGFR2 mutations, though it is not effective against all (Goyal et al., 2019). Besides, further evidence indicates that FGFR inhibitor resistance could be still gained under circumstances of no occurrence of genetic alterations or those caused by other MARK signaling components (Goyal et al., 2021b; Cleary et al., 2021). Pharmacodynamically, almost all FGFR inhibitors are associated with increased phosphate levels; hence, concomitant phosphate-lowering treatment may be necessary to patients experiencing hyperphosphatemia during FGFR inhibitor therapy. Additionally, various degrees of ocular and nail abnormalities have been reported in these studies as side effects of the treatment (Javle et al., 2018; Abou-Alfa et al., 2020b; Goyal et al., 2020; Xie et al., 2020). All the enigma underscores the importance of further investigating FGFR inhibitors resistance mechanisms.

Efforts to explore resistance mechanisms to FGFR inhibitors and ways to overcome this resistance have been proposed and are under exploration. It is currently understood that there are two distinct FGFR2 resistance acquisition types: primary resistance and acquired resistance. Silverman and colleagues reported observations that individuals with FGFR2 fusion, who also possess tumor suppressor gene alterations (including BAP1, CDKN2A/B, PBRM1, and TP53), have shorter progression-free survival (Silverman et al., 2021). Regarding acquired resistance, one study reported the emergence of an FGFR2 V565F gatekeeper mutation in patients with FGFR2 fusion iCCA treated with infigratinib. Furthermore, two patients were reported to have developed multiple polyclonal secondary mutations (Goyal et al., 2017).

To combat resistance, a batch of influential research was conducted in aspects of increasing FGFR inhibitor sensitivity, focusing on potential efficient inhibitors, and presenting molecular landscape in recent few years. Wu and colleagues performed high-throughput combination drug screens (Wu et al., 2022b) on patient-derived xenograft (PDX) cell lines and mouse models. Their results illustrate that adaptive signaling through EGFR plays a key role in lowering FGFR inhibitor sensitivity and developing resistance. In sensitive models, disturbed cell death induction processes are observed. While suppressing wild-type EGFR responses through inhibiting MEK/ERK and mTOR signaling causes cell death and tumor regression. Another influential study was published on Sept. 06, 2023, and it recorded the team's discovery of the highly selective, irreversible, small-molecule RLY-4008s capability of inducing tumor regression and focusing both primary and acquired resistance (Subbiah et al., 2023).

Beyond the exploration of potential mechanisms of resistance, Silverman et al. conducted sequencing on a clinical cohort of cholangiocarcinoma (CCA) with FGFR2 rearrangements, providing direct evidence of the response to Pemigatinib targeted therapy. In this work, a post-treatment clinical genomic landscape was constructed for the FIGHT-202 cohort, examining the response of patients with FGFR2 fusions or rearrangements and those without FGFR2 alterations to Pemigatinib, as well as investigating pathways to acquired resistance to Pemigatinib. These findings offer invaluable insights for the application of Pemigatinib and future FGFR2-targeted therapies via suggesting a wide range of selection and enlightenment of potentiality of FGFR2 inhibitors' resistance acquisition (Silverman et al., 2021).

IDH1/IDH2 inhibitors: clinical insights, and resistance mechanisms

Isocitrate dehydrogenase (IDH) plays a pivotal role in cellular metabolic processes. Alterations in the genes responsible for the metabolic enzymes IDH1 and IDH2 have been linked to the emergence of early-stage biliary lesions with potential malignancy (Valle et al., 2017; Hadfield et al., 2023). Mutations in IDH1/2 are found in approximately 13%–36% of intrahepatic cholangiocarcinoma cases and are less common in extrahepatic cholangiocarcinoma, constituting less than 1% of instances (Valle et al., 2017; Boscoe et al., 2019; Abou-Alfa et al., 2020a). Mutations in Isocitrate Dehydrogenase 1/2 (IDH1/2) are frequently observed in cholangiocarcinoma (CCA). Ivosidenib (AG120), a small molecule and selective inhibitor, has been developed targeting the IDH1 mutation and has recently been approved by the FDA for use in advanced-stage and metastatic intrahepatic cholangiocarcinoma (iCCA) (Norsworthy et al., 2019; Zhu and Kwong, 2022). However, phase III trial reports indicate that the objective response rate (ORR) and disease stability (SD) are only 2% and 51% respectively (Zhu et al., 2021). Moreover, IDH1 mutation-driven mouse models of similar solid tumors do not exhibit tumor regression upon removal of the IDH1 mutation, implying a limited efficacy of IDH1 mutation inhibitors when used as monotherapy in tumors with comparable pathological conditions (Turcan et al., 2018).

In response to the challenges of low sensitivity and resistance faced by IDH mutation inhibitors, Wu and colleagues have engineered mouse models with IDH1 mutations and uncovered that tumor maintenance is mediated by dual (R)-2-hydroxyglutarate activities: suppression of $CD8^+$ T cell activity and the autonomous inactivation of TET2 DNA demethylase within tumor cells (Wu

et al., 2022a). This epigenetic and metabolic shift in the tumor microenvironment, as demonstrated in these mouse models, suggests that immune checkpoint blockade coupled with the IFN-γ-TET2 axis can surmount immunosuppression, thereby providing a strategy to counteract the resistance to IDH1 mutation inhibitors. Kwong et al. have investigated the potential synergistic effects of combining PD-L1 inhibitors with AG120 (ivosidenib) and the pairing of CTLA4 antibodies with AG120 in their research (Zhu and Kwong, 2022). Their findings reveal that while the combination of PD-L1 inhibitors and AG120 does not result in a synergistic effect, such an effect is evident between CTLA4 antibodies and AG120. Building upon previously reported studies, their exploration proposes a new avenue: the combination of CTLA4 inhibitors and IDH1 mutation-targeted treatments as a promising therapeutic strategy.

VEGF inhibitors and PlGF

Vascular endothelial growth factor (VEGF) modulates cancer cell proliferation with its functional role in enhancing angiogenesis. VEGF has been shown to be upregulated in over 75% hepatobiliary malignancies (Valle et al., 2017). VEGF inhibitor sorafenib combinedly used with gemcitabine are proven to provide clinical benefits to unresectable or metastatic BTC patients (Moehler et al., 2014). Several studies have shown that high expression of VEGF receptor (VEGFR) in a hypoxic environment can induce tumor angiogenesis, and Hypoxia inducible factor 1 alpha (HIF-1a) induces the production of multiple mediators in hypoxia. Therefore, inhibitors against VEGF have become an important issue. (Ramakrishnan, 2014 #145).

Placental growth factor (PlGF), a constituent of the vascular endothelial growth factor (VEGF) family, typically engages with Nrp1 and VEGFR1 on the surface of endothelial cells. This interaction promotes crosstalk among Nrp1, VEGFR1, and VEGFR2, thereby amplifying the cellular responses initiated by VEGF (Simons et al., 2016; Aoki et al., 2022). PIGF has been identified as a signal molecule that activates tumor cells, prompting the recruitment of stromal cells and subsequently enhancing angiogenesis and inflammatory responses. PlGF is closely associated with the tumor progression and metastasis (Fischer et al., 2007; Rolny et al., 2011; Heindryckx et al., 2013). As a potential marker for inhibiting the proliferation of cholangiocarcinoma cells, PlGF presents favorable conditions due to its high expression under pathological and hypoxic conditions, which allows for the selective inhibition of pathological angiogenesis (Green et al., 2001; Jain and Xu, 2007).

Zhu and colleagues reported Nrp1's critical role in restricting CCA tumor cell proliferation and migration, and further inhibiting the tumor progression and lung metastasis *in vitro* and vivo experiments (Zhu et al., 2018). A novel study explored the potential mechanisms of PIGF overcoming chemotherapy insensitivity (Aoki et al., 2022). This study demonstrates the potential of Placental Growth Factor (PlGF) blockade in ameliorating the hypoxic conditions within solid tumors, improving blood perfusion, and enhancing the sensitivity to standardized chemotherapy regimens.

Moreover, inhibitors developed for rare mutation-driven targets with low occurrence in the population have also shown objective improvements. Neurotrophic tyrosine receptor kinase (*NTRK*) fusion inhibitors entrectinib and Larotrectinib are approved for advanced-stage solid tumor patients in 2019 (US Food and Drug Administration, 2018; US Food and Drug Administration, 2019). In 2022, the combination of dabrafenib and trametinib (targeting BRAFV600E mutation) is approved for unresectable or metastatic CCA (US Food and Drug Administration, 2022). Molecular therapies targeting various mutation points have been extensively explored through clinical trials and evaluations of therapeutic effects. The next step is to review the current state of Immune Checkpoint Inhibitor (O'Rourke et al.) treatments in recent years, summarize the significant work on studying ICI resistance mechanisms from a single-cell perspective, enhance our understanding of how to overcome resistance, and identify potential paths to improve the response rate.

ICI rechallenge and strategies: clues from the single cell perspective

ICIs current status and efficacy

Immune checkpoint inhibitors (ICIs) primarily refer to agents such as cytotoxic T-Lymphocyte antigen-4 (CTLA-4) and programmed death-1/programmed death-ligand-1 (PD-1/PDL-1), which are antibodies that block immune checkpoint proteins (Hadfield et al., 2023). Immune checkpoint inhibitors therapy represents a novel therapeutic approach, particularly for malignant tumors. Patients with high microsatellite instability (MSI-H) or deficient DNA mismatch repair (dMMR) are especially targeted for this type of treatment. In 2017, the United States Food and Drug Administration (FDA) first approved the PD-1 inhibitor pembrolizumab for the treatment of these two types of tumors. (Wang et al., 2021). In gastrointestinal malignancy realm only, the incidence rates of MSI-H is ranging under 60% among all cases (Williams and Huang, 2013). In this phase II trial that included 22 patients with cholangiocarcinoma (CCA), the ORR for patients with MSI-H or dMMR was 40.9% (Marabelle et al., 2020). MSI-H are commonly found with incidence of chronic cholecystitis and pancreaticobiliary maljunction. Furthermore, pembrolizumab has received expanded approval for its therapeutic indications. In 2020, it was approved for the treatment of patients with high tumor mutational burden (TMB-H) solid tumors (US Food and Drug Administration, 2020). Dostarlimab is approved for the treatment of patients with dMMR who have recurrent or advanced-stage disease in adults. These patients receiving pembrolizumab treatment belong to the group with unresectable or metastatic solid tumors. In this context, pembrolizumab is used as a subsequent therapy to inhibit disease progression (US Food and Drug Administration, 2021).

Evaluation of the efficacy of ICIs in cholangiocarcinoma and other neighboring hepatobiliary carcinomas is ongoing. As previously mentioned, currently using chemotherapy combined with ICIs as a standard treatment strategy is a hot topic. A phase III study evaluated the combination of gemcitabine and cisplatin with the PD-1 inhibitor Durvalumab, which currently reports an ORR of 24.9%. In addition, in the phase II LEAP-005 study,

advanced hepatobiliary carcinoma patients are shown to gain clinical benefits from the second-line treatment of the combination of pembrolizumab and Lenvatinib (Perez-Fidalgo and Martinelli, 2023). In the treatment of advanced hepatobiliary patients, the PD-1 inhibitor nivolumab has also demonstrated objective remission on dMMR patients, and therefore nivolumab can be used as a first-line treatment for future patients with such features.

Diverse cells participate in tumor initiation and interact with the tumor microenvironment

The explosive prosperity of single-cell sequencing technologies has enabled us to observe the heterogeneity of the immune landscape and the metabolic microenvironment presented by cellular behaviors and pathological characteristics in cholangiocarcinoma (CCA) at a single-cell resolution. A series of influential single-cell RNA sequencing and spatial transcriptomics studies have become the key to our in-depth exploration of the resistance mechanisms to immune checkpoint inhibitors (ICIs). Next, we describe the insights into CCA on single cell perspective and give introduction to a batch of influential studies:

Ma and colleagues conducted single-cell transcriptomic sequencing on patients with hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), studying the behavior of different cellular populations within the tumor microenvironment from the perspective of reprogramming in hepatobiliary cancers (Ma et al., 2019). This study identified an axis where the expression of vascular endothelial growth factor A (VEGFA) in malignant cells correlated with higher degrees of hypoxia; in conjunction with other studies, it suggests that the regulation of VEGFA induces levels of hypoxia-inducible factor 1-alpha (HIF1A) mRNA and protein, activating downstream hypoxic signaling pathways (Wiener et al., 1996; Semenza, 2012). The work discovered high expression of T cell toxicology-related genes (GZMA, GZMB, GZMH, and PRF1) and immune checkpoint genes PDCD1, IFNG, and NKG7 in $CD8^+$ and $CD4^+$ cells in the low diversity group (Div-low), indicating that tumors classified in this manner are potential effective targets for ICI therapy. Furthermore, the results imply that anti-angiogenic drug treatments may enhance the efficacy of immunotherapy (Khan and Kerbel, 2018); Another sequencing work targeting vascular cancer-associated fibroblasts (vCAFs) subgrouping reported tumor-infiltrating CD4 regulatory T cells performed high correlation with immune suppression (Zhang et al., 2020); Additionally, we are pleased to see direct comparisons of treatment failures and successes. This work has illustrated changes and distinctions in the tumor evolutionary trajectories post-treatment, providing new evidence for identifying possible causes of therapeutic failure (Ma et al., 2020). Tumor cell clonality is related to the polarization of CD4 T cells and CD8 T cells: memory and cytotoxic CD8 T cells are enriched in low clonality groups, while proliferative pre-exhausted and conventional pre-exhausted T cells are enriched in high clonality groups. CD8 T cells were enriched in downstream pathways associated with immune response. Through these studies we gained basic comprehension of common T cells' functional role under single-cell vision, but it's also important to notice other cell types' function in CCA (Golino et al., 2023).

Accumulated evidence has revealed that not only cholangiocytes and hepatocytes but also other cells such as fibroblasts, endothelial, and tumor-associated macrophages (TAMs) participate in the pathological process and tumor initiation of cholangiocarcinoma in various ways (Sato et al., 2021). While the previous research on tumor initiation and development focused on the immune suppression and the unbalance of the tumor microenvironment, recent studies focus more on reporting how these cells in the iCCA microenvironment interact with the tumor structure and make alterations to the surrounding microenvironment (Zhang et al., 2020; Affo et al., 2021). These cells play a critical role in modulating the balance and pressure in the microenvironment.

In general, CCA tumor structure is a patchwork of fibrotic stroma, inflamed, gliotic tissue. A few types of cells enrich the microenvironment: cancer-associated fibroblasts (CAFs), T cells, B cells, endothelial, lymphatic cells, TAMs, Tregs, and NK cells (Fabris et al., 2019; Fabris et al., 2021) These cells promote CCA invasion and progress via inhibiting immune responses, inducting angiogenesis, or activating migration with certain signaling pathways. For instance, we will introduce a typical pair, CCA cells-CAFs, and the crosstalk in between. CCA cells release platelet-derived growth factor D (PDGF-D) causing CAFs to recruit in the close area of the tumor tissue. CAFs prompt further fibrosis of healthy cells and secrete vascular endothelial growth factors (VEGF) to induct lymphangiogenesis and angiogenesis in CCA (Cadamuro et al., 2019). CAFs' pro-angiogenic effect under special circumstances enables CCA invasive and self-maintaining. Alternatively stated, the power that CCA fuels the maintenance and tumor development is from the tumor microenvironment itself after the formation.

Like the functions of CAF, TAMs play an analogous role in regulating CCA progression. In the tumor microenvironment, TAMs exist as the most enriched immune cells, and they play a pivotal role in modulating the tumor progression through participation in the crosstalk between malignant cells and the tumor microenvironment (Franklin and Li, 2016; Cortés et al., 2017). TAMs promote invasion in several aspects. First, TAMs of activated phase secrete cytokines that promote biliary epithelial proliferation and fibrosis (Sato et al., 2018). Next, TAMs secrete VEGF and other factors that induce angiogenesis (Roy et al., 2019). Besides, TAMs motivate CCA cells proliferation via Wnt/β-catenin signaling pathway (Loilome et al., 2014; Boulter et al., 2015). Last, TAMs inhibit T cells' regular functions of clearing malignant cells and are associated with tumor progression, leading to a poor prognosis for CCA (Doedens et al., 2010).

A latest work was published in Januray 2024. Gao and colleagues conducted single cell transcriptomic sequencing on pre- and post-therapy iCCA patients of combination of gemcitabine with oxaliplatin and lenvatinib and anti-PD1 antibody (Lu et al., 2024). This work performed comparison between poor response group and efficient response group. The proliferation of CD8 and the transition of CD8 GZMB + to CD8 GZMK + improves response when going into the therapy, while Macro CD5L + could reduce the response by increasing CLTA-4 in CD8 GZMB+. This study underscores the impact of $CD8^+$ T cell status transition and

Macro CD5L + induced exhaustion in affecting response in combination treatment.

Tregs typically possess potent immunosuppressive properties and are frequently found in tumor-adjacent regions. These cells can secrete inflammatory cytokines and mediate immunosuppression by metabolizing ATP in the microenvironment (Ohta et al., 2006; Sawant et al., 2019; Guo et al., 2021; Schneider et al., 2021; Moreau et al., 2022). There is evidence that Tregs in CCA express CTLA-4 associated protein genes, which may contribute to their immunosuppressive properties, as CTLA-4 can inhibit the activation of $CD8^+$ T cells by binding with CD80 expressed on antigen-presenting cells (Ma et al., 2019).

Several single-cell RNA (scRNA) sequencings have been performed and reported recently and these scRNA sequencings have revealed the CCA heterogeneity from single cell level. Since single-cell sequencing provides resolution at the individual cell level (Song et al., 2022), it has become an ideal method of analyzing heterogeneity than bulk sequencing. Kwong's lab published their scRNA dataset (Carapeto et al., 2022). Their work involved spatial sequencing technique and therefore depicts the correlation between the immune profiling and genomic mutations. In addition, the roles played by different cell populations in CCA have also been investigated. A study conducted by Beijing University of Technology performed scRNA sequencing on 56,871 cells for 8 cases. This research illustrated the heterogeneity of fibroblasts through the transcriptomic profiles and intercellular interactions and identified fibroblast subgroups according to scRNA clustering analyses (Zhang et al., 2020). Zhang's lab revealed distinct fibroblast subgroups first on the single cell level, and they brought inspirations to future research on this topic.

Therapy resistance implications: spatial transcriptome

The integration of spatial transcriptomics technology has provided valuable spatial insights into the mechanisms of cholangiocarcinoma treatment resistance. A recent study published in Gut focused on patients with intrahepatic cholangiocarcinoma (iCCA) who had undergone chemotherapy, characterizing the transcriptomic landscapes that differentiate rapid progression (RP) from long survival (LS) groups (O'Rourke et al., 2024). The research team conducted diagnostic biopsies and combined these with whole transcriptome sequencing of macrodissected tissue regions from different geographic areas of the tumor for analysis. Tumor tissues were categorized into the tumor core, tumor stroma, invasive fronts, and non-tumor areas, allowing for the explicit capture of spatial expression differences. This approach identified two potential mechanisms undermining chemotherapy efficacy: enhanced immunogenic cell death and metabolic deactivation. The study also highlighted the role of bone marrow cell and T cell communication in forming an immunosuppressive environment within the RP group. The identification of an RPLS signature through spatial transcriptomics was validated across multiple cell lines, single-cell RNA sequencing data, animal models, and transcriptomic datasets, demonstrating that tumor-induced immunotolerance is a decisive factor in determining long-term survival post-chemotherapy.

Additionally, Lin and colleagues reported on the multi-omics analysis of different geographic regions of the tumor, presenting a dynamic classification of iCCA based on diverse levels of immune infiltration and immune escape (Lin et al., 2022). Each patient's four to six primary tumor regions underwent comprehensive analysis through whole exome sequencing (Schneider et al.), RNA sequencing (RNA-seq), T-cell receptor sequencing (TCR-seq), and multiplex immunofluorescence assays. This analysis classified patients into sparse, mixed, and highly immune-infiltrated groups. The study found that highly infiltrated tumors exhibited high levels of immune activation and similar TCR repertoires across regions. However, T cell exhaustion and defects in antigen presentation could offset these factors. It was also noted that FGFR2 fusion was associated with a low tumor mutational burden (TMB) and low levels of immune infiltration. The significance of this work lies in its spatial dissection of iCCA patients' immune heterogeneity, shedding light on its impact on the formation of immune escape mechanisms.

These studies have provided an accurate understanding of cholangiocarcinoma heterogeneity and the diverse roles of cell types, offering background knowledge of the driving forces behind the origin and continued progression of CCA. This may, in turn, assist in further elucidating the potential pathways through which CCA acquires drug resistance.

Conclusion

We introduced the current status of CCA molecular target and ICI treatment and recent discovery of therapy resistance. With the identification of mutation sites and an improved understanding of the cell of origin and the pathway of tumor growth and development, the prospect of gaining better treatment effects for CCA is becoming more realistic and optimistic than ever. The combined use of targeted therapy and traditional treatment methods such chemotherapy provides great prospects for improving the therapeutic effectiveness of CCA. We also comprehensively elucidated the landscape of CCA pathology and the advances in treatment options from a perspective of single cell and spatial transcriptome techniques.

A noteworthy trend in recent years is the efficacy of combination therapy strategies in treating CCA, likely due to the limitations of targeted therapies alone. Most targeted therapies fail to extend progression-free survival (PFS) beyond 6 months, constrained by various resistance mechanisms. Future clinical trials should prioritize evaluating combination strategies that mitigate and overcome these resistance mechanisms. This approach could pave the way for significant advancements in the combined use of targeted drugs and ICI therapy. In addition, the treatment strategy of using specific target inhibitors needs to be further explored and optimized. For example, several clinical trials are evaluating the therapeutic effects of FGFR inhibitors on patients with FGFR2 fusion or rearrangement. Adopting inclusion criteria for people with specific genetic mutations would be of great value in achieving more instructive clinical results. We believe the expansion of novel studies from these perspectives may soon lift the veil on treatment resistance and increase the response rate.

Author contributions

JW: Writing–original draft, Writing–review and editing. SL: Validation, Visualization, Writing–review and editing. YC: Validation, Visualization, Writing–review and editing. YC: Conceptualization, Project administration, Supervision, Validation, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

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

Publisher's note

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

References

Abou-Alfa, G. K., Macarulla, T., Javle, M. M., Kelley, R. K., Lubner, S. J., Adeva, J., et al. (2020a). Ivosidenib in IDH1-mutant, chemotherapy-refractory cholangiocarcinoma (ClarIDHy): a multicentre, randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Oncol.* 21, 796–807. doi:10.1016/S1470-2045(20)30157-1

Abou-Alfa, G. K., Sahai, V., Hollebecque, A., Vaccaro, G., Melisi, D., Al-Rajabi, R., et al. (2020b). Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study. *Lancet Oncol.* 21, 671–684. doi:10.1016/S1470-2045(20)30109-1

Affo, S., Nair, A., Brundu, F., Ravichandra, A., Bhattacharjee, S., Matsuda, M., et al. (2021). Promotion of cholangiocarcinoma growth by diverse cancer-associated fibroblast subpopulations. *Cancer Cell* 39, 883–882. doi:10.1016/j.ccell.2021.05.010

Aoki, S., Inoue, K., Klein, S., Halvorsen, S., Chen, J., Matsui, A., et al. (2022). Placental growth factor promotes tumour desmoplasia and treatment resistance in intrahepatic cholangiocarcinoma. *Gut* 71, 185–193. doi:10.1136/gutjnl-2020-322493

Atkins, M. B., Lee, S. J., Chmielowski, B., Tarhini, A. A., Cohen, G. I., Truong, T. G., et al. (2023). Combination dabrafenib and trametinib versus combination nivolumab and ipilimumab for patients with advanced BRAF-mutant melanoma: the DREAMseq trial-ECOG-ACRIN EA6134. *J. Clin. Oncol.* 41, 186–197. doi:10.1200/JCO.22.01763

Balogh, J., Victor III, D., Asham, E. H., Burroughs, S. G., Boktour, M., Saharia, A., et al. (2016). Hepatocellular carcinoma: a review. *J. Hepatocell. carcinoma* 3, 41–53. doi:10.2147/JHC.S61146

Banales, J. M., Cardinale, V., Macias, R., Andersen, J. B., Braconi, C., Carpino, G., et al. (2019). Cholangiocarcinoma: state-of-the-art knowledge and challenges. *Liver Int.* 39, 5–6. doi:10.1111/liv.14101

Bekaii-Saab, T. S., Valle, J. W., Van Cutsem, E., Rimassa, L., Furuse, J., Ioka, T., et al. (2020). *FIGHT-302: phase III study of first-line (1L) pemigatinib (PEM) versus gemcitabine (GEM) plus cisplatin (CIS) for cholangiocarcinoma (CCA) with FGFR2 fusions or rearrangements*. American Society of Clinical Oncology.

Bertuccio, P., Malvezzi, M., Carioli, G., Hashim, D., Boffetta, P., El-Serag, H. B., et al. (2019). Global trends in mortality from intrahepatic and extrahepatic cholangiocarcinoma. *J. hepatology* 71, 104–114. doi:10.1016/j.jhep.2019.03.013

Boscoe, A. N., Rolland, C., and Kelley, R. K. (2019). Frequency and prognostic significance of isocitrate dehydrogenase 1 mutations in cholangiocarcinoma: a systematic literature review. *J. Gastrointest. Oncol.* 10, 751–765. doi:10.21037/jgo.2019.03.10

Boulter, L., Guest, R. V., Kendall, T. J., Wilson, D. H., Wojtacha, D., Robson, A. J., et al. (2015). WNT signaling drives cholangiocarcinoma growth and can be pharmacologically inhibited. *J. Clin. investigation* 125, 1269–1285. doi:10.1172/JCI76452

Bridgewater, J., Galle, P. R., Khan, S. A., Llovet, J. M., Park, J.-W., Patel, T., et al. (2014). Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma. *J. hepatology* 60, 1268–1289. doi:10.1016/j.jhep.2014.01.021

Brindley, P. J., Bachini, M., Ilyas, S. I., Khan, S. A., Loukas, A., Sirica, A. E., et al. (2021). Cholangiocarcinoma. *Nat. Rev. Dis. Prim.* 7, 65. doi:10.1038/s41572-021-00300-2

Byron, S. A., Chen, H., Wortmann, A., Loch, D., Gartside, M. G., Dehkhoda, F., et al. (2013). The N550K/H mutations in FGFR2 confer differential resistance to PD173074, dovitinib, and ponatinib ATP-competitive inhibitors. *Neoplasia* 15, 975–988. doi:10.1593/neo.121106

Cadamuro, M., Brivio, S., Mertens, J., Vismara, M., Moncsek, A., Milani, C., et al. (2019). Platelet-derived growth factor-D enables liver myofibroblasts to promote tumor lymphangiogenesis in cholangiocarcinoma. *J. Hepatol.* 70, 700–709. doi:10.1016/j.jhep.2018.12.004

Carapeto, F., Bozorgui, B., Shroff, R. T., Chagani, S., Solis Soto, L., Foo, W. C., et al. (2022). The immunogenomic landscape of resected intrahepatic cholangiocarcinoma. *Hepatology* 75, 297–308. doi:10.1002/hep.32150

Chen, X., and Calvisi, D. F. (2014). Hydrodynamic transfection for generation of novel mouse models for liver cancer research. *Am. J. Pathol.* 184, 912–923. doi:10.1016/j.ajpath.2013.12.002

Cleary, J. M., Iyer, G., Oh, D.-Y., Mellinghoff, I. K., Goyal, L., Ng, M. C., et al. (2020). *Final results from the phase I study expansion cohort of the selective FGFR inhibitor Debio 1,347 in patients with solid tumors harboring an FGFR gene fusion*. American Society of Clinical Oncology.

Cleary, J. M., Raghavan, S., Wu, Q., Li, Y. Y., Spurr, L. F., Gupta, H. V., et al. (2021). FGFR2 extracellular domain in-frame deletions are therapeutically targetable genomic alterations that function as oncogenic drivers in cholangiocarcinoma. *Cancer Discov.* 11, 2488–2505. doi:10.1158/2159-8290.CD-20-1669

Cortés, M., Sanchez-Moral, L., De Barrios, O., Fernández-Aceñero, M. J., Martínez-Campanario, M. C., Esteve-Codina, A., et al. (2017). Tumor-associated macrophages (TAMs) depend on ZEB1 for their cancer-promoting roles. *Embo J.* 36, 3336–3355. doi:10.15252/embj.201797345

Doedens, A. L., Stockmann, C., Rubinstein, M. P., Liao, D., Zhang, N., Denardo, D. G., et al. (2010). Macrophage expression of hypoxia-inducible factor-1 alpha suppresses T-cell function and promotes tumor progression. *Cancer Res.* 70, 7465–7475. doi:10.1158/0008-5472.CAN-10-1439

Fabris, L., Perugorria, M. J., Mertens, J., Björkström, N. K., Cramer, T., Lleo, A., et al. (2019). The tumour microenvironment and immune milieu of cholangiocarcinoma. *Liver Int.* 39, 63–78. doi:10.1111/liv.14098

Fabris, L., Sato, K., Alpini, G., and Strazzabosco, M. (2021). The tumor microenvironment in cholangiocarcinoma progression. *Hepatology* 73, 75–85. doi:10.1002/hep.31410

Farshidfar, F., Zheng, S., Gingras, M. C., Newton, Y., Shih, J., Robertson, A. G., et al. (2017). Integrative genomic analysis of cholangiocarcinoma identifies distinct IDH-mutant molecular profiles. *Cell Rep.* 18, 2780–2794. doi:10.1016/j.celrep.2017.02.033

Fischer, C., Jonckx, B., Mazzone, M., Zacchigna, S., Loges, S., Pattarini, L., et al. (2007). Anti-PlGF inhibits growth of VEGF (R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell* 131, 463–475. doi:10.1016/j.cell.2007.08.038

Franklin, R. A., and Li, M. O. (2016). Ontogeny of tumor-associated macrophages and its implication in cancer regulation. *Trends cancer* 2, 20–34. doi:10.1016/j.trecan.2015.11.004

Gao, Q., Zhao, Y. J., Wang, X. Y., Guo, W. J., Gao, S., Wei, L., et al. (2014). Activating mutations in PTPN3 promote cholangiocarcinoma cell proliferation and migration and are associated with tumor recurrence in patients. *Gastroenterology* 146, 1397–1407. doi:10.1053/j.gastro.2014.01.062

Ghedini, G. C., Ronca, R., Presta, M., and Giacomini, A. (2018). Future applications of FGF/FGFR inhibitors in cancer. *Expert Rev. anticancer Ther.* 18, 861–872. doi:10.1080/14737140.2018.1491795

Gingold, J. A., Zhu, D., Lee, D.-F., Kaseb, A., and Chen, J. (2018). Genomic profiling and metabolic homeostasis in primary liver cancers. *Trends Mol. Med.* 24, 395–411. doi:10.1016/j.molmed.2018.02.006

Golino, J. L., Wang, X., Maeng, H. M., and Xie, C. (2023). Revealing the heterogeneity of the tumor ecosystem of cholangiocarcinoma through single-cell transcriptomics. *Cells* 12, 862. doi:10.3390/cells12060862

Goyal, L., Kongpetch, S., Crolley, V. E., and Bridgewater, J. (2021a). Targeting FGFR inhibition in cholangiocarcinoma. *Cancer Treat. Rev.* 95, 102170. doi:10.1016/j.ctrv.2021.102170

Goyal, L., Meric-Bernstam, F., Hollebecque, A., Morizane, C., Valle, J. W., Karasic, T. B., et al. (2021b). Abstract CT010: primary results of phase 2 FOENIX-CCA2: the irreversible FGFR1-4 inhibitor futibatinib in intrahepatic cholangiocarcinoma (iCCA) with FGFR2 fusions/rearrangements. *Cancer Res.* 81, CT010. doi:10.1158/1538-7445.am2021-ct010

Goyal, L., Meric-Bernstam, F., Hollebecque, A., Valle, J. W., Morizane, C., Karasic, T. B., et al. (2020). *FOENIX-CCA2: a phase II, open-label, multicenter study of futibatinib in patients (pts) with intrahepatic cholangiocarcinoma (iCCA) harboring FGFR2 gene fusions or other rearrangements*. American Society of Clinical Oncology.

Goyal, L., Meric-Bernstam, F., Hollebecque, A., Valle, J. W., Morizane, C., Karasic, T. B., et al. (2023). Futibatinib for FGFR2-rearranged intrahepatic cholangiocarcinoma. *N. Engl. J. Med.* 388, 228–239. doi:10.1056/NEJMoa2206834

Goyal, L., Saha, S. K., Liu, L. Y., Siravegna, G., Leshchiner, I., Ahronian, L. G., et al. (2017). Polyclonal secondary FGFR2 mutations drive acquired resistance to FGFR inhibition in patients with FGFR2 fusion–positive cholangiocarcinoma. *Cancer Discov.* 7, 252–263. doi:10.1158/2159-8290.CD-16-1000

Goyal, L., Shi, L., Liu, L. Y., Fece De La Cruz, F., Lennerz, J. K., Raghavan, S., et al. (2019). TAS-120 overcomes resistance to ATP-competitive FGFR inhibitors in patients with FGFR2 fusion–positive intrahepatic cholangiocarcinoma. *Cancer Discov.* 9, 1064–1079. doi:10.1158/2159-8290.CD-19-0182

Green, C. J., Lichtlen, P., Huynh, N. T., Yanovsky, M., Laderoute, K. R., Schaffner, W., et al. (2001). Placenta growth factor gene expression is induced by hypoxia in fibroblasts: a central role for metal transcription factor-1. *Cancer Res.* 61, 2696–2703.

Guo, Y., Xie, Y.-Q., Gao, M., Zhao, Y., Franco, F., Wenes, M., et al. (2021). Metabolic reprogramming of terminally exhausted CD8+ T cells by IL-10 enhances anti-tumor immunity. *Nat. Immunol.* 22, 746–756. doi:10.1038/s41590-021-00940-2

Hadfield, M. J., Decarli, K., Bash, K., Sun, G., and Almhanna, K. (2023). Current and emerging therapeutic targets for the treatment of cholangiocarcinoma: an updated review. *Int. J. Mol. Sci.* 25, 543. doi:10.3390/ijms25010543

Harding, J. J., Khalil, D. N., Fabris, L., and Abou-Alfa, G. K. (2023). Rational development of combination therapies for biliary tract cancers. *J. Hepatology* 78, 217–228. doi:10.1016/j.jhep.2022.09.004

Heindryckx, F., Coulon, S., Terrie, E., Casteleyn, C., Stassen, J.-M., Geerts, A., et al. (2013). The placental growth factor as a target against hepatocellular carcinoma in a diethylnitrosamine-induced mouse model. *J. hepatology* 58, 319–328. doi:10.1016/j.jhep.2012.09.032

Jain, R. K., and Xu, L. (2007). alphaPlGF: a new kid on the antiangiogenesis block. *Cell* 131, 443–445. doi:10.1016/j.cell.2007.10.023

Javle, M. M., Roychowdhury, S., Kelley, R. K., Sadeghi, S., Macarulla, T., Waldschmidt, D. T., et al. (2021b). *Final results from a phase II study of infigratinib (BGJ398), an FGFR-selective tyrosine kinase inhibitor, in patients with previously treated advanced cholangiocarcinoma harboring an FGFR2 gene fusion or rearrangement*. American Society of Clinical Oncology.

Javle, M., Lowery, M., Shroff, R. T., Weiss, K. H., Springfeld, C., Borad, M. J., et al. (2018). Phase II study of BGJ398 in patients with FGFR-altered advanced cholangiocarcinoma. *J. Clin. Oncol.* 36, 276–282. doi:10.1200/JCO.2017.75.5009

Javle, M., Roychowdhury, S., Kelley, R. K., Sadeghi, S., Macarulla, T., Weiss, K. H., et al. (2021a). Infigratinib (BGJ398) in previously treated patients with advanced or metastatic cholangiocarcinoma with FGFR2 fusions or rearrangements: mature results from a multicentre, open-label, single-arm, phase 2 study. *Lancet Gastroenterology Hepatology* 6, 803–815. doi:10.1016/S2468-1253(21)00196-5

Kelley, R. K., Bridgewater, J., Gores, G. J., and Zhu, A. X. (2020). Systemic therapies for intrahepatic cholangiocarcinoma. *J. Hepatol.* 72, 353–363. doi:10.1016/j.jhep.2019.10.009

Khan, K. A., and Kerbel, R. S. (2018). Improving immunotherapy outcomes with anti-angiogenic treatments and vice versa. *Nat. Rev. Clin. Oncol.* 15, 310–324. doi:10.1038/nrclinonc.2018.9

Krook, M. A., Bonneville, R., Chen, H.-Z., Reeser, J. W., Wing, M. R., Martin, D. M., et al. (2019). Tumor heterogeneity and acquired drug resistance in FGFR2-fusion-positive cholangiocarcinoma through rapid research autopsy. *Mol. Case Stud.* 5, a004002. doi:10.1101/mcs.a004002

Lamarca, A., Barriuso, J., Mcnamara, M. G., and Valle, J. W. (2020a). Molecular targeted therapies: ready for "prime time" in biliary tract cancer. *J. hepatology* 73, 170–185. doi:10.1016/j.jhep.2020.03.007

Lamarca, A., Edeline, J., and Goyal, L. (2022). How I treat biliary tract cancer. *ESMO open* 7, 100378. doi:10.1016/j.esmoop.2021.100378

Lamarca, A., Edeline, J., Mcnamara, M. G., Hubner, R. A., Nagino, M., Bridgewater, J., et al. (2020b). Current standards and future perspectives in adjuvant treatment for biliary tract cancers. *Cancer Treat. Rev.* 84, 101936. doi:10.1016/j.ctrv.2019.101936

Lin, Y., Peng, L., Dong, L., Liu, D., Ma, J., Lin, J., et al. (2022). Geospatial immune heterogeneity reflects the diverse tumor-immune interactions in intrahepatic cholangiocarcinoma. *Cancer Discov.* 12, 2350–2371. doi:10.1158/2159-8290.CD-21-1640

Liu, P. C., Koblish, H., Wu, L., Bowman, K., Diamond, S., Dimatteo, D., et al. (2020). INCB054828 (pemigatinib), a potent and selective inhibitor of fibroblast growth factor receptors 1, 2, and 3, displays activity against genetically defined tumor models. *PLoS One* 15, e0231877. doi:10.1371/journal.pone.0231877

Loilome, W., Bungkanjana, P., Techasen, A., Namwat, N., Yongvanit, P., Puapairoj, A., et al. (2014). Activated macrophages promote Wnt/β-catenin signaling in cholangiocarcinoma cells. *Tumor Biol.* 35, 5357–5367. doi:10.1007/s13277-014-1698-2

Lu, J. C., Wu, L. L., Sun, Y. N., Huang, X. Y., Gao, C., Guo, X. J., et al. (2024). Macro CD5L(+) deteriorates CD8(+)T cells exhaustion and impairs combination of Gemcitabine-Oxaliplatin-Lenvatinib-anti-PD1 therapy in intrahepatic cholangiocarcinoma. *Nat. Commun.* 15, 621. doi:10.1038/s41467-024-44795-1

Ma, L., Hernandez, M. O., Zhao, Y., Mehta, M., Tran, B., Kelly, M., et al. (2019). Tumor cell biodiversity drives microenvironmental reprogramming in liver cancer. *Cancer Cell* 36, 418–430. doi:10.1016/j.ccell.2019.08.007

Ma, L., Wang, L., Chang, C.-W., Heinrich, S., Dominguez, D., Forgues, M., et al. (2020). Single-cell atlas of tumor clonal evolution in liver cancer. *bioRxiv*. doi:10.1016/j.jhep.2021.06.028

Marabelle, A., Le, D. T., Ascierto, P. A., Di Giacomo, A. M., De Jesus-Acosta, A., Delord, J.-P., et al. (2020). Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair–deficient cancer: results from the phase II KEYNOTE-158 study. *J. Clin. Oncol.* 38, 1–10. doi:10.1200/JCO.19.02105

Moehler, M., Maderer, A., Schimanski, C., Kanzler, S., Denzer, U., Kolligs, F., et al. (2014). Gemcitabine plus sorafenib versus gemcitabine alone in advanced biliary tract cancer: a double-blind placebo-controlled multicentre phase II AIO study with biomarker and serum programme. *Eur. J. Cancer* 50, 3125–3135. doi:10.1016/j.ejca.2014.09.013

Moeini, A., Sia, D., Zhang, Z., Camprecios, G., Stueck, A., Dong, H., et al. (2017). Mixed hepatocellular cholangiocarcinoma tumors: cholangiolocellular carcinoma is a distinct molecular entity. *J. Hepatol.* 66, 952–961. doi:10.1016/j.jhep.2017.01.010

Montal, R., Sia, D., Montironi, C., Leow, W. Q., Esteban-Fabró, R., Pinyol, R., et al. (2020). Molecular classification and therapeutic targets in extrahepatic cholangiocarcinoma. *J. Hepatol.* 73, 315–327. doi:10.1016/j.jhep.2020.03.008

Moreau, J. M., Velegraki, M., Bolyard, C., Rosenblum, M. D., and Li, Z. (2022). Transforming growth factor–β1 in regulatory T cell biology. *Sci. Immunol.* 7, eabi4613. doi:10.1126/sciimmunol.abi4613

Norsworthy, K. J., Luo, L., Hsu, V., Gudi, R., Dorff, S. E., Przepiorka, D., et al. (2019). FDA approval summary: ivosidenib for relapsed or refractory acute myeloid leukemia with an isocitrate dehydrogenase-1 mutation. *Clin. Cancer Res.* 25, 3205–3209. doi:10.1158/1078-0432.CCR-18-3749

Oh, D.-Y., He, A. R., Qin, S., Chen, L.-T., Okusaka, T., Vogel, A., et al. (2022). *A phase 3 randomized, double-blind, placebo-controlled study of durvalumab in combination with gemcitabine plus cisplatin (GemCis) in patients (pts) with advanced biliary tract cancer (BTC): topaz-1*. American Society of Clinical Oncology.

Ohta, A., Gorelik, E., Prasad, S. J., Ronchese, F., Lukashev, D., Wong, M. K., et al. (2006). A2A adenosine receptor protects tumors from antitumor T cells. *Proc. Natl. Acad. Sci.* 103, 13132–13137. doi:10.1073/pnas.0605251103

O'Rourke, C. J., Salati, M., Rae, C., Carpino, G., Leslie, H., Pea, A., et al. (2024). Molecular portraits of patients with intrahepatic cholangiocarcinoma who diverge as rapid progressors or long survivors on chemotherapy. *Gut* 73, 496–508. doi:10.1136/gutjnl-2023-330748

Palmer, W. C., and Patel, T. (2012). Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. *J. hepatology* 57, 69–76. doi:10.1016/j.jhep.2012.02.022

Park, J. O., Feng, Y.-H., Chen, Y.-Y., Su, W.-C., Oh, D.-Y., Shen, L., et al. (2019). *Updated results of a phase IIa study to evaluate the clinical efficacy and safety of erdafitinib in Asian advanced cholangiocarcinoma (CCA) patients with FGFR alterations*. American Society of Clinical Oncology.

Perez-Fidalgo, J., and Martinelli, E. (2023). Lenvatinib plus pembrolizumab a new effective combination of targeted agents. *ESMO open* 8, 101157. doi:10.1016/j.esmoop.2023.101157

Primrose, J. N., Fox, R. P., Palmer, D. H., Malik, H. Z., Prasad, R., Mirza, D., et al. (2019). Capecitabine compared with observation in resected biliary tract cancer (BILCAP): a randomised, controlled, multicentre, phase 3 study. *Lancet Oncol.* 20, 663–673. doi:10.1016/S1470-2045(18)30915-X

Razumilava, N., and Gores, G. J. (2014). Cholangiocarcinoma. *Lancet* 383, 2168–2179. doi:10.1016/S0140-6736(13)61903-0

Rolny, C., Mazzone, M., Tugues, S., Laoui, D., Johansson, I., Coulon, C., et al. (2011). HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF. *Cancer Cell* 19, 31–44. doi:10.1016/j.ccr.2010.11.009

Roy, S., Glaser, S., and Chakraborty, S. (2019). Inflammation and progression of cholangiocarcinoma: role of angiogenic and lymphangiogenic mechanisms. *Front. Med.* 6, 293. doi:10.3389/fmed.2019.00293

Sato, K., Meng, F., Giang, T., Glaser, S., and Alpini, G. (2018). Mechanisms of cholangiocyte responses to injury. *Biochimica Biophysica Acta (BBA)-Molecular Basis Dis.* 1864, 1262–1269. doi:10.1016/j.bbadis.2017.06.017

Sato, K., Zhang, W., Safarikia, S., Isidan, A., Chen, A. M., Li, P., et al. (2021). Organoids and spheroids as models for studying cholestatic liver injury and cholangiocarcinoma. *Hepatology* 74, 491–502. doi:10.1002/hep.31653

Sawant, D. V., Yano, H., Chikina, M., Zhang, Q., Liao, M., Liu, C., et al. (2019). Adaptive plasticity of IL-10+ and IL-35+ Treg cells cooperatively promotes tumor T cell exhaustion. *Nat. Immunol.* 20, 724–735. doi:10.1038/s41590-019-0346-9

Schneider, E., Winzer, R., Rissiek, A., Ricklefs, I., Meyer-Schwesinger, C., Ricklefs, F. L., et al. (2021). CD73-mediated adenosine production by CD8 T cell-derived extracellular vesicles constitutes an intrinsic mechanism of immune suppression. *Nat. Commun.* 12, 5911. doi:10.1038/s41467-021-26134-w

Semenza, G. L. (2012). Hypoxia-inducible factors in physiology and medicine. *Cell* 148, 399–408. doi:10.1016/j.cell.2012.01.021

Sia, D., Hoshida, Y., Villanueva, A., Roayaie, S., Ferrer, J., Tabak, B., et al. (2013). Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. *Gastroenterology* 144, 829–840. doi:10.1053/j.gastro.2013.01.001

Silverman, I. M., Hollebecque, A., Friboulet, L., Owens, S., Newton, R. C., Zhen, H., et al. (2021). Clinicogenomic analysis of FGFR2-rearranged cholangiocarcinoma identifies correlates of response and mechanisms of resistance to pemigatinib. *Cancer Discov.* 11, 326–339. doi:10.1158/2159-8290.CD-20-0766

Simons, M., Gordon, E., and Claesson-Welsh, L. (2016). Mechanisms and regulation of endothelial VEGF receptor signalling. *Nat. Rev. Mol. Cell Biol.* 17, 611–625. doi:10.1038/nrm.2016.87

Song, G., Shi, Y., Meng, L., Ma, J., Huang, S., Zhang, J., et al. (2022). Single-cell transcriptomic analysis suggests two molecularly subtypes of intrahepatic cholangiocarcinoma. *Nat. Commun.* 13, 1642. doi:10.1038/s41467-022-29164-0

Subbiah, V., Sahai, V., Maglic, D., Bruderek, K., Touré, B. B., Zhao, S., et al. (2023). RLY-4008, the first highly selective FGFR2 inhibitor with activity across FGFR2 alterations and resistance mutations. *Cancer Discov.* 13, 2012–2031. doi:10.1158/2159-8290.CD-23-0475

Turcan, S., Makarov, V., Taranda, J., Wang, Y., Fabius, A. W., Wu, W., et al. (2018). Mutant-IDH1-dependent chromatin state reprogramming, reversibility, and persistence. *Nat. Genet.* 50, 62–72. doi:10.1038/s41588-017-0001-z

Turner, N., and Grose, R. (2010). Fibroblast growth factor signalling: from development to cancer. *Nat. Rev. Cancer* 10, 116–129. doi:10.1038/nrc2780

US Food and Drug Administration (2018). *FDA approves an oncology drug that targets a key genetic driver of cancer, rather than a specific type of tumor*. Silver Spring, MD: Case Medical Research.

US Food and Drug Administration (2019). *FDA approves entrectinib for NTRK solid tumors and ROS-1 NSCLC*. Silver Spring, MD: FDA.

US Food and Drug Administration (2020). *FDA approves pembrolizumab for adults and children with TMB-H solid tumors*. Silver Spring, MD: US Food and Drug Administration.

US Food and Drug Administration (2021). *FDA grants accelerated approval to dostarlimab-gxly for dMMR advanced solid tumors*. Silver Spring, MD: US Food and Drug Administration.

US Food and Drug Administration (2022). *FDA grants accelerated approval to dabrafenib in combination with trametinib for unre-sectable or metastatic solid tumors with BRAF V600E mutation*. Silver Spring, MD, USA: US Food and Drug Administration.

Valle, J. W., Lamarca, A., Goyal, L., Barriuso, J., and Zhu, A. X. (2017). New horizons for precision medicine in biliary tract cancers. *Cancer Discov.* 7, 943–962. doi:10.1158/2159-8290.CD-17-0245

Varghese, A. M., Patel, J., Janjigian, Y. Y., Meng, F., Selcuklu, S. D., Iyer, G., et al. (2021). Noninvasive detection of polyclonal acquired resistance to FGFR inhibition in patients with cholangiocarcinoma harboring FGFR2 alterations. *JCO Precis. Oncol.* 5, 44–50. doi:10.1200/PO.20.00178

Wang, Z., Ren, Z., Li, R., Ge, J., Zhang, G., Xin, Y., et al. (2021). Multi-omics integrative bioinformatics analyses reveal long non-coding RNA modulates genomic integrity via competing endogenous RNA mechanism and serves as novel biomarkers for overall survival in lung adenocarcinoma. *Front. Cell Dev. Biol.* 9, 691540. doi:10.3389/fcell.2021.691540

Wiener, C. M., Booth, G., and Semenza, G. L. (1996). In vivoexpression of mRNAs encoding hypoxia-inducible factor 1. *Biochem. biophysical Res. Commun.* 225, 485–488. doi:10.1006/bbrc.1996.1199

Williams, A. S., and Huang, W.-Y. (2013). The analysis of microsatellite instability in extracolonic gastrointestinal malignancy. *Pathology* 45, 540–552. doi:10.1097/PAT.0b013e3283653307

Wu, M. J., Shi, L., Dubrot, J., Merritt, J., Vijay, V., Wei, T. Y., et al. (2022a). Mutant IDH inhibits ifnγ-TET2 signaling to promote immunoevasion and tumor maintenance in cholangiocarcinoma. *Cancer Discov.* 12, 812–835. doi:10.1158/2159-8290.CD-21-1077

Wu, Q., Zhen, Y., Shi, L., Vu, P., Greninger, P., Adil, R., et al. (2022b). EGFR inhibition potentiates FGFR inhibitor therapy and overcomes resistance in FGFR2 fusion-positive cholangiocarcinoma. *Cancer Discov.* 12, 1378–1395. doi:10.1158/2159-8290.CD-21-1168

Xie, Y., Su, N., Yang, J., Tan, Q., Huang, S., Jin, M., et al. (2020). FGF/FGFR signaling in health and disease. *Signal Transduct. Target. Ther.* 5, 181. doi:10.1038/s41392-020-00222-7

Xue, R., Chen, L., Zhang, C., Fujita, M., Li, R., Yan, S. M., et al. (2019). Genomic and transcriptomic profiling of combined hepatocellular and intrahepatic cholangiocarcinoma reveals distinct molecular subtypes. *Cancer Cell* 35, 932–947. doi:10.1016/j.ccell.2019.04.007

Zhang, M., Yang, H., Wan, L., Wang, Z., Wang, H., Ge, C., et al. (2020). Single-cell transcriptomic architecture and intercellular crosstalk of human intrahepatic cholangiocarcinoma. *J. hepatology* 73, 1118–1130. doi:10.1016/j.jhep.2020.05.039

Zhu, A. X., Macarulla, T., Javle, M. M., Kelley, R. K., Lubner, S. J., Adeva, J., et al. (2021). Final overall survival efficacy results of ivosidenib for patients with advanced cholangiocarcinoma with IDH1 mutation: the phase 3 randomized clinical ClarIDHy trial. *JAMA Oncol.* 7, 1669–1677. doi:10.1001/jamaoncol.2021.3836

Zhu, H., Jiang, X., Zhou, X., Dong, X., Xie, K., Yang, C., et al. (2018). Neuropilin-1 regulated by miR-320 contributes to the growth and metastasis of cholangiocarcinoma cells. *Liver Int.* 38, 125–135. doi:10.1111/liv.13495

Zhu, Y., and Kwong, L. N. (2020). Insights into the origin of intrahepatic cholangiocarcinoma from mouse models. *Hepatology* 72, 305–314. doi:10.1002/hep.31200

Zhu, Y., and Kwong, L. N. (2022). IDH1 inhibition reawakens the immune response against cholangiocarcinoma. *Cancer Discov.* 12, 604–605. doi:10.1158/2159-8290.CD-21-1643

frontiers | Frontiers in Cell and Developmental Biology

TYPE Original Research
PUBLISHED 05 August 2024
DOI 10.3389/fcell.2024.1391873

OPEN ACCESS

EDITED BY
Sambhavi Animesh,
Massachusetts General Hospital, United States

REVIEWED BY
Emanuele Giurisato,
University of Siena, Italy
Aditya Yashwant Sarode,
Columbia University, United States

*CORRESPONDENCE
Weiping Li,
question12397@163.com
Xinghe Liao,
fdzlxinghe@163.com

†These authors have contributed equally to this work

RECEIVED 26 February 2024
ACCEPTED 18 July 2024
PUBLISHED 05 August 2024

CITATION
Chen H, Du Y, Kong Z, Liao X and Li W (2024), PRNP is a pan-cancer prognostic and immunity-related to EMT in colorectal cancer. *Front. Cell Dev. Biol.* 12:1391873. doi: 10.3389/fcell.2024.1391873

PRNP is a pan-cancer prognostic and immunity-related to EMT in colorectal cancer

Haifeng Chen[1†], Yao Du[2†], Zhiyuan Kong[3], Xinghe Liao[4,5*] and Weiping Li[3*]

[1]Department of Gastroenterology, The First People's Hospital of Taicang City, Taicang Affiliated Hospital of Soochow University, Taicang, Jiangsu, China, [2]Department of Gastrointestinal Surgery, The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China, [3]Department of Gastrointestinal Surgery, The First People's Hospital of Taicang City, Taicang Affiliated Hospital of Soochow University, Taicang, Jiangsu, China, [4]Department of Integrated Therapy, Fudan University Shanghai Cancer Center, Shanghai, China, [5]Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China

Background: Prion protein gene (PRNP) is widely expressed in a variety of tissues. Although the roles of PRNP in several cancers have been investigated, no pan-cancer analysis has revealed its relationship with tumorigenesis and immunity.

Methods: Comprehensive analyses were conducted on The Cancer Genome Atlas (TCGA) Pan-Cancer dataset from the University of California Santa Cruz (UCSC) database to determine the expression of PRNP and its potential prognostic implications. Immune infiltration and enrichment analysis methods were used to ascertain correlations between PRNP expression levels, tumor immunity, and immunotherapy. Additionally, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) methods were employed to examine possible signaling pathways involving PRNP. *In vitro* experiments using CCK-8 assay, Wound healing assay, and Transwell assay to detect the effect of Cellular prion protein (PrPC) on proliferation, migration, and invasion in colorectal cancer (CRC) cells. The expression levels of epithelial-mesenchymal transition (EMT)-related proteins (N-cadherin, E-cadherin, Vimentin and Snail) were detected by western blot.

Results: Among most cancer types, PRNP is expressed at high levels, which is linked to the prognosis of patients. PRNP expression is strongly associated with immune infiltrating cells, immunosuppressive cell infiltration and immune checkpoint molecules. In addition to tumor mutation burden (TMB), substantial correlations are detected between PRNP expression and microsatellite instability (MSI) in several cancers. *In vitro* cell studies inferred that PrPC enhanced the proliferation, migration, invasion, and EMT of CRC cells.

Conclusion: PRNP serves as an immune-related prognostic marker that holds promise for predicting outcomes related to CRC immunotherapy while simultaneously promoting cell proliferation, migration, and invasion activities. Furthermore, it potentially plays a role in governing EMT regulation within CRC.

KEYWORDS

PRNP, pan-cancer, prognostic, immunity, EMT

1 Introduction

Cancer is a widespread cause of mortality and significant debilitation, exerting a negative impact on the quality of life globally (Bray et al., 2018). Presently, definitive cures for cancer remain unavailable. Despite considerable progress in treatment modalities, the overall survival (OS) rate for patients after 5 years has remained unsatisfactory despite the use of therapies such as targeted therapy, immunotherapy, and radiation therapy. In 2022, the United States reported new cancer cases of 1,918,030 and 609,360 deaths according to cancer statistics data (Siegel et al., 2022). Conventional strategies have yielded disappointing long-term outcomes despite continuous efforts. However, immune checkpoint blockade therapy has achieved remarkable success as an immunotherapeutic approach to treating cancer (Gao et al., 2020; Herbst et al., 2020). The efficacy of immunotherapy hinges largely upon the identification of specific tumor antigens (Li and Ding, 2020); unfortunately, trials matching immunotherapy-related biomarkers remain limited across most cancers (Khemlina et al., 2017). Fortunately, with the ongoing refinement and development of public databases, like TCGA, the discovery of novel targets for immunotherapy has become more convenient via pan-cancer examination of individual genes, assessing their association with the following aspects; immune infiltration patterns, clinical prognosis, and associated signaling pathways.

Hence, it is crucial to create innovative diagnostic as well as prognostic biomarkers tailored specifically for different types of cancers, given their complexity involving intricate interactions between tumors and the immunological system within the tumor microenvironment (TME). The TME encompasses various cellular components including a substantial proportion of infiltrating immune cells that have a role in initiating and advancing human cancers' progression (Bindea et al., 2013). The four prion-gene family members constitute PRNP (PrPC), PRND (Doppel), PRNT (PRT), and SPRN (Shadoo). The gene that is widely studied in this family is PRNP(PrPC) (Allais-Bonnet and Pailhoux, 2014). PRNP(PrPC) is predominantly distributed in the central nervous system (CNS), followed by the gastrointestinal tract (Tang et al., 2016; Go and Lee, 2020). Previous studies have confirmed a link between PRNP(PrPC) and the occurrence and development of gastric cancer, CRC, lung cancer, and breast cancer, with its expression being linked to drug resistance, proliferation, apoptosis, migration, and invasion of various malignant tumor cells (Gil et al., 2016; Castle and Gill, 2017; Luo et al., 2017; Atkinson et al., 2019; Lin et al., 2020). The theory is founded on the observation that PRNP(PrPC) can promote anti-apoptosis, invasion, proliferation, and metastasis of cancer cells via different signal transduction pathways and a series of cascading reactions (Li et al., 2010).

We used various publicly available databases to assess the expression of PRNP(PrPC) and the prognostic implications it poses on various cancer types. Next, we investigated potential correlations between PRNP(PrPC) expression levels and both immune infiltrations and the expression of immune checkpoint markers. Additionally, we specifically validated our findings in CRC to examine the associations between PRNP(PrPC) expression levels and the EMT process. The core aim of this research was to determine the potential of PRNP(PrPC) being utilized as a biomarker for predicting the prognosis and the treatment response to immune checkpoint inhibitors, while also elucidating its involvement in CRC's EMT process. These discoveries provide new insights aimed at improving response rates to immunotherapy.

2 Materials and methods

2.1 Bioinformatics data and resources

We obtained the TCGA Pan-Cancer dataset, which is a harmonized and standardized collection of data from various cancer types in UCSC (https://xenabrowser.net/). The data on the expression of the PRNP gene (ENSG00000171867) was extracted from this dataset for each sample. Subsequently, the samples were filtered based on their source, and we applied a log2 (x+1) transformation to normalize the expression values. A criterion where the expression level was 0 for the samples was utilized to exclude samples. Furthermore, we performed the same log2 (x+1) transformation and excluded cancer types that were affirmed to have <3 available samples.

2.2 Survival analysis

The R packages "survminer" and "survival" were utilized to perform Kaplan-Meier and Cox regression analyses in order to investigate the impact of PRNP on the prognosis of patients. The evaluation focused on OS. A univariate Cox proportional hazards regression model was employed to determine the hazard ratio (HR) for mortalities linked to the expression of PRNP. The adjusted HR for the expression of PRNP was estimated by the constructed multivariate Cox model. P-values < 0.05 denoted statistical significance.

2.3 Protein-protein interaction (PPI), GO, and KEGG analyses

The PPI network of PRNP was constructed by the STRING database (https://string-db.org/) and enrichment analysis, applying a 0.4 threshold of the minimum interaction score. For the analysis of molecules associated with PRNP, ClusterProfiler packages were used to perform GO and KEGG analyses.

2.4 Immune landscape analysis

The level of immune cell infiltration for each tumor was examined depending on gene expression using the "donvo_CIBERSOR" method from the R package IOBR. Infiltration scores of cancer-associated fibroblasts (CAFs), B cells, CD4_T cells, CD8_T cells, Macrophages, NK cells, Endothelial cells, and other cells were reassessed in each tumor using the "donvo_epic" method from the R package IOBR. Furthermore, the B cell infiltration score, T cell CD4 infiltration score, T cell CD8 infiltration score, Neutrophil infiltration score, Macrophage infiltration score, and DC (Dendritic Cell) infiltration score were re-

examined for each patient's tumor as per gene expression using the "Timer" approach that is provided by the R package IOBR. PRNP expression data along with 60 marker genes representing 24 Inhibitory and 36 Stimulatory immune checkpoint pathway genes were analyzed in every sample. The "TMB function" provided by the R package was used to compute the TMB of each tumor. An analysis was then performed to calculate individual tumors' TMB value by combining TMB and gene expression data from samples through the TMB function that is integrated into the R package. Moreover, a previous study availed MSI scores for each tumor (Bonneville et al., 2017).

2.5 Materials and reagents

The National Collection of Authenticated Cell Cultures (Shanghai, China) Supplied the Human CRC cell lines HCT116 and HT29. CCK-8 was purchased from GlpBio (United States), and GEM HCl was obtained from Sigma-Aldrich Chemical Co., Ltd. Materials vital in cell culture, including McCoy's 5A and fetal bovine serum (FBS) were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China) and Serana (Germany). Piperstreptomycin and the Transwell chamber were obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China) and Merck Millipore (Billerica, Massachusetts, United States), respectively. Cytosine arabinoside (Ara-C) was obtained from Ararat (Canton) Biotechnology Co., Ltd. (Guangzhou, China). PRNP overexpression lentivirus (LV-PRNP), blank control vector lentivirus (LV-control), shRNAs targeting the PRNP gene (shRNA-PRNP), and negative control (shRNA-control) were purchased from Shanghai Nuobai Biotechnology Co., Ltd (NM_000311.5). Supplementary Table S1 shows the sequences of shRNAs. The other reagents used met the analytical grade.

2.6 Cell culture

The human CRC cell lines HCT116 and HT29, which exhibit varying levels of PrPC expression, were acquired from the National Collection of Authenticated Cell Cultures and were cultured in McCoy's 5A medium supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) (P/S). The cells were maintained in the following conditions: a temperature of 37°C and a 5% CO2 humidified atmosphere.

2.7 Transfection of recombinant lentivirus with different expression levels of PrPC in tumor cells

HCT116 in conjunction with HT29 tumor cells was cultured after attaining the logarithmic growth phase. Cells were introduced to each well of a 6-well plate at a density of 5×10^4 cells/mL. The wells were then grouped into four: PrPC-, PrPC+, PrPC- control, and PrPC+ control. Following a 12-h culture period, the cells were infected with the lentiviral vector (PrPC- group add shRNA-PRNP lentiviral vector; PrPC+ group add LV-PRNP lentiviral vector; PrPC- control group add shRNA-control lentiviral vector; PrPC+ control group add LV-control lentiviral vector) when they reached approximately 30%–50% confluence. To ensure consistent infection rates, a multiplicity of infection ratios of 100, 10, 10, and 10 were respectively used for each group. Equal amounts of virus particles, empty vector constructs, and culture medium were added to their corresponding wells. After incubating for another 12 h, 0.5 ug/mL concentration of puromycin was added to each group of supernatant. And then after 12 h of cultivation, the supernatant was discarded and replaced with a complete medium. Four days post-transfection, cell counting was performed using bright field microscopy while the count of green fluorescent protein-positive cells was carried out under fluorescent microscopy. Transfection efficiency was calculated based on these counts. Protein detection analysis was conducted once the transfection efficiency exceeded >80% (Wang S. et al., 2012).

2.8 CCK-8 assay for cell proliferation

The human CRC cell lines HCT116 and HT29, with different expression levels of PrPC (5×10^5 cells per well) were seeded onto 96-well plates. They were seeded when they were in their logarithmic growth phase and incubated in McCoy's 5A containing 10% FBS and P/S at 37°C with 5% CO2 saturation. After being cultured in a medium for 6, 12, and 24 h, these CRC cell lines were incubated with 20 μL CCK8 solution for 4 h at 37°C, and the absorbance value at 490 nm was then recorded.

2.9 Wound healing assay

The human CRC cell lines HCT116 and HT29, which exhibited varying levels of PrPC expression, were cultured in six-well plates at a density of 5×10^5 cells per well. After completing 24 h of incubation at 37°C, the cells were subjected to mechanical injury by creating two acellular lanes measuring 1 mm in width using plastic pipette tips and Ara-C (0.2 ug/mL) was added to the medium. Subsequently, any detached cell residue was rinsed from the wells. Changes in the wound area over time were monitored utilizing an inverted microscope, and scratch width was taken at the 24-h mark by ImageJ software as per a previously established protocol (Miyazaki et al., 2015).

2.10 Cell migration and invasion assay

The experiments on cell migration assays were conducted following previously described methods (Jia et al., 2017). HCT116 and HT29 cells were cultured for 24 h in a medium that did not have serum. To investigate the migratory behavior of human CRC cell lines HCT116 and HT29 with varying levels of PrPC expression, Transwell cell culture chambers with an 8 mm pore size (BD Bioscience) were employed. In brief, 100 μL of serum-free medium with 80,000 cells and Ara-C (0.2 ug/mL) was introduced to the upper chamber, while 600 μL of culture

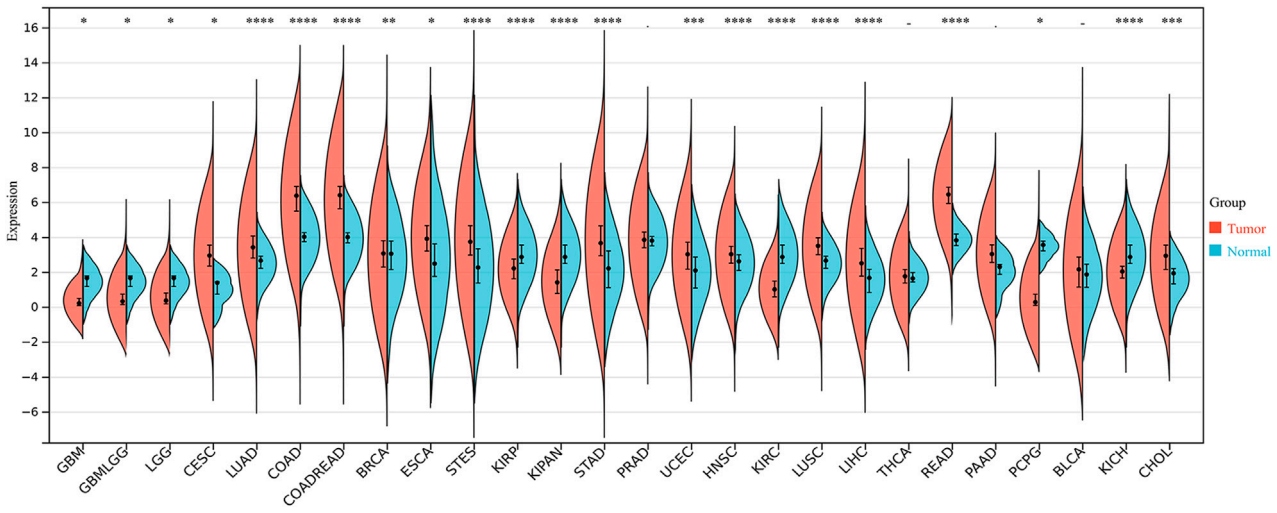


FIGURE 1
Expression levels of PRNP mRNA in pan-cancer: Comparison of PRNP expression between tumor and normal samples in 26 cancer types obtained from the TCGA database (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; -, not significant).

medium with 20% FBS and Ara-C (0.2 ug/mL) was introduced into the lower chamber. Following a 24-h incubation period, the upper chamber was taken off, and any nonmigrating cells from its surface were gently removed by wiping them off using a cotton swab. The cells that migrated were then fixed utilizing methanol for 20 min, stained with Giemsa for 1 h, and subsequently counted under a microscope. In the invasion experiment, the upper chamber, seeded with 5 $\times 10^4$ cells and 1% FBS-supplemented medium with Ara-C (0.2 ug/mL), was coated with Matrigel (BD Bioscience); meanwhile, the lower chamber had a 20% FBS-supplemented medium with Ara-C (0.2 ug/mL). After being incubated at 37°C with a CO2 concentration of 5% for 24 h, the Transwell chamber was retrieved, and its contents were discarded before being rinsed with calcium-free PBS solution. Afterward, the cells were treated using methanol and to ensure fixation the treatment was allowed to last for 30 min, followed by staining utilizing a solution containing 0.1% crystal violet for 20 min. Any cells that did not migrate were gently eliminated using a cotton swab and subsequently enumerated under microscopic observation.

2.11 Western blot analysis

As previously described, N-cadherin, E-cadherin, Vimentin and Snail proteins in the CRC cells (HCT116 and HT29) were determined using western blot analysis (Subramanian et al., 2012). SDS-PAGE separated equivalent amounts of proteins, followed by transferring them onto a 4 PVDF membrane. A 5% skimmed milk blockaded the proteins, along with incubation of the proteins with the primary antibody (PrPC, 1:2,000; Cat. No: 12555-1-AP, Proteintech, United States and N-cadherin, 1:1,000, Cat. No: AF5239, Affinity Biosciences, United States and E-cadherin, 1:1,000, Cat. No: AF0131, Affinity Biosciences, United States and Vimentin, 1:1,000, Cat. No: AF7013, Affinity Biosciences, United States and Snail, 1:1,000, Cat. No: AF6032, Affinity Biosciences, United States) in PBST for 2 h at room temperature. The membrane was incubated with the secondary antibody (Goat Anti-Rabbit IgG HRP, 1:10,000, Cat. No: SA00001-2, Proteintech, United States) in PBST for 1 h. β-actin (1:10,000, Cat. No: 20536-1-AP, Proteintech, United States) was the internal control. ECL kit from Beyotime was utilized to visualize the proteins.

3 Results

3.1 Prognostic value and aberrant expression of PRNP in pan-cancer

The levels of PRNP mRNA expression in 26 distinct types of cancer were initially examined to assess the significance of PRNP in malignancies. This analysis was conducted using a comprehensive dataset obtained from the TCGA Pan-Cancer (PANCAN) project within the UCSC database. Our findings revealed that PRNP is significantly upregulated in most cancers, with only a few exceptions (Figure 1). To further ascertain the prognostic value of PRNP expression across various cancer types, a survival analysis was conducted employing a Cox proportional hazards model. The results affirmed that heightened levels of PRNP expression were linked to poorer OS rates, specifically in COAD, READ, and COADREAD (Figure 2A). These observations were subsequently confirmed through Kaplan-Meier survival analysis (Figures 2B–D).

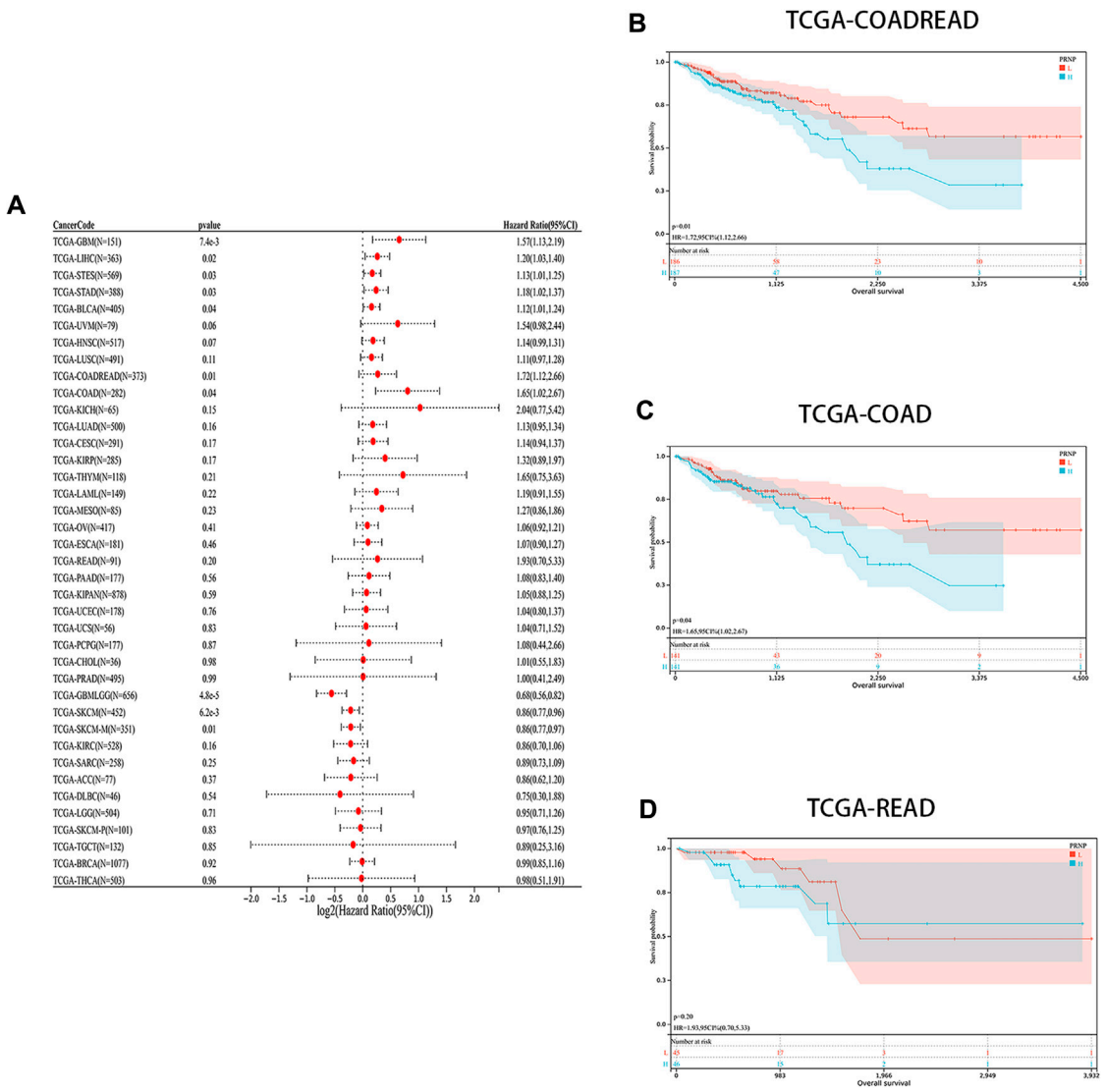


FIGURE 2
Prognostic analysis of PRNP in different cancer types: **(A)** Forest plot showing the correlation between PRNP expression and OS in various cancers; **(B–D)** Kaplan-Meier survival analysis results show that OS is decreased in COADREAD, COAD, and READ patients with higher PRNP expression levels.

3.2 Immune infiltration examination of PRNP in pan-cancer

We aimed to explore the potential of PRNP serving as a therapeutic target for immunotherapy in cancer by investigating its association with tumor immunity. Recognizing the crucial role of TME in tumorigenesis and progression, various algorithms (CIBERSORT, EPIC, and TIMER) were utilized to evaluate the correlation between PRNP expression and immune cell infiltration levels. Findings from these algorithms indicated a positive relationship between PRNP expression and several cell types, including macrophages, neutrophils, CAFs, and $CD8^+$ T cells. Conversely, a negative link was observed with plasma cells and regulatory T cells (Figure 3).

Immune checkpoints, a crucial mechanism used by tumor cells to avoid detection and attack by T cells, were the focus of our study. We aimed to explore the associations between PRNP and 60 common immune checkpoint expressions (24 inhibitory and 36 stimulatory) across various cancers. Interestingly, positive correlations were found between PRNP and a majority of immune checkpoint molecules in nearly all types of cancer, particularly with CD274 and C10orf54, as shown in Figure 4A. Strong associations were observed between the expression level of PRNP and MSI as well as TMB across distinct cancer types. Specifically, a positive correlation was identified between PRNP and TMB in HNSC and COAD, while in KIRC, STAD, READ, KIPA, and GBML, it showed a negative correlation. Furthermore, a positive link between PRNP and MSI was found in GBML, THCA, HNSC, and UCEC; conversely, a negative correlation with MSI was observed in STAD, PRAD, LUSC, STES, KIPA, ESCA, PAAD, and LUAD, as depicted in Figures 4B, C.

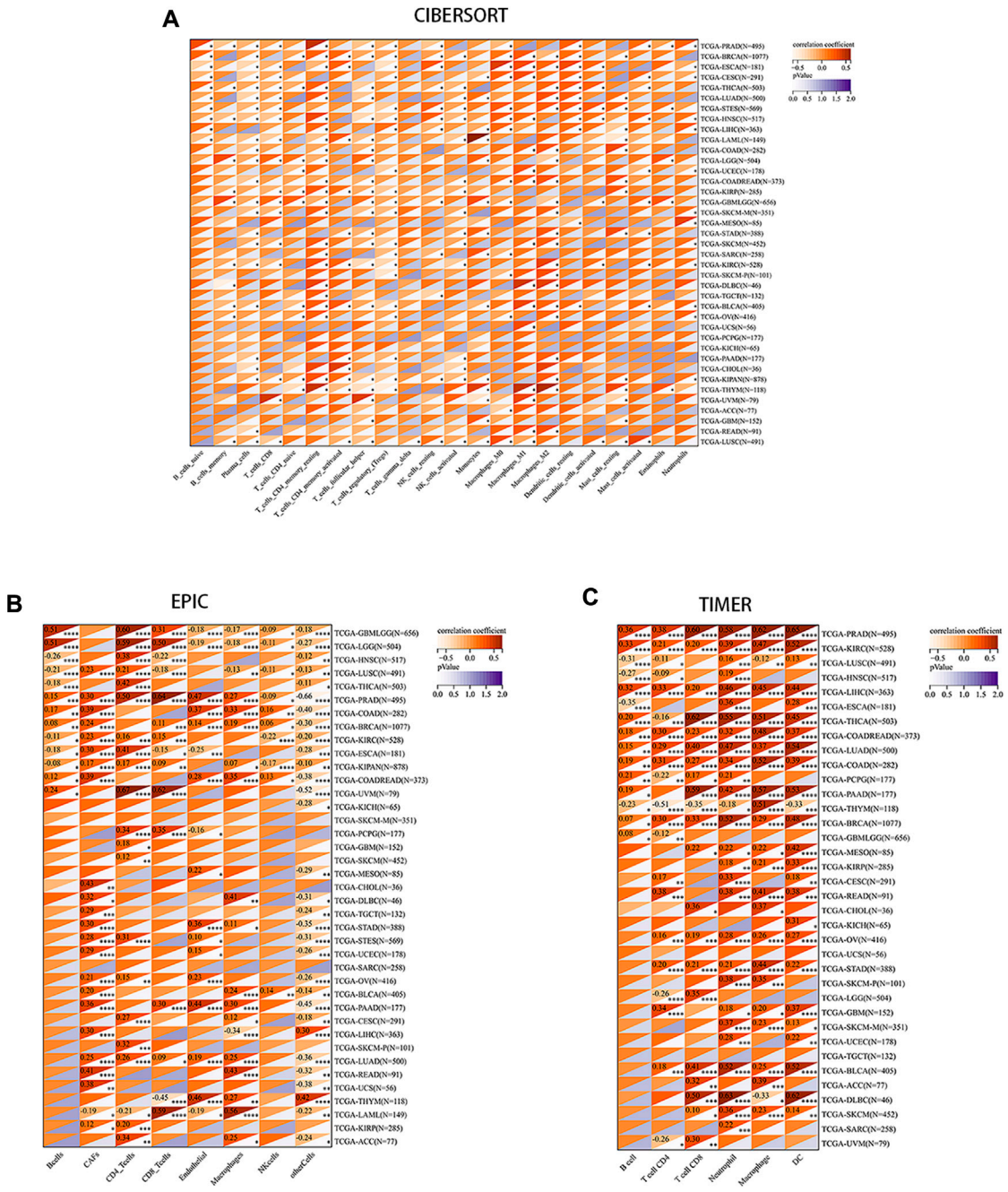


FIGURE 3
Correlation between PRNP expression levels and immune cell infiltration in various pan-cancer types using: **(A)** The CIBERSORT algorithm; **(B)** The EPIC algorithm; **(C)** The TIMER algorithm.

3.3 Evaluation of enrichment of PRNP-related genes in pan-cancer

A PPI network for PRNP was generated, and the STRING was utilized to detect the top 10 associated genes (Figure 5A). GO and KEGG analyses for the genes followed. The KEGG analysis affirmed a significant enrichment of these genes in pathways such as Prion diseases, Viral myocarditis, and Phospholipase D signaling pathway, among others (Figure 5B). Additionally, the GO analysis demonstrated the enrichment of these genes in cellular responses to organonitrogen compounds, nitrogen compounds, and cellular calcium ion homeostasis, among others (Figure 5C).

3.4 Validation of the effect of knockdown and overexpression of PrPC in CRC cell lines

The transfection efficiency of PrPC- and PrPC+ was analyzed by western blot (Figure 6A). Results showed a statistically substantial decline in the expression of PrPC in HCT116 cells of the PrPC-

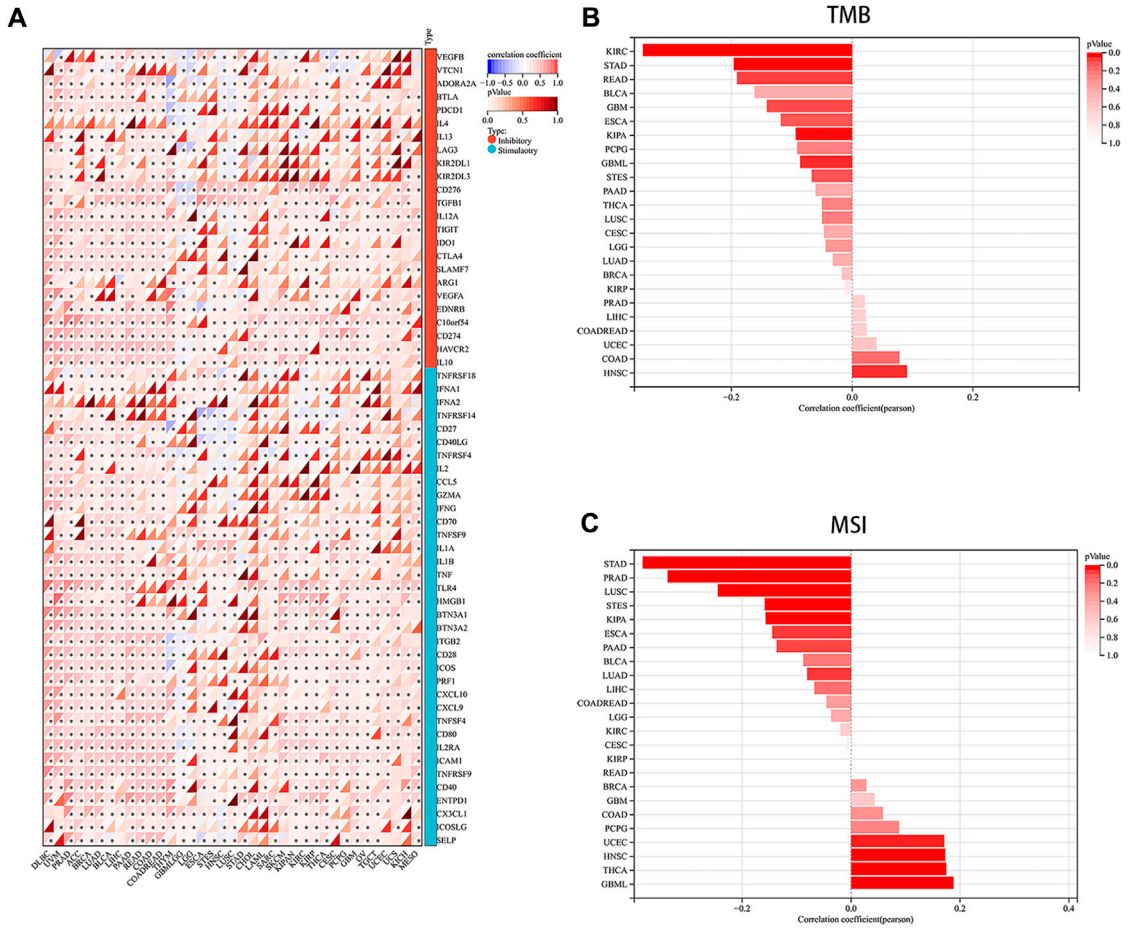


FIGURE 4
Overexpressed PRNP is positively correlated with immune checkpoint molecules in multiple cancers: **(A)** Heatmap showing correlations between PRNP expression and immune checkpoint genes' RNA levels in the TCGA pan-cancer database using Spearman's correlation test; **(B)** Histogram showing the correlation between PRNP expression and TMB in different cancers; **(C)** Histogram showing the correlation between PRNP expression and MSI in different cancers. *$p < 0.05$.

group compared with the PrPC- control group ($p < 0.05$, Figure 6B). Moreover, a statistically substantial increase in the expression level of PrPC was observed in the PrPC+ group compared to the PrPC+ control group ($p < 0.01$). No significant variation was deduced between the PrPC- control group (or PrPC+ control group) and the blank control group ($p > 0.05$). Similarly, the expression of PrPC in HT29 cells (Figure 6C) decreased remarkably in the PrPC- group ($p < 0.001$) and increased significantly in the PrPC+ group when compared to the corresponding control group ($p < 0.01$).

3.5 Effects of different PrPC expression levels on the proliferation ability of CRC cell lines

HCT116 and HT29 human CRC cell lines were cultured for 6, 12, and 24 h. A CCK-8 assay explored the influence of different PrPC expression levels on CRC proliferation. As shown in Figure 7A, after 6 h of culturing HCT116 cells, no significant difference in cell proliferation was affirmed between the PrPC- control group and the PrPC- group ($p > 0.05$). Nevertheless, after 12 and 24 h of culturing, the proliferation capacity decreased by 28.54% and 30.74% in PrPC- group compared to control group ($p < 0.05$, $p < 0.01$). Conversely, the proliferation ability of HCT116 cell lines significantly increased after 6, 12, and 24 h of culturing when the PrPC gene was overexpressed, and the proliferation capacity increased by 27.05%, 16.72%, and 15.07% in PrPC+ group compared to control group ($p < 0.01$, $p < 0.01$, $p < 0.05$). Similar findings were noted in HT29 cells, after 12 and 24 h of culturing, the proliferation capacity decreased by 29.33% and 33.46% in PrPC- group compared to control group ($p < 0.01$, $p < 0.05$). On the contrary, the proliferation capacity increased by 180.9%, 113.27%, and 39.89% in PrPC+ group compared to control group ($p < 0.001$, $p < 0.001$, $p < 0.01$) (Figure 7B).

3.6 Effect of different PrPC expression levels on migration ability of CRC cell lines

In-vitro cell migration assays were utilized to explore the effect of different PrPC expression levels on CRC migration (Figure 8A). As shown in Figure 8B, results from the Wound healing assay indicated a significant decrease in the migration ability of HCT116 cell lines

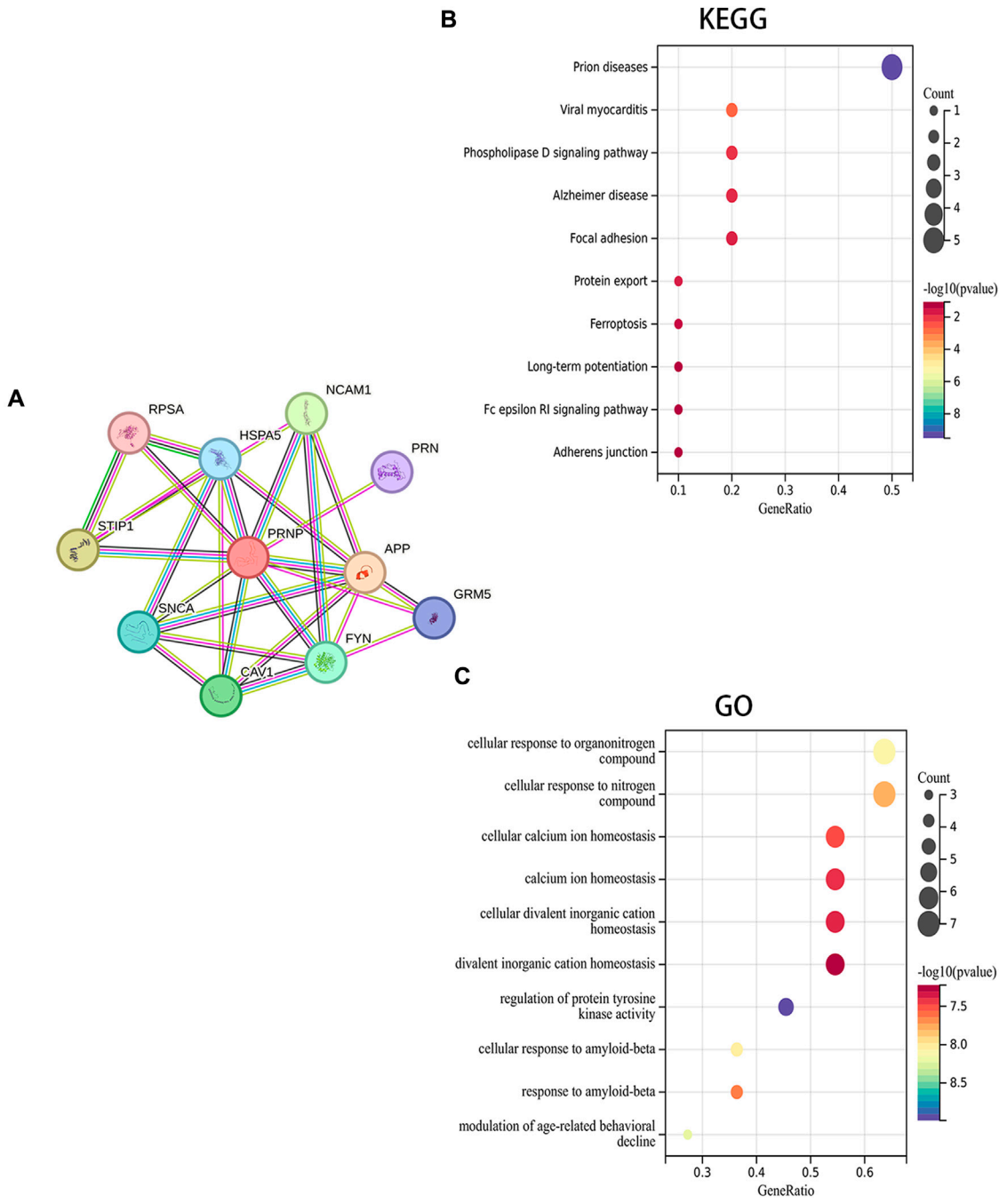


FIGURE 5
Fuctional enrichment analysis of PRNP-related genes: **(A)** PPI network for PRNP using the STRING datase; **(B)** KEGG and **(C)** GO enrichment analyses of the PRNP-related genes.

after 12 and 24 h of cell culture when the PrPC gene was silenced, and the migration ability decreased by 11.72% and 19.99%, respectively ($p < 0.05$, $p < 0.01$). Conversely, the migration ability of HCT116 cell lines significantly increased after 12 and 24 h of cell culture when the PrPC gene was overexpressed, and the migration ability increased by 37.31% and 57.42%, respectively ($p < 0.05$, $p < 0.001$). Similar outcomes were found in HT29 cells, after 12 and 24 h of cell culture, the migration ability decreased by 11.49% and 26.68% in PrPC- group compared to control group ($p < 0.01$, $p < 0.05$). On the contrary, the migration ability increased by 38.06% and 60.40% in PrPC+ group compared to control group (both $p < 0.01$) (Figure 8C). The Transwell cell migration assay was employed to validate the findings of the Wound healing assay regarding the effects of PrPC on CRC (Figure 9A). Data analysis revealed a substantial decline in the number of migrating HCT116 cells in the PrPC- group in comparison to that in the PrPC- control group, and the migration ability decreased by 26% ($p < 0.001$). Conversely, the number of cells migrating in the PrPC+ group was markedly heightened compared to that in the PrPC+ control group, and the migration ability increased by 138% ($p < 0.001$) (Figure 9B). Similar

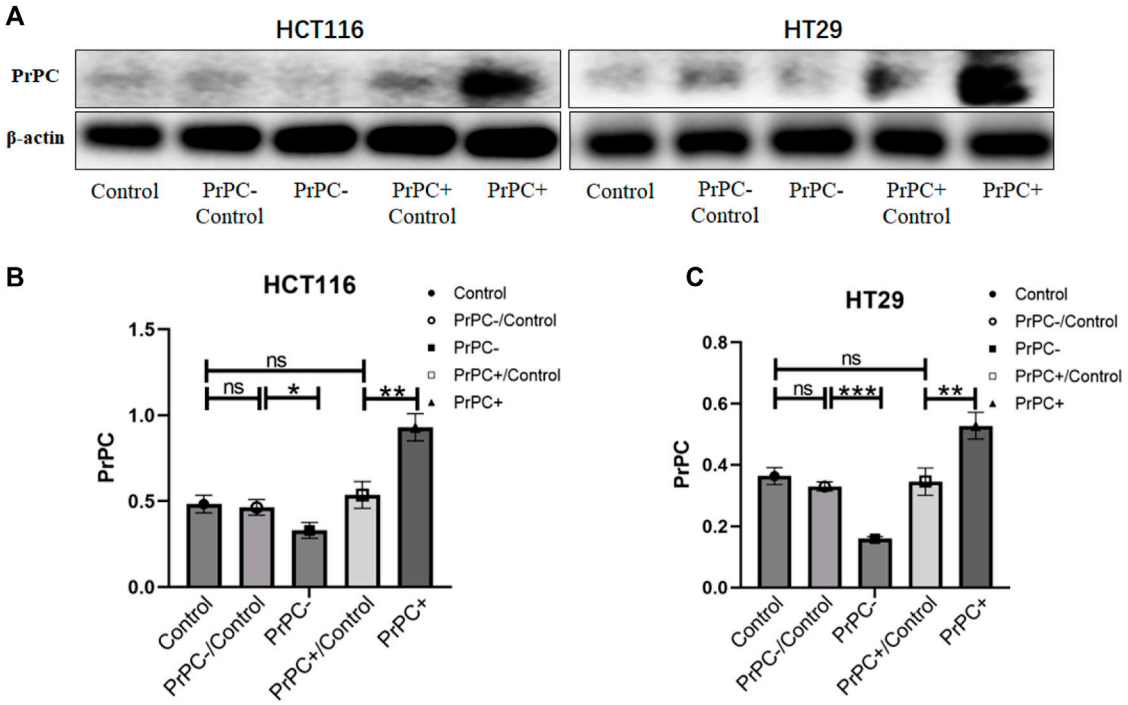


FIGURE 6
The expression levels of PrPC on CRC cell lines (HCT116 and HT29) and the control group were detected by western blot analysis. ns, no significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for comparison with the control group in **(A–C)**.

trends were observed in HT29 cells, the migration ability decreased by 17.30% in PrPC- group compared to control group ($p < 0.05$), and the migration ability increased by 75.50% in PrPC+ group compared to control group ($p < 0.01$) (Figure 9C).

3.7 Effects of different PrPC expression levels on the invasion ability of CRC cell lines

In-vitro cell invasion assays were conducted to explore the influence of varying PrPC expression levels on CRC invasion (Figure 10A). As presented in Figure 10B, the results demonstrated a decrease that is significant in the invasion ability of HCT116 cells in the PrPC- group compared to that in the PrPC- control group, and the invasion ability decreased by 6% ($p < 0.01$). In contrast, the invasion ability of the PrPC+ group was remarkedly increased compared to that of the PrPC+ control group, and the invasion ability increased by 42% ($p < 0.01$). Similar patterns were observed in HT29 cells, the invasion ability decreased by 6.33% in PrPC- group compared to control group ($p < 0.01$), and the migration ability increased by 56% in PrPC+ group compared to control group ($p < 0.05$) (Figure 10C).

3.8 Effects of different PrPC expression levels on EMT-related protein expression in CRC cell lines

In Figure 11A, the expression of EMT-related proteins (N-cadherin, E-cadherin, Vimentin and Snail) in CRC cell lines with varying PrPC expression levels was detected using western blot. The results (Figures 11B–I) indicated that silencing the PrPC gene led to a declined expression of N-cadherin (both $p < 0.001$), Vimentin (both $p < 0.05$) and Snail (both $p < 0.001$) and a surge in the expression level of E-cadherin ($p < 0.05$, $p < 0.01$) in HCT116 and HT29 cells. Conversely, when the PrPC gene was overexpressed, the expression of N-cadherin ($p < 0.01$, $p < 0.001$), Vimentin (both $p < 0.05$) and Snail ($p < 0.01$, $p < 0.001$) increased, and E-cadherin decreased (both $p < 0.01$) in both CRC cell lines. No significant difference was observed in the expression of N-cadherin, E-cadherin, Vimentin and Snail between the blank group and the control group.

4 Discussion

The PrPC, encoded by the gene PRNP, is responsible for producing the major prion protein and exhibits widespread expression in various tissues, with specifically high levels found in the CNS (Tang et al., 2016; Go and Lee, 2020). Recently, remarkable studies have affirmed a high expression of PRNP(PrPC) in different types of cancers (Gil et al., 2016; Luo et al., 2017; Atkinson et al., 2019; Lin et al., 2020), consistent with our findings. Our investigation of PRNP(PrPC) expression in pan-cancer revealed significant upregulation in various cancer types, such as BRCA, COADREAD, ESCA, and STAD. The role and function of PRNP(PrPC) in influencing the proliferation, apoptosis, invasion, and metastasis of different cancer types indicate its potential as a promising therapeutic target involved in the therapeutic management of cancer.

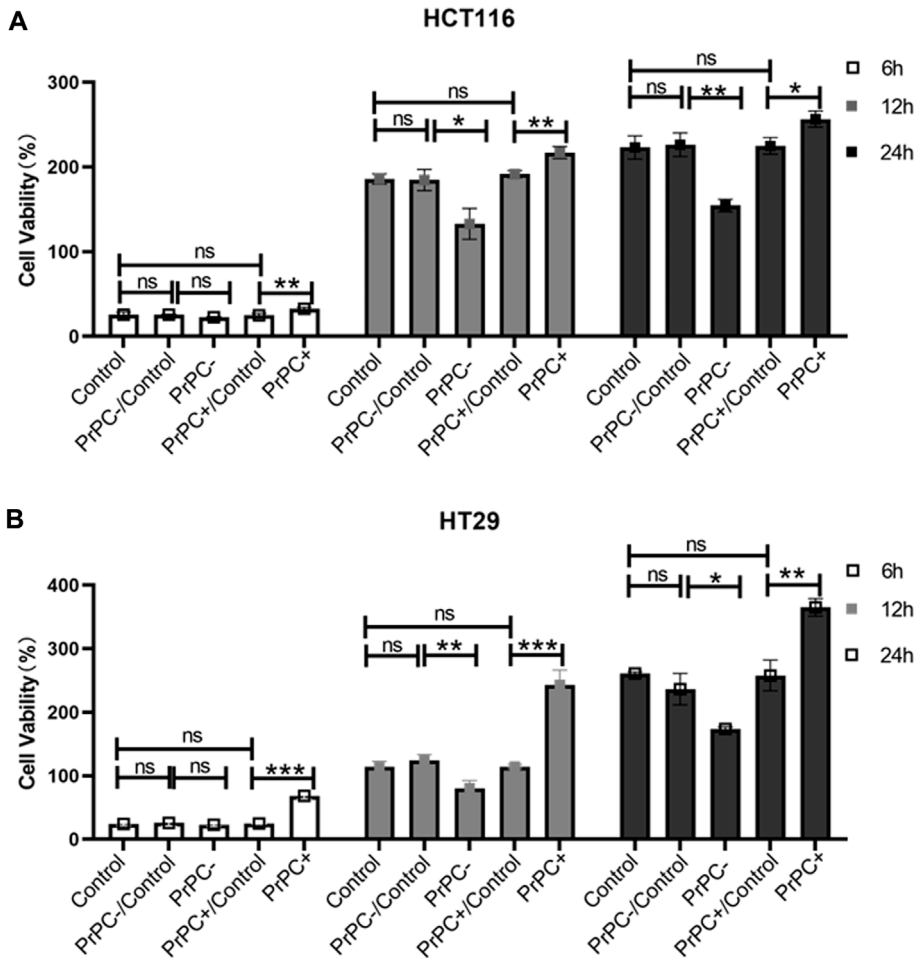


FIGURE 7
The proliferation of PrPC on CRC cell lines and the control group were detected by the CCK-8 assay. ns, no significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for comparison with the control group in **(A, B)**.

Furthermore, we conducted an analysis to determine correlations between PRNP(PrPC) expression and TME, immune cell infiltration, and immune checkpoints. The TME comprises multiple immune cell types that function in either clearing tumor cells or promoting tumor immune escape, significantly impacting tumor prognosis (Bindea et al., 2013; Huntington et al., 2020). We found a positive link between PRNP(PrPC) expression and several cell types, particularly macrophages, neutrophils, CAFs, and $CD8^+$ T cells while observing a negative correlation with plasma cells and regulatory T cells. With the rapid development of immune checkpoint inhibitors-based immunotherapies, there is an increasing need to develop biomarkers capable of predicting patient responsiveness to these treatments (Ribas and Wolchok, 2018). CTLA-4 and PD-1 have been identified as crucial regulators of T-cell reactions and exhibit promising potential as therapeutic targets for cancer treatment (Anderson et al., 2016; Korman et al., 2022). Recently, Giacomelli et al. (2023) found that immune suppressive microenvironment of mismatch repair proficient (pMMR) CRC characterized by dense infiltration of TAMs, occurrence of TANs, T-cell exhaustion, and interferon-γ unresponsiveness by host and tumor cells. Notably, we observed a significant association between PRNP(PrPC) and the majority of immune checkpoint molecules across various cancer types, particularly robust correlations with CD274 and C10orf54.

The expression of PRNP(PrPC) was observed to vary in both CRC tissues and their corresponding normal colorectal tissues, with a significantly higher positive expression rate noted in CRC compared to normal colorectal tissues. Moreover, we identified an association between the level of PRNP(PrPC) expression in CRC tissues and various clinicopathological features such as TNM stage, tumor invasion depth, tumor differentiation degree, presence of vascular invasion, and lymph node metastasis. Importantly, our findings also affirm a correlation between high PRNP(PrPC) expression levels and poor prognosis among CRC patients (Du et al., 2022). Subsequent research has revealed that the downregulation of PRNP(PrPC) expression can increase the sensitivity of HT29 cells to the chemotherapy drug cisplatin and promote cisplatin-induced apoptosis. The mechanism may be linked to the upregulation of apoptotic proteins Bax as well as caspase-3, along with the downregulation of the anti-apoptotic protein Bcl-2 (Du et al., 2019). Considering that sustained proliferative ability is considered one of the fundamental characteristics of cancer cells, extensive research has been

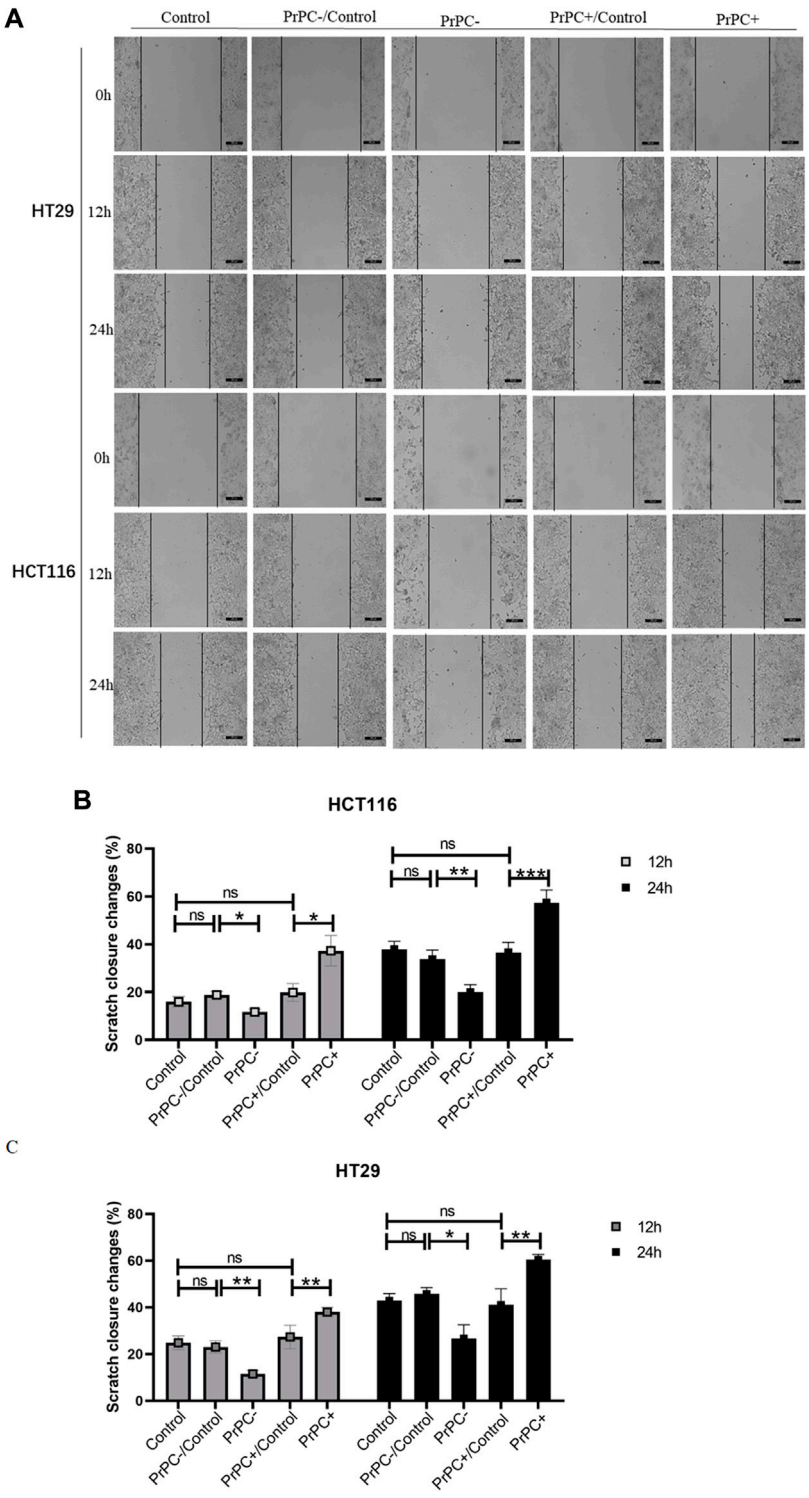


FIGURE 8
The migration of PrPC on CRC cell lines and the control group were detected by the Wound healing assay. ns, no significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for comparison with the control group in **(A–C)**.

conducted on the role of PrPC in promoting cancer cell proliferation. Early evidence supporting the involvement of PrPC in driving cancer cell growth was provided by Daiming Fan's team through their investigation using gastric cancer cell lines SGC7901 and AGS (Liang et al., 2007). Chieng and Say (2015) demonstrated that overexpression of PrPC in LS 174T cells promotes cell growth and proliferation, while the proliferation of colon cancer cells significantly reduced after siRNA knockdown of PrPC in DLD-1 and SW480 CRC

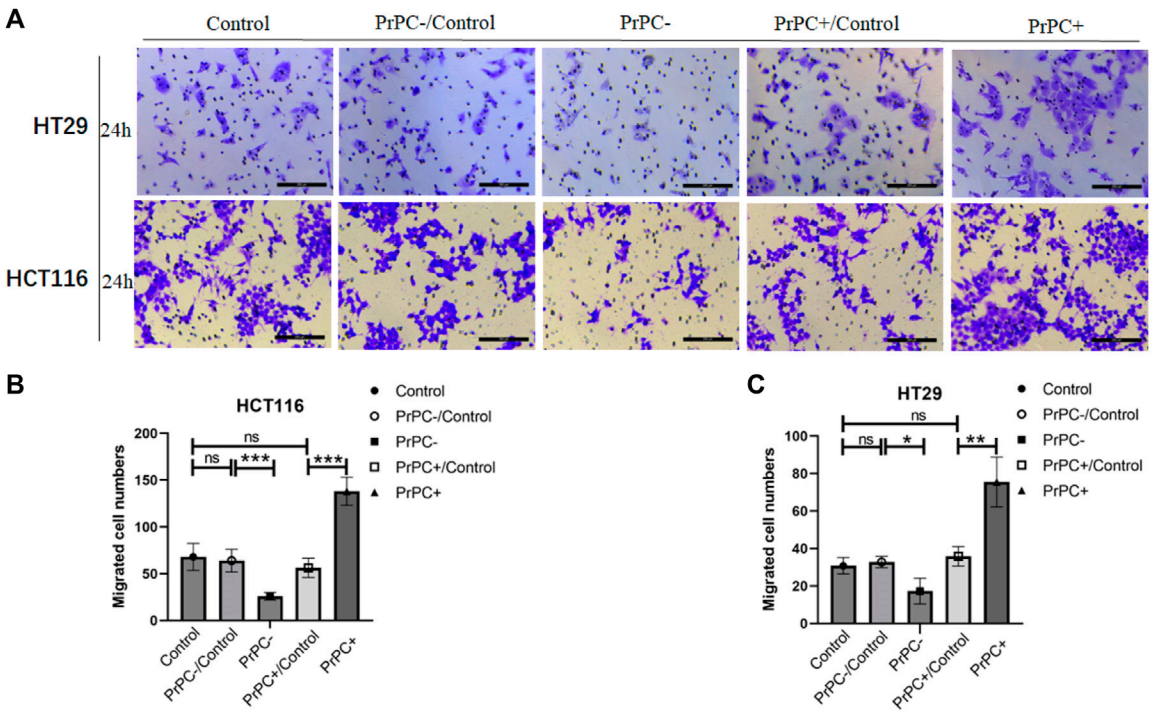


FIGURE 9
The migration of PrPC on CRC cell lines and the control group were detected by the Transwell assay. ns, no significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for comparison with the control group in **(A–C)**.

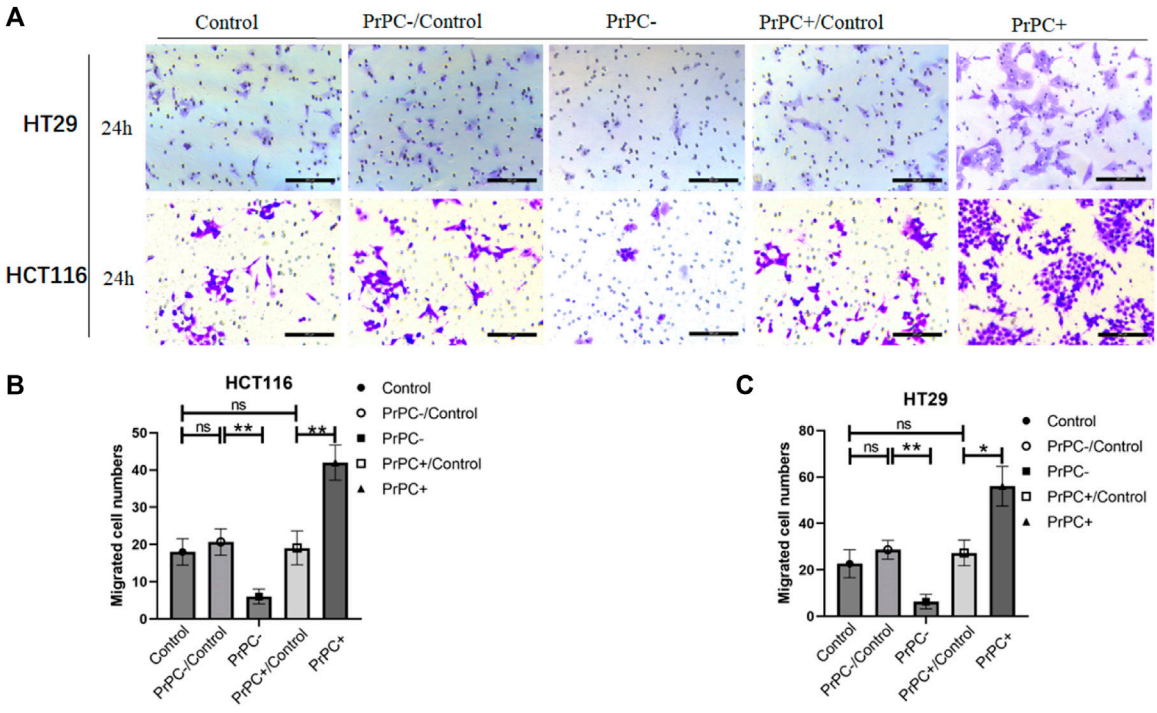


FIGURE 10
The invasion of PrPC on CRC cell lines and the control group were detected by the cell invasion assay. ns, no significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for comparison with the control group in **(A–C)**.

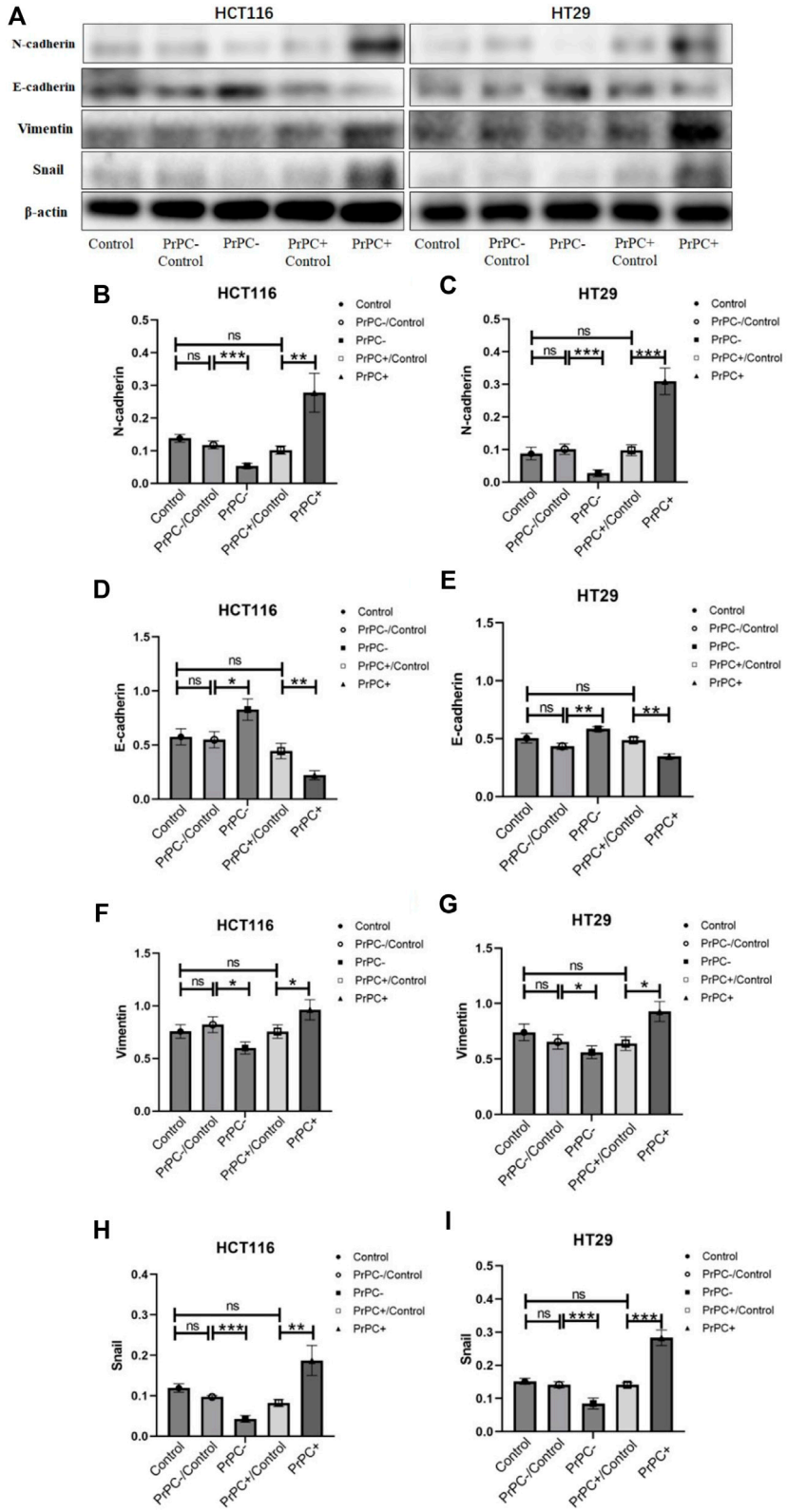


FIGURE 11
N-cadherin, E-cadherin, Vimentin, and Snail were detected by western blot in different PrPC expression levels of CRC cell lines and the control group. ns, no significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for comparison with the control group in **(A–I)**.

cell lines. Yun et al. (2016) found that HT29 cells treated with fucoidan exhibited decreased cell proliferation, diminished levels of the anti-apoptotic protein Bcl-2, and heightened levels of pro-apoptotic proteins Bax, cleaved caspase-3, and cleaved PARP1. Furthermore, the fucoidan-induced alterations in cell proliferation, apoptosis, and migration were further enhanced by downregulating PrPC expression using si-PRNP. Administering si-PRNP along with fucoidan via intraperitoneal injection resulted in reduced tumor volume and proliferation in Balb/c nude mice. These findings imply that the improved antitumor efficacy detected may be attributed to a decrease in angiogenesis. Similarly, we observed that the overexpression of PrPC in HCT116 and HT29 cell lines can promote the proliferation of CRC cells, and with prolonged cell culture time, the proliferation capacity of both CRC cells increased. This suggests a close relationship between PrPC expression levels and the proliferation of CRC cell lines.

Metastasis, the primary cause of death in cancer patients, signifies an advanced stage of malignancy. This progression encompasses various mechanisms, including the movement and infiltration of malignant cells, which are characteristic traits (Tahtamouni et al., 2019). EMT, a crucial factor in tumor invasion and metastasis as well as embryonic development, serves as a primary molecular mechanism facilitating the enhancement of metastasis and invasion during cancer promotion (Hodge et al., 2018). The orchestration of the EMT program involves key transcription factors consisting of Slug, Snail, Twist, as well as ZEB1 or ZEB2 (Lu and Kang, 2019). Du et al. (2013) found that mesenchymal genes (Twist and N-cadherin) were significantly upregulated, while the epithelial marker E-cadherin was downregulated in PrPC overexpressing colorectal cancer stem cells (CCSCs), whereas knockdown of PrPC resulted in a reversed expression pattern. In addition, their results further confirmed the correlation of PrPC with the expression of EMT-related molecules by using double immunofluorescence staining and western blot analysis. Through further research, the authors concluded that PrPC regulated the EMT phenotype by modulating Twist, and it was positively correlated with the mesenchymal properties of cells from CRC patients. Moreover, they found that PrPC promoted EMT via the ERK2 (MAPK1) signaling pathway and conferring high metastatic capacity. In our study, Wound healing assay and Transwell assay were employed to explore the impact of PrPC expression on the migration ability of CRC cell lines. It was found that high PrPC expression can promote increased migration ability of HCT116 and HT29 cell lines and may be related to EMT-related proteins (N-cadherin, E-cadherin, Vimentin and Snail). Notably, an increase in PrPC expression led to elevated N-cadherin, Vimentin and Snail expression and decreased E-cadherin expression. Conversely, knockdown of PrPC reversed EMT and reduced cell proliferation, migration, and invasion, while down-regulating N-cadherin, Vimentin and Snail and up-regulating E-cadherin. The EMT and TGF-β axes are the two main pathways to distinguish the C4 and CMS4 subgroups in CRC, and they also found that EMT and TGF-β signaling pathway feature among those that are correlated with PRNP gene expression (Le Corre et al., 2019). De Lacerda et al. observed that the migration as well as the invasion of CRC cell lines were stimulated by HOP in a PrPC-dependent manner, and the impact of HOP on cell migration and invasion is mediated through the phosphorylation of the ERK1/2 pathway (de Lacerda et al., 2016). These results propose that the ERK1/2 pathway participates in the HOP-driven invasion of CRC cells. In a study on PrPC's role in EMT formation, Wang Q. et al. (2012) found that reducing the transcription of PrPC could down-regulate the expression of special AT-rich sequence-binding protein-1 (SATB1) through the Fyn-SP1-SATB1 pathway, thereby reducing the metastatic ability of CRC cells and decreasing their distant metastasis *in vivo*. Based on these findings, it is observed that PrPC may function in the process of distant metastasis of CRC, providing a potential new therapeutic target for patients with distant metastasis of CRC.

5 Conclusion

In summary, the results of our study inferred that PRNP(PrPC) is an immune-related prognostic biomarker and has the potential to serve as a prognostic indicator for CRC immunotherapy, while also promoting proliferation, migration, and invasion. Additionally, the overexpression of PRNP(PrPC) can promote the occurrence of EMT in CRC cells. The mechanism may be linked to the upregulation of N-cadherin, Vimentin and Snail and downregulation of E-cadherin. However, its specific signaling pathway is still unclear and warrants further investigation.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

HC: Validation, Writing–original draft. YD: Data curation, Writing–original draft, Writing–review and editing. ZK: Data curation, Writing–original draft. XL: Writing–original draft, Writing–review and editing. WL: Funding acquisition, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was funded by the Suzhou "Science and Education and Health" Youth Science and Technology Projects (Grant No. KJXW2020071) and "National Tutor System" Training Program for Health Youth Key Talents in Suzhou (Grant No. Qngg2023042).

Conflict of interest

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

Publisher's note

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2024.1391873/full#supplementary-material

References

Allais-Bonnet, A., and Pailhoux, E. (2014). Role of the prion protein family in the gonads. *Front. Cell Dev. Biol.* 2, 56. doi:10.3389/fcell.2014.00056

Anderson, A. C., Joller, N., and Kuchroo, V. K. (2016). Lag-3, tim-3, and TIGIT: Co-inhibitory receptors with specialized functions in immune regulation. *Immunity* 44 (5), 989–1004. doi:10.1016/j.immuni.2016.05.001

Atkinson, C. J., Kawamata, F., Liu, C., Ham, S., Győrffy, B., Munn, A. L., et al. (2019). EGFR and Prion protein promote signaling via FOXO3a-KLF5 resulting in clinical resistance to platinum agents in colorectal cancer. *Mol. Oncol.* 13 (4), 725–737. doi:10.1002/1878-0261.12411

Bindea, G., Mlecnik, B., Tosolini, M., Kirilovsky, A., Waldner, M., Obenauf, A. C., et al. (2013). Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* 39 (4), 782–795. doi:10.1016/j.immuni.2013.10.003

Bonneville, R., Krook, M. A., Kautto, E. A., Miya, J., Wing, M. R., Chen, H. Z., et al. (2017). Landscape of microsatellite instability across 39 cancer types. *JCO Precis. Oncol.* 2017, PO.17.00073. doi:10.1200/PO.17.00073

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

Castle, A. R., and Gill, A. C. (2017). Physiological functions of the cellular prion protein. *Front. Mol. Biosci.* 4, 19. doi:10.3389/fmolb.2017.00019

Chieng, C. K., and Say, Y. H. (2015). Cellular prion protein contributes to LS 174T colon cancer cell carcinogenesis by increasing invasiveness and resistance against doxorubicin-induced apoptosis. *Tumour Biol.* 36 (10), 8107–8120. doi:10.1007/s13277-015-3530-z

de Lacerda, T. C., Costa-Silva, B., Giudice, F. S., Dias, M. V., de Oliveira, G. P., Teixeira, B. L., et al. (2016). Prion protein binding to HOP modulates the migration and invasion of colorectal cancer cells. *Clin. Exp. Metastasis* 33 (5), 441–451. doi:10.1007/s10585-016-9788-8

Du, L., Rao, G., Wang, H., Li, B., Tian, W., Cui, J., et al. (2013). CD44-positive cancer stem cells expressing cellular prion protein contribute to metastatic capacity in colorectal cancer. *Cancer Res.* 73 (8), 2682–2694. doi:10.1158/0008-5472.CAN-12-3759

Du, Y., Chen, H. F., Li, W. P., Kong, Z. Y., Zhu, L. L., and Wang, Y. (2022). Expression of cellular prion protein and its relationship with prognosis in colorectal cancer. *Med. J. Chin. PLA* 47 (9), 902–907. doi:10.11855/j.issn.0577-7402.2022.09.0902

Du, Y., Li, W. P., Xu, H. X., Wang, Y., Lu, Y. C., Chen, Z. H., et al. (2019). Effect of cellular prion protein on the cell apoptosis and expression of Caspase-3, Bcl-2 and Bax of gastric cancer cell line SGC7901 and colon cancer cell line HT29. *Chin. J. CANCER Prev. Treat.* 26 (11), 1587–1592. doi:10.16073/j.cnki.cjcpt.2019.21.02

Gao, J., Navai, N., Alhalabi, O., Siefker-Radtke, A., Campbell, M. T., Tidwell, R. S., et al. (2020). Neoadjuvant PD-L1 plus CTLA-4 blockade in patients with cisplatin-ineligible operable high-risk urothelial carcinoma. *Nat. Med.* 26 (12), 1845–1851. doi:10.1038/s41591-020-1086-y

Giacomelli, M., Monti, M., Pezzola, D. C., Lonardi, S., Bugatti, M., Missale, F., et al. (2023). Immuno-contexture and immune checkpoint molecule expression in mismatch repair proficient colorectal carcinoma. *Cancers (Basel)* 15 (12), 3097. doi:10.3390/cancers15123097

Gil, M., Kim, Y. K., Kim, K. E., Kim, W., Park, C. S., and Lee, K. J. (2016). Cellular prion protein regulates invasion and migration of breast cancer cells through MMP-9 activity. *Biochem. Biophys. Res. Commun.* 470 (1), 213–219. doi:10.1016/j.bbrc.2016.01.038

Go, G., and Lee, S. H. (2020). The cellular prion protein: a promising therapeutic target for cancer. *Int. J. Mol. Sci.* 21 (23), 9208. doi:10.3390/ijms21239208

Herbst, R. S., Giaccone, G., de Marinis, F., Reinmuth, N., Vergnenegre, A., Barrios, C. H., et al. (2020). Atezolizumab for first-line treatment of PD-L1-selected patients with NSCLC. *N. Engl. J. Med.* 383 (14), 1328–1339. doi:10.1056/NEJMoa1917346

Hodge, D. Q., Cui, J., Gamble, M. J., and Guo, W. (2018). Histone variant MacroH2A1 plays an isoform-specific role in suppressing epithelial-mesenchymal transition. *Sci. Rep.* 8 (1), 841. doi:10.1038/s41598-018-19364-4

Huntington, N. D., Cursons, J., and Rautela, J. (2020). The cancer-natural killer cell immunity cycle. *Nat. Rev. Cancer* 20 (8), 437–454. doi:10.1038/s41568-020-0272-z

Jia, S., Lu, J., Qu, T., Feng, Y., Wang, X., Liu, C., et al. (2017). MAGI1 inhibits migration and invasion via blocking MAPK/ERK signaling pathway in gastric cancer. *Chin. J. Cancer Res.* 29 (1), 25–35. doi:10.21147/j.issn.1000-9604.2017.01.04

Khemlina, G., Ikeda, S., and Kurzrock, R. (2017). The biology of Hepatocellular carcinoma: implications for genomic and immune therapies. *Mol. Cancer* 16 (1), 149. doi:10.1186/s12943-017-0712-x

Korman, A. J., Garrett-Thomson, S. C., and Lonberg, N. (2022). The foundations of immune checkpoint blockade and the ipilimumab approval decennial. *Nat. Rev. Drug Discov.* 21 (7), 509–528. doi:10.1038/s41573-021-00345-8

Le Corre, D., Balogoun, R., Pilati, C., Aparicio, T., Martin-Lannerée, S., Marisa, L., et al. (2019). The cellular prion protein controls the mesenchymal-like molecular subtype and predicts disease outcome in colorectal cancer. *EBioMedicine* 46, 94–104. doi:10.1016/j.ebiom.2019.07.036

Li, C., Xin, W., and Sy, M. S. (2010). Binding of pro-prion to filamin A: by design or an unfortunate blunder. *Oncogene* 29 (39), 5329–5345. doi:10.1038/onc.2010.307

Li, Q., and Ding, Z. Y. (2020). The ways of isolating neoantigen-specific T cells. *Front. Oncol.* 10, 1347. doi:10.3389/fonc.2020.01347

Liang, J., Pan, Y., Zhang, D., Guo, C., Shi, Y., Wang, J., et al. (2007). Cellular prion protein promotes proliferation and G1/S transition of human gastric cancer cells SGC7901 and AGS. *FASEB J.* 21 (9), 2247–2256. doi:10.1096/fj.06-7799com

Lin, S. C., Lin, C. H., Shih, N. C., Liu, H. L., Wang, W. C., Lin, K. Y., et al. (2020). Cellular prion protein transcriptionally regulated by NFIL3 enhances lung cancer cell lamellipodium formation and migration through JNK signaling. *Oncogene* 39 (2), 385–398. doi:10.1038/s41388-019-0994-0

Lu, W., and Kang, Y. (2019). Epithelial-mesenchymal plasticity in cancer progression and metastasis. *Dev. Cell* 49 (3), 361–374. doi:10.1016/j.devcel.2019.04.010

Luo, G., Wang, W., Wu, Q., Lu, Y., Su, T., Gu, N., et al. (2017). MGr1-Antigen/37 kDa laminin receptor precursor promotes cellular prion protein induced multi-drug-resistance of gastric cancer. *Oncotarget* 8 (42), 71630–71641. doi:10.18632/oncotarget.17795

Miyazaki, Y., Yoshida, N., Nozaki, T., Inoue, H., Kikuchi, K., and Kusama, K. (2015). Telomerase activity in the occurrence and progression of oral squamous cell carcinoma. *J. Oral Sci.* 57 (4), 295–303. doi:10.2334/josnusd.57.295

Ribas, A., and Wolchok, J. D. (2018). Cancer immunotherapy using checkpoint blockade. *Science* 359 (6382), 1350–1355. doi:10.1126/science.aar4060

Siegel, R. L., Miller, K. D., Fuchs, H. E., and Jemal, A. (2022). Cancer statistics, 2022. *CA Cancer J. Clin.* 72 (1), 7–33. doi:10.3322/caac.21708

Subramanian, V. S., Subramanya, S. B., and Said, H. M. (2012). Relative contribution of THTR-1 and THTR-2 in thiamin uptake by pancreatic acinar cells: studies utilizing Slc19a2 and Slc19a3 knockout mouse models. *Am. J. Physiol. Gastrointest. Liver Physiol.* 302 (5), G572–G578. doi:10.1152/ajpgi.00484.2011

Tahtamouni, L., Ahram, M., Koblinski, J., and Rolfo, C. (2019). Molecular regulation of cancer cell migration, invasion, and metastasis. *Anal. Cell Pathol. (Amst)* 2019, 1356508. doi:10.1155/2019/1356508

Tang, Z., Ma, J., Zhang, W., Gong, C., He, J., Wang, Y., et al. (2016). The role of prion protein expression in predicting gastric cancer prognosis. *J. Cancer* 7 (8), 984–990. doi:10.7150/jca.14237

Wang, Q., Qian, J., Wang, F., and Ma, Z. (2012b). Cellular prion protein accelerates colorectal cancer metastasis via the Fyn-SP1-SATB1 axis. *Oncol. Rep.* 28 (6), 2029–2034. doi:10.3892/or.2012.2025

Wang, S., Zeng, X., Liu, Y., Liang, C., Zhang, H., Liu, C., et al. (2012a). Construction and characterization of a PDCD5 recombinant lentivirus vector and its expression in tumor cells. *Oncol. Rep.* 28 (1), 91–98. doi:10.3892/or.2012.1756

Yun, C. W., Yun, S., Lee, J. H., Han, Y. S., Yoon, Y. M., An, D., et al. (2016). Silencing prion protein in HT29 human colorectal cancer cells enhances anticancer response to fucoidan. *Anticancer Res.* 36 (9), 4449–4458. doi:10.21873/anticanres.10989

frontiers | Frontiers in Cell and Developmental Biology

TYPE Review
PUBLISHED 28 August 2024
DOI 10.3389/fcell.2024.1397945

OPEN ACCESS

EDITED BY
Lucio Miele,
Louisiana State University, United States

REVIEWED BY
Deepak Parashar,
Medical College of Wisconsin, United States
Laurence A. Marchat,
Instituto Politécnico Nacional, Mexico

*CORRESPONDENCE
Ayoub Al Othaim,
ay.alothaim@mu.edu.sa
Fahad Khan,
fahadintegralian@gmail.com

†These authors have contributed equally to this work

RECEIVED 08 March 2024
ACCEPTED 08 August 2024
PUBLISHED 28 August 2024

CITATION
Chauhan P, Pramodh S, Hussain A, Elsori D, Lakhanpal S, Kumar R, Alsaweed M, Iqbal D, Pandey P, Al Othaim A and Khan F (2024) Understanding the role of miRNAs in cervical cancer pathogenesis and therapeutic responses.
Front. Cell Dev. Biol. 12:1397945.
doi: 10.3389/fcell.2024.1397945

Understanding the role of miRNAs in cervical cancer pathogenesis and therapeutic responses

Prashant Chauhan[1†], Sreepoorna Pramodh[2†], Arif Hussain[3], Deena Elsori[4], Sorabh Lakhanpal[5], Rahul Kumar[6], Mohammed Alsaweed[7], Danish Iqbal[8], Pratibha Pandey[9,10], Ayoub Al Othaim[7]* and Fahad Khan[11]*

[1]MPA Research, Greater Noida, Uttar Pradesh, India, [2]Department of Biomedical Sciences, University of Birmingham Dubai, Dubai, United Arab Emirates, [3]School of Life Sciences, Manipal Academy of Higher Education, Dubai, United Arab Emirates, [4]Faculty of Resilience, Rabdan Academy, Abu Dhabi, United Arab Emirates, [5]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, [6]Chitkara Centre for Research and Development, Chitkara University, Baddi, Himachal Pradesh, India, [7]Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Majmaah University, Al-Majmaah, Saudi Arabia, [8]Department of Health Information Management, College of Applied Medical Sciences, Buraydah Private Colleges, Buraydah, Saudi Arabia, [9]Centre for Research Impact and Outcome, Chitkara University Institute of Engineering and Technology, Chitkara University, Rajpura, Punjab, India, [10]Centre for Research Impact and Outcome, Chitkara College of Pharmacy, Chitkara University, Rajpura, Punjab, India, [11]Center for Global Health Research, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India

Cervical cancer (CC) is the most common cancer in women and poses a serious threat to health. Despite familiarity with the factors affecting its etiology, initiation, progression, treatment strategies, and even resistance to therapy, it is considered a significant problem for women. However, several factors have greatly affected the previous aspects of CC progression and treatment in recent decades. miRNAs are short non-coding RNA sequences that regulate gene expression by inhibiting translation of the target mRNA. miRNAs play a crucial role in CC pathogenesis by promoting cancer stem cell (CSC) proliferation, postponing apoptosis, continuing the cell cycle, and promoting invasion, angiogenesis, and metastasis. Similarly, miRNAs influence important CC-related molecular pathways, such as the PI3K/AKT/mTOR signaling pathway, Wnt/β-catenin system, JAK/STAT signaling pathway, and MAPK signaling pathway. Moreover, miRNAs affect the response of CC patients to chemotherapy and radiotherapy. Consequently, this review aims to provide an acquainted summary of onco miRNAs and tumor suppressor (TS) miRNAs and their potential role in CC pathogenesis and therapy responses by focusing on the molecular pathways that drive them.

KEYWORDS

tumour suppressor, miRNAs, cervical cancer, oncogenic mirnas, signalling pathway

1 Introduction

CC is one of the most prevalent gynecological carcinomas, posing a significant threat to the female reproductive system and is the primary cause of cancer-related mortality worldwide (Holcakova et al., 2021). According to epidemiological research on CC, there are an estimated 569,847 new cases and 311,365 deaths worldwide, annually. Alarmingly, projections indicate that by 2030, the incidence of CC is expected to increase by approximately 50% (Kakotkin et al., 2023), underscoring the urgency of understanding its underlying causes. Human papillomaviruses (HPV) is a DNA virus known to infect epithelial cells, and it's the most

common sexually transmitted infection globally. While many HPV infections resolve on their own without causing any symptoms or long-term effects, persistent infection with high-risk HPV types, such as HPV-16 and HPV-18, can lead to the development of CC (Liu et al., 2019). The relationship between HPV and cervical cancer is multifaceted. HPV contributes to cancer progression through various mechanisms, including the modulation of cellular processes and the interference with key regulatory proteins within the host cell (Oyervides-Muñoz et al., 2018). The oncoproteins E5, E6, and E7 encoded by HPV play critical roles in this process. Oncoprotein E5 modulates cellular signaling pathways like EGFR, promoting increased cell proliferation and survival, thus aiding in maintaining the transformed phenotype of HPV-infected cells (Pal and Kundu, 2020). E6 facilitates the degradation of the tumor suppressor protein p53, inhibiting apoptosis and enabling HPV-infected cells to evade cell cycle control, thereby promoting proliferation. E7 interacts with and promotes the degradation of the retinoblastoma (Rb) tumor suppressor protein, disrupting normal cell cycle regulation and leading to uncontrolled cell division and tumor formation (Taghizadeh et al., 2019). Additionally, Epigenetic alterations induced by HPV oncoproteins contribute significantly to the oncogenic process. HPV oncoproteins, such as E6 and E7, can disrupt normal epigenetic regulation in several ways. For instance, E6 and E7 can directly interact with cellular proteins involved in epigenetic regulation, altering their function and leading to aberrant DNA methylation patterns or histone modifications (Sen et al., 2018). Additionally, these oncoproteins can indirectly affect epigenetic processes by promoting the expression of specific non-coding RNAs, such as microRNAs or long non-coding RNAs, which can further modulate gene expression patterns. This disruption of epigenetic regulation by HPV oncoproteins can lead to the silencing of tumor suppressor genes or the activation of oncogenes, ultimately driving the development and progression of HPV-associated cancers like cervical cancer (Ferreira and Esteller, 2018). Although CC is commonly linked with HPV infection, not all diagnosed cases test positive for HPV (Saslow et al., 2012), suggesting the involvement of additional factors in CC development. Despite notable progress in surgical, chemotherapy, and radiation treatments, CC remains complex. Recent studies have highlighted specific dysregulated microRNAs (miRNAs) in CC, suggesting their potential as diagnostic and prognostic biomarkers (He et al., 2016). These findings hold promise for advancing more targeted and personalized strategies to address CC.

miRNAs, small non-coding RNAs, regulate gene expression and are associated with tumorigenesis and other biological processes. The first description of the role of miRNAs in cancer was published in 2002 (Acunzo et al., 2015). Studies have indicated that particular miRNAs are dysregulated in CC, implying that they may serve as biomarkers for diagnosis, prognosis, and therapeutic targets. Recent research has uncovered a multitude of miRNAs that play critical roles in CC pathogenesis and therapeutic response (Abbas et al., 2021). These miRNAs have emerged as potent regulators of gene expression, influencing key cellular processes, such as cell proliferation, apoptosis, angiogenesis, and metastasis (Sharma et al., 2014). Additionally, studies have revealed their crucial involvement in the dysregulation of key signaling pathways involved in the evolution of CC, including the PI3K/AKT/mTOR, Wnt/β-catenin, MAPK, and JAK/STAT pathways (Hemmat et al., 2020). Their complex effects highlight their potential as therapeutic targets in the fight against CC. miRNAs, with their compact size (~22 nucleotides), possess unique qualities that make them readily isolable and easily identifiable in various bodily samples, including tissues, blood, and bodily fluids, and are relatively resistant to degradation (Chevillet et al., 2014). Moreover, their intricacies are intertwined with those of cancer stem cells (CSCs), a subset of cancer cells endowed with distinctive self-renewal and differentiation capabilities. Within CSCs, specific miRNAs act as catalysts, fuelling self-renewal, migration, and resistance to therapy, thus garnering attention for their pivotal roles in both tumor initiation and progression (Khan et al., 2019). Furthermore, the discovery of miRNAs associated with therapeutic response in CC offers new avenues for personalized treatment strategies. By understanding how these miRNAs influence the sensitivity or resistance to different therapeutic approaches, clinicians can tailor treatment regimens to individual patients, potentially improving overall outcomes (Figure 1) (Gandellini et al., 2011; Gong et al., 2014). Therefore, this comprehensive review aims to shed light on the intricate role of miRNAs in the biogenesis of CC and elucidate their impact on dysregulated signaling pathways critical to CC development. Furthermore, it underscores the pivotal roles played by specific oncogenic and suppressor miRNAs in either hindering or promoting therapeutic interventions for CC, positioning miRNAs as promising diagnostic and prognostic markers, and warranting further investigation. By consolidating these insights, this study not only advances our understanding of the molecular complexities underlying CC progression but also lays the foundation for more targeted and precise management strategies. The implications of this review are poised to significantly shape future CC research endeavours, driving the formulation of more efficacious approaches for its treatment and care.

2 Canonical and non-canonical pathway of miRNAs biogenesis

miRNA biogenesis is a complicated set of events that result in the generation of mature miRNAs capable of influencing gene expression. miRNAs are involved in many biological processes and are essential for balanced human growth. Abnormalities in miRNA expression have been linked to a variety of diseases, emphasizing their importance in disease pathophysiology (Peng and Croce, 2016). Furthermore, miRNAs are not restricted to cells but are also released into extracellular fluids. This extracellular release of miRNAs adds another degree of complexity to their regulatory actions while also emphasizing their potential as diagnostic and prognostic indicators for diverse illnesses (Ortiz-Quintero, 2020). Extracellular miRNAs act as signaling molecules, facilitating cell-to-cell communication and serving as promising biomarkers for various illnesses, notably cancer (Conti et al., 2020). Hence, understanding the intricacies of miRNA biogenesis is crucial to effectively managing the onset and progression of severe diseases. miRNA biogenesis begins with the processing of RNA polymerase II/III transcripts, either post-transcriptionally or co-transcriptionally, which initiates the production of miRNAs (Panda and Prajapati, 2024). Around half of the identified miRNAs arise from within genes, mainly from introns, with a smaller portion originating from exons. The other half, however, are intergenic, meaning they are transcribed independently, regulated by their own promoters (Bofill-De Ros and Vang Ørom, 2024). miRNAs are typically grouped as families unless they are transcribed into clusters. These clusters were defined as

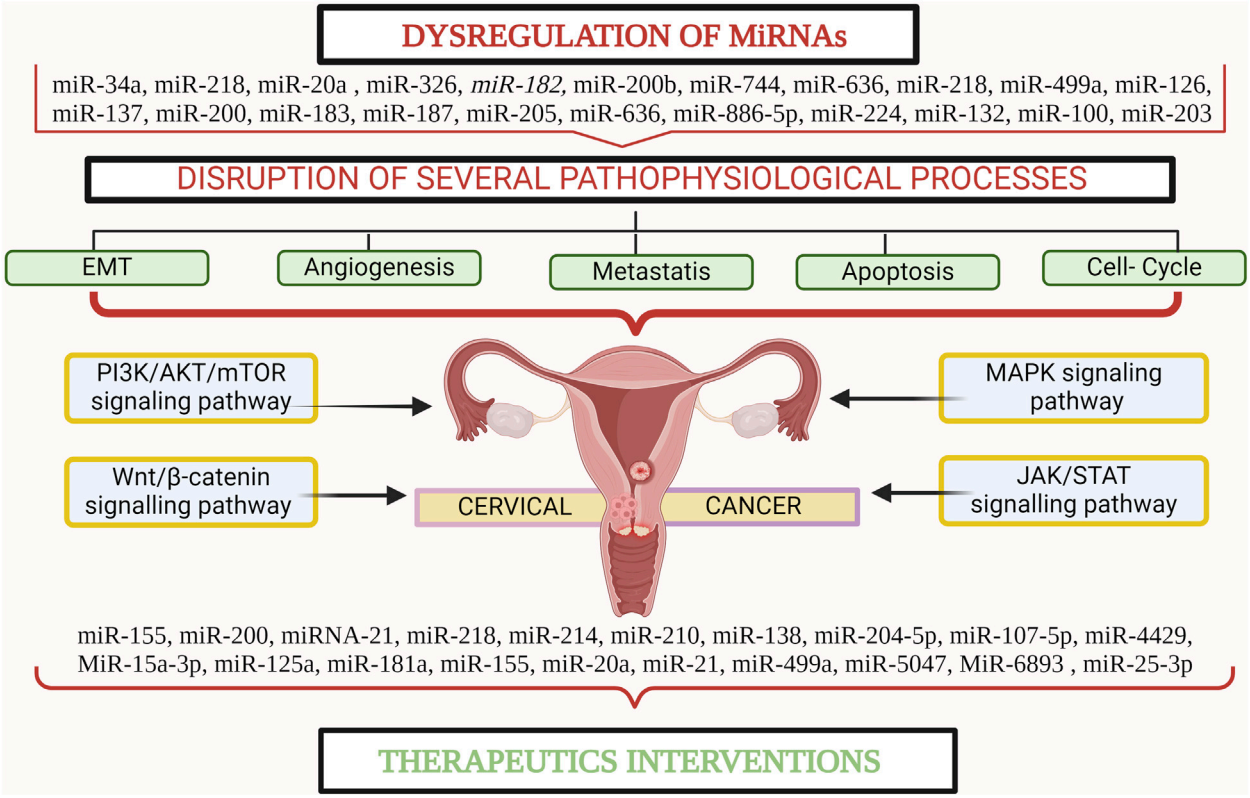


FIGURE 1
miRNA plays a crucial role in the pathogenesis and progression of cervical cancer. Created with BioRender.com.

single-long transcripts that might share seed regions. The biogenesis of miRNAs can be divided into two categories: canonical and non-canonical pathways (Figure 2) (Abdelfattah et al., 2014). The canonical biogenesis pathway is the primary route for miRNA processing. In this pathway, primary miRNAs (pri-miRNAs) are transcribed from their respective genes and subsequently transformed into precursor miRNAs (pre-miRNAs) by the action of the microprocessor complex (MacFarlane and R Murphy, 2010). This complex comprises the RNA binding protein DiGeorge Syndrome Critical Region 8 (DGCR8) and the ribonuclease III enzyme Drosha. DGCR8 identifies specific motifs, including an N6-methyladenylated GGAC, within pri-miRNA (Rani and Sengar, 2022). Drosha subsequently cleaves the pri-miRNA duplex at the base of its characteristic hairpin structure, leading to the formation of a 2-nucleotide 3 overhang on the pre-miRNA. Following their generation, pre-miRNAs undergo export to the cytoplasm, facilitated by the exportin 5 (XPO5)/RanGTP complex (Shirley et al., 2022). In the cytoplasm, to process pre-miRNAs, Dicer binds with a double-stranded RNA-binding domain (dsRBD) protein known as TAR RNA-binding protein (TRBP), eliminating the terminal loop and forming miRNA duplexes (Ergin and Çetinkaya, 2022). The miRNA duplex, produced by Dicer, is loaded onto an Argonaute (AGO) protein, resulting in an effector complex known as the RNA-induced silencing complex (RISC). TRBP and AGO proteins go through post-translational changes, which affect their capacity to control Dicer processing, RISC formation, and miRNA activity. The guide strand of the miRNA-RISC complex directs RISC to target mRNAs by forming complementary base pairs, resulting in mRNA destabilization, translational repression, or cleavage (MacFarlane and R Murphy, 2010). This process, mediated by AGO proteins, is central to the functionality of RISC, enabling it to recognize and interact with target mRNAs, thus initiating post-transcriptional gene silencing, which plays a pivotal role in directing target mRNA recognition and gene regulation. Precise function of the miRNA-RISC complex in fine-tuning gene expression (Iwakawa and Tomari, 2022). This intricate process ultimately regulates gene expression and precisely orchestrates diverse cellular functions. Notably, the selection between 5p and 3p strands of the mature miRNA duplex for loading into AGO is a dynamic process influenced by factors such as thermodynamic stability and nucleotide composition (Zaporozhchenko et al., 2020). The guide strand, chosen for its ability to recognize target miRNAs and regulate genes, earns its name. Meanwhile, its counterpart, the passenger strand, is discarded. This meticulous selection showcases the miRNA machinery's adaptability to the cellular environment (Treiber et al., 2019). Alternatively, researchers have uncovered an additional noncanonical pathway for miRNA biogenesis. Some miRNAs bypass Drosha-mediated processing by undergoing direct cleavage by Dicer or by splicing-independent processing of introns (Yang and Lai, 2011). In certain scenarios, miRNAs emerge independently of Dicer with the help of endonucleases or exonucleases. Mirtrons, for instance, are intriguing miRNAs generated from spliced introns that form hairpin structures akin to pre-miRNAs, effectively circumventing Drosha processing (Cipolla, 2014). Additionally, small nucleolar RNAs (snoRNAs) and transfer RNAs (tRNAs) emerge as unlikely contributors to non-canonical miRNA biogenesis, unveiling the diverse origins of these regulatory molecules. Moreover, certain pre-miRNAs produce pre-miRNAs with suboptimal structures, necessitating monouridylation

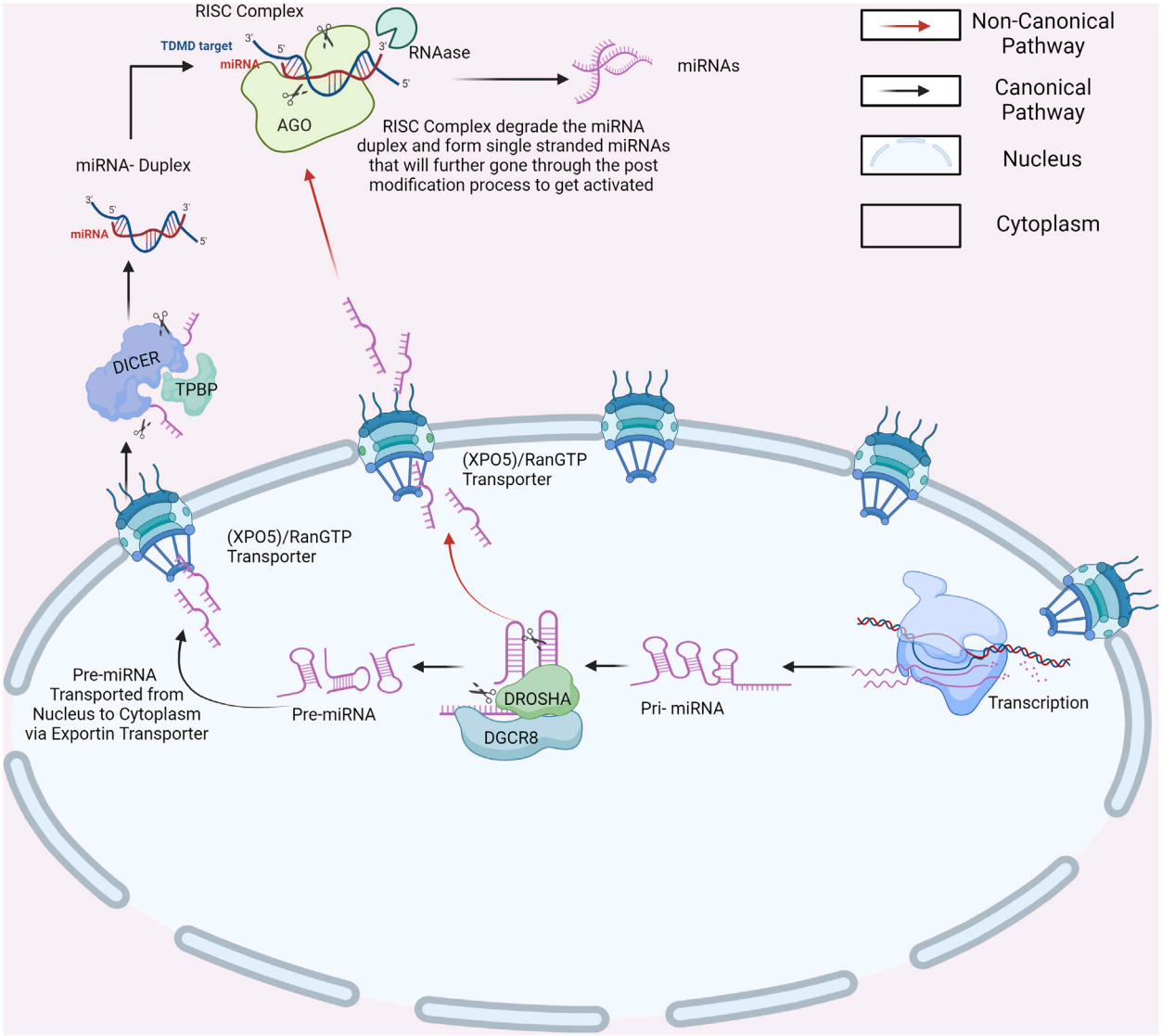


FIGURE 2
Canonical and Non-Canonical Pathways of miRNA Biogenesis. Created with BioRender.com.

by TUTase for efficient processing (Havens et al., 2012). In this intricate pathway, a truncated pre-miRNA is generated by Drosha, shuttled to the cytoplasm, and loaded onto Argonaute 2 (AGO2) without Dicer involvement. Subsequent cleavage by AGO2 and trimming by the exonuclease PARN further refined the miRNA, highlighting the multifaceted nature of miRNA biogenesis (Abdelfattah et al., 2014; Santovito and Weber, 2022). These diverse pathways underscore the complexity and adaptability of cellular mechanisms for generating functional miRNAs for precise post-transcriptional gene regulation. As miRNAs undergo biogenesis, they have the potential to influence a variety of cellular processes including cell proliferation, differentiation, and apoptosis (Herrera-Carrillo and Berkhout, 2017). Altered miRNA function can disturb gene regulation, fueling abnormal cell behavior and advancing various cancers, including CC (Iorio and Croce, 2012). In cancer cells, miRNAs may act as oncogenes, promoting tumor growth and metastasis, or as TS, inhibiting tumorigenesis and metastasis. Dysregulated miRNA expression can affect the functionality of genes involved in essential cancer-related processes such as cell cycle control, apoptosis, DNA repair, and epithelial-mesenchymal transition (EMT) (Medina and Slack, 2008). Therefore, the complex connection between miRNA biogenesis and cancer pathogenesis emphasizes the critical role of miRNAs in driving oncogenic processes as well as their possible use as diagnostic and therapeutic targets in the management of cancer.

3 Role of miRNAs in initiation, proliferation, and progression of cervical cancer

miRNAs have emerged as important participants in the development, proliferation, and progression of CC. These short non-coding RNA molecules have been shown to control gene expression at the post-transcriptional level, impacting a variety of cellular processes implicated in cancer formation. Numerous studies have discovered distinct miRNAs that are dysregulated in CC, resulting in the disruption of essential signaling networks (Ortiz

et al., 2023). Furthermore, miRNAs have been shown to influence the expression of genes involved in cell cycle regulation, apoptosis, angiogenesis, and metastasis, all of which play key roles in tumor initiation, proliferation, and progression (Doghish et al., 2023). Moreover, miRNAs have been implicated in the regulation of EMT, a process that is crucial for metastasis and cancer progression. Certain miRNAs act as either suppressors or promoters of EMT-associated pathways, thereby affecting the invasive potential of CC cells. Overall, miRNAs play a significant role in the initiation, proliferation, and progression of CC by regulating gene expression and influencing key cellular processes involved (Jafri et al., 2017). Moreover, dysregulation of specific miRNAs in CC can lead to the activation of critical signaling pathways, contributing to tumor progression. Several signaling pathways such as the PI3K/AKT/mTOR pathway, the Wnt/β-catenin pathway, the MAPK/ERK pathway, and JAT/STAT pathways are regulated by miRNAs in CC, further highlighting the importance of miRNAs in cancer development (Hasan et al., 2023).

Furthermore, alterations in miRNA expression levels disrupt the balance between oncogenic and tumor-suppressor proteins, thereby influencing cellular development and promoting tumorigenesis (Sharma et al., 2014). The intricate interplay between these factors contributes to the initiation and progression of cancer by dysregulating critical cellular processes such as proliferation, deletion, and mutation of miRNA loci (Jafri et al., 2017). This dysregulation is often attributed to perturbations in the transcription factors and epigenetic silencing mechanisms. Moreover, a distinct subset of miRNAs known as epi-miRNAs exert regulatory control over tumor suppressor genes indirectly by manipulating epigenetic machinery effectors, including DNA methyltransferases, histone deacetylases, and genes within the polyoma suppressor complex (Chengizkhan et al., 2024). This complex regulatory landscape underscores the multifaceted role of miRNAs in cancer development and highlights their potential as targets for therapeutic interventions (Sharma et al., 2014). Additionally, the mechanisms driving CC have unveiled a deeper understanding of the role of miRNAs. Interestingly, a study examines pri-miR-34a levels in various cervical tissues, revealing significant reduction in cervical intraepithelial neoplasia (CIN) and CC compared to normal epithelium, even in early-stage lesions. Additionally, HR-HPV infection correlates with lower pri-miR-34a expression. Experimental findings suggest that HR-HPV E6 induces pri-miR-34a downregulation, potentially via a p53-dependent pathway, observed in transfected cells. These results underscore pri-miR-34a's role as an early indicator of CC development, implicating HR-HPV E6 in its pathogenesis (Li et al., 2010). Moreover, another study demonstrated that miR-20a is significantly upregulated in CC patients, correlating with lymph node metastasis, histological grade, and tumor size. Inhibiting miR-20a with stable anti-miR-20a cell lines suppresses tumor progression by affecting cell cycle, apoptosis, and metastasis *in vitro* and *in vivo*. Additionally, miR-20a directly targets TIMP2 and ATG7, suggesting its role in regulating CC proliferation, migration, and invasion. These findings propose miRNAs as potential therapeutic agents for CC (Bao et al., 2013). Furthermore, a study investigated the role of miR-499a in CC development, finding its significant upregulation in CC cells. Overexpression of miR-499a promotes cancer cell proliferation, migration, invasion, and resistance to apoptosis, while inhibition suppresses these effects. The study identifies SOX6 as a direct target of miR-499a, mediating its oncogenic effects. Inhibiting miR-499a enhances the anticancer effects of cisplatin in a mouse model. These findings propose miR-499a as a potential therapeutic target in cervical cancer (Chen Y. et al., 2020). Moreover, a study investigates microRNA-137 (miR-137) in CC, assessing its expression, clinical relevance, and functional role. MiR-137 downregulation is found in CC cells and tumors, correlating with shorter overall survival in patients. Upregulation of miR-137 inhibits CC proliferation and migration *in vitro* and *in vivo*, potentially by targeting enhancer of zeste homolog 2 (EZH2). EZH2 overexpression reverses miR-137-induced tumor suppression, suggesting its involvement in miR-137-mediated CC regulation. This highlights miR-137 as a negative biomarker for CC prognosis and a potential therapeutic target (Zhang et al., 2018). The study investigates the role of miR-187, a newly identified cancer-related microRNA, in CC. It reveals decreased miR-187 levels in CC tissues and cell lines, with low miR-187 associated with decreased overall survival and progression-free survival rates in patients. Overexpression of miR-187 inhibits proliferation and promotes apoptosis in CC cells, while knockdown enhances proliferation and inhibits apoptosis. Forced expression of miR-187 suppresses subcutaneous tumor growth in mice. Additionally, FGF9 is identified as a downstream target of miR-187, and targeting FGF9 is crucial for miR-187s tumor-suppressive effects in CC cells (Liang et al., 2023). In addition to this (Table 1), highlights several miRNAs that play pivotal roles in the initiation, proliferation, and progression of CC, emphasizing their critical impact on key aspects of CC progression.

3.1 Role of miRNAs in cervical cancer progression

miRNAs contribute to dynamic and complex processes involved in CC progression. It may cover various aspects, including modulation of signalling pathways and regulation of key cellular processes. The interplay between miRNAs and specific molecular targets suggests the potential role of miRNAs in CC progression (Melo and Esteller, 2011). Approximately half of all miRNAs are located within genomic regions implicated in cancer, underscoring their dual roles as oncogenes, which promote cancer development, and TS genes, which inhibit tumor growth. This distinct nature underlines the complex regulatory processes of miRNAs in CC progression (Xu et al., 2016). Dysregulation of specific miRNAs has been associated with various aspects of CC progression, including cell proliferation, invasion, and metastasis (Pardini et al., 2018). The expression profiles of miRNAs vary significantly depending on the histological type of the tissue and pathological or non-pathological conditions. This discrepancy is evident in the distinct expression patterns observed in normal and cancerous tissues (Causin et al., 2021). Therefore, understanding the expression patterns of several miRNAs in the context of CC is crucial for understanding their implications in cancer progression.

Interestingly, a study retrieved data from 24 studies to determine the role of miRNAs in CC and found that the upregulation and downregulation of miR-29a and miR-21 were significantly linked with the progression of CC (Xu et al., 2016). Similarly, another study has been conducted and revealed that miR-132 is a pivotal regulator in the progression of CC, exerting a significant influence on tumor growth and advancement. It plays a crucial role in suppressing the

TABLE 1 miRNAs affects the progression of cervical cancer.

miRNA	*In vitro*/ In silico/*In vivo*	Mechanism of action	Target genes	Oncomirna/ suppressor	Reference
miR-34a	*In vitro*	• P53-dependent pathway • Cell cycle progression • Cellular senescence	P18Ink4c, CDK4, CDK6, Cyclin A, E2, E2F1, BCL2, BIRC3	Oncomirna	Li et al. (2010)
miR-218	*In vitro*	• Focal adhesion	LAMB3	suppressor	Yamamoto et al. (2013)
miR-20a	*In vivo* and *In vitro*	• lymph node metastasis	ATG7 and TIMP2	Oncomirna	Bao et al. (2013)
miR-326	*In vivo* and *In vitro*	• Cell proliferation	TCF4	suppressor	Zhang et al. (2020)
miR-182	*In vivo* and *In vitro*	• Disrupting cell proliferation • Apoptosis • Cell cycle pathways	FOXO1	Oncomirna	Tang et al. (2013)
miR-200b	*In vitro*	• Induced the decrease of cell ability • Increased cell apoptosis • Attenuated ability of cell migration and invasion	FoxG1	Oncomirna	Choi et al. (2022)
miR-744	*In vitro*	• Apoptosis induction	Bcl-2,	suppressor	Chen and Liu (2016)
miR-636	*In vitro*	• Represses cell survival	CDK6/Bcl-2	suppressor	Hu et al. (2020)
miR-218	*In vitro*	• Reduced the proliferation	cisplatin (CDDP)	suppressor	Li et al. (2012)
miR-499a	*In vivo*	• Enhanced the proliferation • Cell cycle progression • Colony formation • Apoptosis resistance • Migration and invasion	SOX6	Oncomirna	Chen et al. (2020a)
miR-137	*In vitro* and *In vivo*	• Cell proliferation and migration	EZH2	Oncomirna	Zhang et al. (2018)
miR-133b	*In vitro*	• Enhances cell proliferation • Colony formation	MST2, CDC42, ERK1 and ERK2, RHOA, AKT1	Oncomirna	Qin et al. (2012)
miR-200	*In vitro*	• Metastatic inhibit (EMT)	ZEB1, ZEB2, Sip 1	suppressor	Gregory et al. (2008)
miR-372	*In vitro*	• Cell growth induce arrest in S/G2 phase of cycle	CDK1, Cyclin A1	Oncomirna	Tian et al. (2011)
miR-187	*In vitro*	• Inhibition of the growth of CC cells by targeting FGF9	FGF9	suppressor	Liang et al. (2023)
miR-205	*In vitro*	• Promotion of angiogenesis • Tumour progression by activating the Akt signalling pathway via TSLC1 upregulation	TSLC1	suppressor	Zhang et al. (2019a)
miR-636	*In vitro*	• Inhibition of cell proliferation • induction of cell apoptosis by targeting CDK6 and Bcl-2	CDK6 and Bcl-2	suppressor	Hu et al. (2020)
miR-145	*In vitro*	• Cell motility	IGF-1	suppressor	Wang et al. (2015)
miR-886-5p	*In vitro*	• Cell transformation and B progression	AX	Oncomirna	Li et al. (2011a)
miR-873-5p	In silico and *In vitro*	• Suppressed the expressions of Jag1, Maml2 and Hey1	ZEB1	Oncomirna	Wen et al. (2021)
miR-224	*In vitro*	• Associated with aggressive progression and poor prognosis	RASSF8	Oncomirna	Huang et al. (2016)
miR-126	*In vitro*	• Promotion of CC advancement through PI3K/AKT/mTOR pathway activation	ZEB1	suppressor	Xu et al. (2019)
miR-214	*In vitro*	• Promotion of CC advancement through PI3K/AKT/mTOR pathway activation	Bcl2l2	suppressor	Wang et al. (2013)
miR-100	*In vitro*	• Growth, cell cycle and apoptosis	PLK1	suppressor	Li et al. (2011b)

expression of RDX oncogene, thereby facilitating the proliferation and progression of CC cells. This mechanism underscores the intricate involvement of miR-132 in driving the aggressive nature of CC and highlights its potential as a therapeutic target for intervention strategies aimed at impeding disease progression (Pardini et al., 2018). Furthermore, to investigate the clinical importance of miR-224, a study performed an experiment using 126 pairs of CC cell lines along with normal cells and observed upregulation of miR-224, which was aggressively associated with the progression of CC (Shen et al., 2013). Additionally, a study investigated the clinical relevance of miR-145 in CC by analysing 114 pairs of human CC tissue samples and adjacent normal tissues. Using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays, observed a significant downregulation of miR-145 expression in CC cell lines. This suggests a potential association between decreased miR-145 expression and the progression of CC (Wang et al., 2015). RT-qPCR was used to assess the expression levels of miRNA-873-5p in CC specimens and cell lines. These findings provided compelling evidence indicating that decreased expression of miRNA-873-5p serves as an unfavorable prognostic factor for CC patients (Wen et al., 2021). To uncover a unique miRNA fingerprint for CC and cervical intraepithelial neoplasia (CIN), a study employed a cutting-edge miRNA microarray was used to delve into the intricate world of miRNA expression profiles, comparing CC, CIN, and normal cervical tissues, and Real-time RT-PCR was used to validate the expression of miRNAs. Interestingly, functional studies were performed to determine the downregulation of miR-218 and upregulation of miR-21 among all miRNAs and concluded that these miRNAs may be involved in the progression of cervical neoplasm (Zeng et al., 2015). Furthermore, to delve deeper into the functional significance of miR-200b in CC progression, a study was conducted to elucidate its involvement in CC development. This study analyzed 30 paired CC samples to explore the role of miR-200b in CC. Remarkably, these findings revealed a notable upregulation of miR-200b in cancer tissues, accompanied by the downregulation of FoxG1, which was subsequently identified as a target gene of miR-200b (Zeng et al., 2016). Moreover, to unveil the pathogenic mechanisms underlying CC, an intriguing investigation revealed the upregulation of miR-182 and miR-183 in CC cell lines. Conversely, the expression of nine mRNAs (miR-211, 145, 223, 150, 142-5p, 36, 328, 195, 199b, and 142-3p) was consistently identified across CC cell lines. Particularly, heightened expression of miRNA-182 was also observed in primary CC, correlating with the progression of advanced CC. These findings implicate miR-182 as an onco-miRNA in CC progression, and its dysfunction is linked to CC pathogenesis by disrupting cell proliferation (Tang et al., 2013).

3.2 miRNAs affect the cell cycle during cervical cancer progression

The intricate interplay between miRNAs and the cell cycle stands as a pivotal determinant in the progression of CC. Therefore, understanding the consequences of miRNAs on the complicated procedure of cell cycle regulation is crucial as errors in this pathway play an integral part in the emergence and progression of CC (Choi et al., 2022). However, emerging research has shed light on the multifaceted roles of miRNAs in modulating the cell cycle dynamics in CC. Through their regulatory effects on the cell cycle, miRNAs wield significant influence over the progression and proliferation of CC cells (Shen et al., 2020).

In this context, examining the intricate interaction between miRNAs and the cell cycle reveals unique insights into the pathophysiology of CC. As a result, researchers have delved into the complex web of CC development to determine the functional significance of miRNAs in interrupting the cell cycle. Amidst this exploration, Baicalein, known for its anti-cancer properties in CC treatment, was investigated for its mechanism of action involving circular RNA (circRNA) hippocampus abundant transcript 1 (circHIAT1) and miRNA-19a-3p (miR-19a-3p) (Hu et al., 2021). Cell viability and colony formation assays revealed baicalein's inhibition of CC cell growth and cell cycle progression, coupled with enhanced apoptosis. Notably, miR-19a-3p downregulation in baicalein-treated CC cells and its overexpression mitigated baicalein-induced inhibition of CC progression (Hu et al., 2021). Furthermore, a study focused on unravelling the role and molecular mechanism of miRNA-29a in CC progression. Through a series of experiments utilizing various molecular and cellular techniques, the researchers found that miR-29a expression was diminished in CC tissues and cells, correlating negatively with hypermethylation of the p16 promoter. Additionally, functional assays revealed that miR-29a inhibited cell proliferation and induced cell cycle arrest in CC cells. Mechanistically, miR-29a was found to modulate the methylation pattern of the p16 gene by targeting DNA methyltransferases (DNMT)3A and (DNMT)3B (Robaina et al., 2015). These findings shed light on the epigenetic regulation of the tumour suppressor p16 by miR-29a, presenting a novel mechanism in CC progression (Robaina et al., 2015). A study, through a comprehensive analysis of the cancer genome atlas (TCGA) data, unveiled a significant reduction in miR-140-3p levels in CC tissues. Subsequently, RT-qPCR assays confirmed this negative correlation between miR-140-3p expression and both CC tissues with various cell lines. To elucidate the specific role of miR-140-3p, researchers employed miRNA mimics to enforce its expression in Caski and C33A cells. Remarkably, the overexpression of miR-140-3p notably hindered CC cell proliferation, as evidenced by Cell Counting Kit (CCK-8) assays. Further validation through Western blot analysis revealed a consequential induction of cell cycle arrest, supported by decreased levels of cell cycle-related proteins Cyclin A, Cyclin B1, and Cyclin D1 (Ma et al., 2020). Moreover, the downregulation of miR-372 was observed in cervical carcinoma tissues adjacent to normal cervical tissues. An investigation using growth curve and Fluorescence-Activated Cell Sorting (FACS) tests showed that aberrant expression of miR-372 causes arrest in the S/G2 stages of the cell cycle and decreases cell growth in HeLa cells. The study used bioinformatics predictions to identify cyclin-dependent kinase 2 (CDK2) and cyclin A1 as putative targets of miR-372. A fluorescence reporter test was used to confirm this hypothesis (Tian et al., 2011).

3.3 miRNAs affect apoptosis during cervical cancer progression

The influence of miRNAs on apoptosis appears to be a critical factor in the intricate domain of CC development and requires further exploration (Wang and Chen, 2019). Interestingly, several

studies have demonstrated the crucial role of miRNAs in modulating apoptosis in CC development. Apoptosis is a basic mechanism that maintains homeostasis and eliminates aberrant cells, including cancer cells (Miao et al., 2020). Therefore, dysregulation of this fundamental mechanism is a hallmark of the progression of numerous carcinomas such as CC. Hence, outlining the intricate relationships between miRNAs and apoptosis is essential for understanding the molecular processes that drive the advancement of CC. Meanwhile, a study investigated the oncogenic role of miRNA in nine CC cell lines and observed that elevated expression of miR-181a significantly modulated apoptosis in cell lines. Later, the inhibited expression of miR-181a promoted apoptosis in CC cells, suggesting that miR-181a might be an oncogene in CC cells (Xu et al., 2016). The mRNA and protein expression levels of Bcl-2 in HeLa cells were increased by miR-34a-5p suppression, but decreased by miR-34a-5p overexpression. Bcl-2 is a direct target gene of miR-34a-5p and participates in the effects of miR-34a-5p on HeLa cell viability, migration, invasion, and apoptosis. Suppression of miR-34a-5p promoted the viability, migration, and invasion of HeLa cells by increasing the expression of Bcl-2 (Wang X. et al., 2018). Moreover, overexpression of Bcl-2 significantly promoted cell viability, migration, and invasion and had no influence on cell apoptosis. The suppression of Bcl-2 showed the opposite effect, with an increase in apoptosis. Therefore, Bcl-2 expression is downregulated when miR-34a-5p is overexpressed, which prevents human CC cells from proliferating and promotes their death (Wang X. et al., 2018).

Similarly, another study demonstrated the intricate role of miR-7 in HeLa and C-33A cell lines, shedding light on its profound impact on cell viability and apoptosis. The results revealed a remarkable association. Overexpression of miR-7 significantly suppressed cell viability coupled with a marked increase in apoptosis. In contrast, miR-7 inhibition had the opposite effects, emphasizing its role in cellular dynamics. Additionally, an X-linked inhibitor of apoptosis protein (XIAP), an oncogene, was shown to be a novel target of miR-7 in HeLa and C-33A cells, its effects of miR-7 were mitigated by the ectopic production of XIAP. These findings suggest that miR-7 regulates apoptosis and malignancy by targeting XIAP, thereby offering therapeutic avenues for cancer treatment (Liu et al., 2013). Furthermore, miR-148a plays a crucial role in regulating the growth, apoptosis, invasion, and migration of CC cells by targeting the regulator of ribosome synthesis 1 (RRS1). The expression levels of miR-148a and RRS1 were analyzed in CC tissues and cell lines. Downregulation of miR-148a and upregulation of RRS1 have been observed in CC tissues and cells, correlating with poor clinicopathological characteristics. Functional assays revealed that upregulation of miR-148a inhibited cell proliferation, migration, and invasion while promoting apoptosis in CC cells. Furthermore, RRS1 was identified as a direct target of miR-148a, and miR-148a negatively regulated RRS1 expression. These findings underscore the tumor-suppressive function of miR-148a in CC development by modulating RRS1 expression, suggesting its potential as a therapeutic target for CC (Zhang Y. et al., 2019). miR-150 exerts a significant influence on CC cell survival and growth, and its inhibition leads to the suppression of these actions. Furthermore, miR-150 drives cell cycle progression from G1/G0 to S phase, thereby enhancing cell growth. Notably, miR-150 modulated the expression of key cell cycle- and apoptosis-related genes, including CyclinD1, p27, BIM, and FASL. Additionally, miR-150 directly targets the 3′-UTR of FOXO4, a regulator of CyclinD1, p27, BIM, and FASL expression, thereby reducing FOXO4 expression levels. This multifaceted regulatory role underscores the significance of miR-150 in CC progression (Li et al., 2015).

Moreover, *in vitro* investigations showed that a decreased level of miR-200b lowered cell ability and cell apoptosis and altered the cell migration ability in both C33A and HeLa cells (Choi et al., 2022). Additionally, to distinguish the role of miR-636 in CC progression or inhibition, a study has shown that miR-636 is significantly downregulated in CC cells, and *in vitro* results suggested that the upregulation of miR-636 can inhibit cell proliferation and induce cell apoptosis. Moreover, cyclin-dependent kinase 6 (CDK6) and B-cell lymphoma 2 (Bcl-2) are targets of miR-636 (Hu et al., 2020). Bioinformatic analysis of miR-122 identified RAD21 as its target gene. A study revealed that overexpression of miR-122 induces cell cycle arrest and promotes apoptosis by targeting RAD21 (Yang et al., 2022). Regulation of apoptosis showed that miR-146a exerts an effect on the regulation of Th17 cell differentiation, and further studies have revealed that miR-146a enables its target gene TRAF6 to regulate CC cell growth and apoptosis through the NF-kB signalling pathway (Li T. et al., 2019).

3.4 miRNAs affect migration, EMT, invasion, and metastasis during cervical cancer progression

Numerous investigations have unveiled the critical involvement of miRNAs in CC invasion and metastasis through their regulation of key pathways, notably the Notch, Wnt/β-catenin, and phosphoinositide-3 kinase (PI3K)-Akt pathways (Wang et al., 2016). Additionally, miRNAs play a crucial role in modulating EMT, further contributing to the metastatic cascade in CC. Several findings offer novel perspectives on the complex role of miRNAs in driving the pathogenesis of metastatic CC, underscoring their potential as therapeutic targets and prognostic markers in combating this disease (Wang and Chen, 2019). As a result, a study experimented to determine the relationship between miR-20a and progression of CC and analysed the upregulated expression of miR-20a in CC cells in comparison to normal cells by *in vitro* and *in vivo* analysis (Zhao et al., 2015). Furthermore, inhibition of miR-20a effectively halted tumour progression by influencing cell cycle regulation, apoptosis, and metastasis both *in vitro* and *in vivo*. Additionally, TIMP2 and ATG7 were identified as direct targets of miR-20a through luciferase assays and western blot analysis. These findings underscore the role of miR-20a in cervical tumorigenesis, particularly in lymph node metastasis (Zhao et al., 2015). Moreover, an investigation experimented with elucidating the correlation of miR-218 with CC and discovered the downregulation of miR-218 especially in metastatic cancer tissues. Furthermore, miR-218 expression was discovered to be related to the clinicopathological features of patients with CC. *In vitro*, overexpression of miR-218 inhibited the motility, invasion, and EMT of CC cells. Moreover, miR-218 directly suppressed the expression of SFMBT1 and DCUN1D1 mRNAs by targeting their

3′UTRs. While DCUN1D1 overexpression boosted migration and invasion without producing EMT, elevated levels of SFMBT1 produced EMT and increased migration and invasion (Li et al., 2012). Moreover, miR-204-5p displays reduced expression levels in CC cells, leading to diminished cellular functionalities such as proliferation, invasion, migration, and EMT. Through functional assays, it was established that the upregulation of miR-204-5p exerts a suppressive effect on these cancerous processes. Notably, transcription factor AP-2 alpha (TFAP2A) emerged as the primary target gene affected by miR-204-5p, with TFAP2A found to transcriptionally repress miR-204-5p in CC cells. Molecular mechanism assays confirmed the reciprocal regulation between TFAP2A and miR-204-5p. Subsequent rescued-function assays revealed that overexpression of TFAP2A could reverse the inhibitory effects of miR-204-5p upregulation on cellular processes. Altogether, the miR-204-5p/TFAP2A feedback loop enhances the proliferative and motility capabilities of CC cells, underscoring a novel regulatory mechanism with potential implications for CC therapy (Bao et al., 2013). In CC patients, reduced expression of miR-125a is observed, correlating inversely with tumour size, FIGO stage, and preoperative metastasis. Kaplan-Meier analysis indicates that higher miR-125a expression predicts better outcomes for patients. Through dual luciferase assays, the STAT3 gene is identified as a direct target of miR-125a. Functional investigations demonstrate that overexpression of miR-125a inhibits growth, invasion, and EMT of CC cells both *in vitro* and *in vivo* by downregulating STAT3 expression. Furthermore, miR-125a induces G2/M cell cycle arrest and inhibits several G2/M checkpoint proteins, suggesting its potential as a biomarker and therapeutic target in CC (Fan et al., 2015). Similarly, a study employed microarray analysis to identify molecular alterations in CC cells treated with TGFβ1 and observed a significant downregulation of miR-374c-5p compared to parental cell lines. Subsequent experiments revealed that ectopic expression of miR-374c-5p suppressed invasion and migration of TGFβ1-treated CC cells, while its knockdown enhanced these processes in parental cell lines. Mechanistically, miR-374c-5p targeted the 3′-UTR of FOXC1, resulting in decreased FOXC1 expression and subsequent suppression of snails. Clinically, low miR-374c-5p expression correlated with poor patient survival and increased lymph node metastasis in CC samples (Huang et al., 2017). Moreover, miR-374c-5p levels negatively correlated with FOXC1 expression, which was elevated in cervical cancers with lymph node metastasis. These findings underscore the pivotal role of miR-374c-5p in regulating CC metastasis via FOXC1 targeting (Huang et al., 2017). Additionally, the change in miR-499a expression either upregulation or downregulation was also associated with change on CC proliferation, formation, progression, migration, and invasion. The increased expression of miR-499a was significantly linked with cell progression. Further, experimentation revealed that the sex-determining region Y box was directly associated with a target of miR-499a. miR-499a-induced SOX6 downregulation mediated the oncogenic effects of miR-499a in CC (Chen Y. et al., 2020). Recently, a study was performed to identify the role of miR-218 and revealed that the overexpression of miR-218 plays a crucial role in tumour metastasis and reduces the proliferation in human CC cell lines HeLa and induces cell apoptosis via AKT-mTOR signalling pathway. Furthermore, miR-218 increased chemosensitivity to cisplatin *in vitro*. This intricate interplay underscores miRNAs' pivotal role in the pathological development of CC. Regulation of EMT revealed that miR-663b can directly target the 3′UTR of monoacylglycerol acyltransferase 3 (MGAT3) and can participate in the EMT regulatory process (Li et al., 2012).

3.5 MiRNAs affect angiogenesis during cervical cancer progression

miRNAs play a significant role in regulating angiogenesis during CC progression. In the context of CC, exosome miRNAs, such as miR-221-3p, have been shown to promote angiogenesis by targeting specific genes involved in this process (Xu et al., 2023). Exosomal miRNAs are secreted by CC cells and taken up by microvascular endothelial cells (MVECs). These exosomal miRNAs can modulate gene expression in MVECs, leading to changes in angiogenic processes (Aslan et al., 2019). Additionally, mir-221-3p downregulates Mitogen-Activated Protein Kinase 10 (MAPK10) expression, which in turn affects downstream factors such as Cellular-Fibrosarcoma Oncogene (c-FOS), Cellular-Jun Proto-Oncogene (c-JUN), Jun B Proto-Oncogene (JUNB), and Vascular Endothelial Growth Factor (VEGF). This cascade of events enhances the abilities of migration, invasion, and angiogenesis in CC cells (Zhang L. et al., 2019). Specifically, miR-205 has been identified as a key player in inhibiting angiogenesis in CC through various mechanisms. Downregulation of miR-205 has been shown to promote angiogenesis by activating the Akt signalling pathway via Tumour Suppressor in Lung Cancer 1 (TSLC1) upregulation. Additionally, miR-205 has been reported to inhibit proliferation, invasion, migration, and angiogenesis in CC by targeting TSLC1 (Zhang F. et al., 2019).

Furthermore, other miRNAs such as miR-187 have also been implicated in inhibiting the growth of CC cells by targeting FGF9. These findings highlight the intricate regulatory roles of miRNAs in modulating angiogenesis and tumour progression in CC (Liang et al., 2023). Moreover, by targeting the hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) mRNA, miR-296 significantly contributes to angiogenesis by lowering HGS levels and slowing down the degradation of growth factor receptors Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) and Platelet-Derived Growth Factor Receptor (PDGFR) beta (Würdinger et al., 2008). A study investigated that miR-129-5p affects cell angiogenesis, invasion, and migration by targeting the Zinc Finger Protein of the Cerebellum 2 (ZIC2) via the Hedgehog signalling pathway in CC. Tissues from 87 CC patients were analysed for miR-129-5p levels, ZIC2 mRNA and protein levels, and Hedgehog pathway components. *In vitro* assays assessed angiogenesis, invasion, and migration, while *in vivo* tumour formation in nude mice analysed angiogenesis and tumour growth (Wang YF. et al., 2018). Upregulating miR-129-5p decreased ZIC2, Shh, Gli1, Gli2, Chemokine (C-X-C Motif) Ligand 1 (CXCL1), VEGF, and Ang2 levels, inhibiting angiogenesis, migration, and invasion in CC cells. Similarly, nude mice showed inhibited tumour growth and angiogenesis (Wang YF. et al., 2018).

Furthermore, a research looked at the involvement of cervical squamous cell carcinoma (CSCC) cell-secreted exosomal miR-221-3p in tumour angiogenesis. Clinical specimens revealed a strong connection between miR-221-3p expression and microvascular density in CSCC. Experiments with CSCC cell lines and miR-221-3p modification verified its accumulation in CSCC exosomes and transferred to human umbilical vein endothelial cells (HUVECs) (Wu et al., 2019). Functional experiments have shown that CSCC exosomal miR-221-3p stimulates angiogenesis *in vitro* and tumour development *in vivo*. Bioinformatic prediction and experimental confirmation revealed thrombospondin-2 (THBS2) as a direct miR-221-3p target, and THBS2 overexpression in HUVECs inhibited miR-221-3p′s angiogenic impact. These findings indicate that CSCC-derived exosomal miR-221-3p might be used as a diagnostic biomarker and therapeutic target for CSCC progression (Wu et al., 2019). Additionally, regulation of angiogenesis observed that upregulation of miR-129–5p inhibits CC cell growth and angiogenesis in naked mice via suppression of the Hedgehog signalling pathway and negative targeting of ZIC2 (Xu et al., 2022).

4 Role of miRNAs in cervical cancer resistance to therapeutic regimens

MiRNAs have been shown to play a crucial role in CC resistance to therapeutic regimens. Studies have revealed that dysregulation of specific miRNAs can contribute to the development of resistance to promising therapies such as chemotherapy, immunotherapy, hormonal therapy and radiation therapy in CC patients. These miRNAs function by modulating the expression of target genes involved in drug efflux, DNA repair, and apoptotic pathways, thereby conferring resistance to treatment (Geretto et al., 2017). Furthermore, the dysregulation of miRNAs has been associated with the resistance of CC cells to targeted therapies, such as inhibitors of the epidermal growth factor receptor (EGFR) and VEGF. Research has also indicated that miRNAs can serve as potential biomarkers for predicting the response of CC patients to specific therapeutic interventions. Identifying the expression patterns of these miRNAs in CC tissues may offer valuable insights into the likelihood of treatment success and aid in the personalized management of patients (Wang et al., 2019). Drug resistance poses a formidable obstacle to successful cancer chemotherapy, significantly impacting patient outcomes. Indeed, more than 90% of cancer-related deaths are attributed to the development of drug resistance, underscoring the urgent need for innovative strategies to overcome this challenge (Vasan et al., 2019). Moreover, ongoing investigations are exploring the potential of utilizing miRNA-based therapeutics to overcome resistance mechanisms in CC. By targeting specific dysregulated miRNAs, researchers aim to sensitize cancer cells to conventional therapies and enhance treatment efficacy (Si et al., 2019). The evolving understanding of miRNAs and their intricate involvement in CC resistance underscores the promising prospects for integrating miRNA-based approaches into the comprehensive management of this disease. Continued research in this field holds great potential for advancing the development of innovative strategies to combat therapeutic resistance and improve outcomes for CC patients (Si et al., 2019). Numerous studies underscore the intricate role of miRNAs in orchestrating drug resistance in tumour cells. These miRNAs exert their influence by targeting genes associated with drug resistance or by modulating crucial cellular processes (Si et al., 2019). Notably, a single miRNA can target multiple genes, and its regulatory impact is often tissue-specific, underscoring the complexity of miRNA-mediated drug resistance mechanisms. Additionally (Table 2), offers a thorough summary of the miRNAs that are predominantly responsible for influencing therapeutic treatments in cancer patients. These miRNAs specifically target genes and proteins, providing insight into their complex functions in the mechanisms behind treatment resistance. But other miRNAs also show promise for advancing therapeutic approaches, underscoring their complex influence on CC therapy.

4.1 Chemotherapy

Resistance to chemotherapeutic medicines is a significant challenge for cancer therapy. Aberrant miRNA expression is linked to chemoresistance, with other variables including reduced drug absorption, enhanced DNA damage repair, apoptotic inactivation, EMT activation, and epigenetic modifications. However some miRNAs play a dual role in both contributing to chemoresistance and aiding in its overcoming (Karimi and Mollaei, 2021). Recent research illustrates the role of miRNAs in chemoresistance and chemosensitivity in CC. Chemoresistance cell lines, created by increasing chemotherapeutic drug concentrations, are extensively used to examine the function of miRNAs in chemoresistance (Masadah et al., 2021). According to the findings, overexpression of miR-499a and miR-181a in CC cells can lead to chemoresistance to cisplatin (CDDP) by targeting SRY-Box Transcription Factor 6 (SOX6) and Protein Kinase C Delta (PRKCD) (Ke et al., 2013; Chen Y. et al., 2020). Moreover, the study revealed that inhibiting the expression of miR-499a and miR-181a heightened the chemosensitivity to CDDP (Ke et al., 2013). Conversely, previous research has highlighted the role of SOX9 in activating miR130a expression by binding to its promoter (Feng et al., 2018). This activation, in turn, fosters CDDP chemoresistance by downregulating the expression of copper transporter protein 1 (CTR1), a downstream target of miR-130a. The investigation further elucidated the involvement of the SOX9/miR-130a/CTR1 axis in mediating chemoresistance to CDDP in CC (Feng et al., 2018). Moreover, it has been observed that hsc-circ-0023404 suppresses autophagy-induced apoptosis and targets miR-5047, contributing to chemoresistance (Mitra and Elangovan, 2021). Furthermore, cancer cells can develop chemoresistance by triggering EMT. Regulating EMT might help overcome chemoresistance. CC cells develop resistance to paclitaxel by overexpressing miR375, which promotes EMT by targeting E-cadherin (Banno et al., 2014). Overexpression of oncogenic miRNAs, such as miR-20a and miR-21, led to chemoresistance to CDDP and paclitaxel in CC cell lines and tissues (Xiong et al., 2017). The interaction of SMAD7, Bcl-2, survivin, c-myc, Bax, and PDCD4 with miR-21 has been linked to CDDP and paclitaxel resistance in CC cells (Mitra and Elangovan, 2021). Furthermore, depleting or silencing miR-20a in inhibitor of apoptosis-stimulating protein of p53 (iASPP) could potentially restore the expression of

TABLE 2 miRNAs implicated in therapeutic regimens of cervical cancer.

miRNA	Effect on treatments	Target genes/proteins	*In vitro*/ In silico/*In vivo*	Reference
miR-499a, miR-181a	• lead to chemoresistance to CDDP	SOX6 and PRKCD	*In vitro*	Masadah et al. (2021)
MiR-6893	• CDDP resistance through autophagy signalling in cancer cells	EMT	*In vitro*	Chen et al. (2019)
miR375	• develop resistance to paclitaxel and promotes EMT	E-cadherin	*In vitro* and *in vivo*	Shen et al. (2014)
miR-21	• chemoresistance to CDDP and paclitaxel in CC cell lines and tissues	SMAD7, Bcl-2, survivin, c-myc, Bax, and PDCD4	*In vitro*	Du et al. (2017)
miR-20a	• restore FBXL5 and BTG3 expression and overcome chemoresistance	SMAD7, Bcl-2, survivin, c-myc, Bax, and PDCD4	*In vitro*	Xiong et al. (2017)
miR-25-3p	• enhanced EMT, migration, and invasion capacities in Caski and Hela cells, as well as resistance to chemotherapy	Sema4c and snail expression	*In vitro*	Song and Li (2017)
miR-155	• greater chemosensitivity to CDDP	TP53, SMAD2 and CCND1	*In vitro*	Bayraktar and Van Roosbroeck (2018)
miR-218	• tumour development	cyclin D1 and CDK4	*In vitro* and *In vivo*	Li et al. (2022)
miR-181a	• radio-resistant CC specimens and cell lines	pro-apoptotic PRKCD gene and protein	*In vitro* and *In vivo*	Ke et al. (2013)
miR-125a	• sensitized CC cells to radiation therapy	p21 (CDKN1A)	*In vitro*	Pedroza-Torres et al. (2018)
MiR-15a-3p	• exposure to radiation • sensitivity to radiation therapy by targeting	TPD52	*In vitro* and *In vivo*	Wu et al. (2018)
miR-4429	• radio-resistant CC cells	RAD51 recombinase (RAD51)	*In vitro* and In silico	Sun et al. (2020)
miR-130a-3p	• contributes to tumour progression	ERα and AR	*In vitro* and *In vivo*	Fan et al. (2021)
miR-107-5p	• promote tumour proliferation and invasion	ERα	*In vitro* and *In vivo*	Kaur and Khatik (2020)
miR-204-5p	• control proliferation and invasion of endometrial carcinoma cells	TrkB-STAT3-	*In vitro*, *In vivo* and In silico	Bao et al. (2013)
miR-138, miR-210, and miR-744	• enhancing the effectiveness of chemotherapy w	ACA and cisplatin	*In vitro*	Shen et al. (2020)
miR-214	• increase sensitivity to cisplatin	Bcl2l2	*In vitro*	Wang et al. (2013)
miR-218	• enhance sensitivity to cisplatin	AKT-mTOR	*In vitro*	Li et al. (2012)
miRNA-21	• regulating T cell function and immune response	PD-1 and PD-L1, PTEN	*In vitro*	Deng et al. (2022)
miR-200	• modulate the response to immunotherapy	EMT	*In vitro* and *In vivo*	Klicka et al. (2022)
miR-155	• modulate the response to immunotherapy	T cells and dendritic cells	*In vitro* and *In vivo*	Kalkusova et al. (2022)

F-box and leucine-rich repeat protein 5 (FBXL5) and B-cell translocation gene 3 (BTG3), thereby overcoming chemoresistance (Xiong et al., 2017). Moreover, it was found that downregulation of miR-25-3p is linked to enhanced EMT, migration, and invasion capacities in Caski and Hela cells, as well as resistance to chemotherapy. miR25-3p-mimic therapy can increase chemosensitivity by reducing Sema4c and snail expression and enhancing E-cadherin expression (Song and Li, 2017). Similarly, Overexpression of miR-155 inhibits cell growth and increases chemosensitivity to CDDP via targeting EMT. Overexpressing miR-155 boosted TP53 expression while inhibiting SMAD2 and CCND1, leading to greater chemosensitivity to CDDP (Kalkusova et al., 2022). Lower expression of miR-125a, miR-144, miR-218, miR-506, and miR-1284 was linked to poor prognosis and chemoresistance in CC cells. Inhibiting miR-218 expression was linked to tumour development. MiR-218 mimics increased miR-218 levels and sensitised CC cells to carboplatin by inhibiting cyclin D1 and CDK4 activity, resulting in decreased tumour development and weight (Dong et al., 2014).

4.2 Radiotherapy

The correlation between miRNAs and resistance mechanisms is a substantial barrier to improving radiation efficacy CC treatment. Recently several studies have demonstrated the aberrant expression of numerous miRNAs linked to the resistance in radiotherapy in CC treatment (Lizano et al., 2024). A recent *in vitro* and *in vivo* study has unveiled a significant upregulation of miR-181a in radio-resistant CC specimens and cell lines compared to their radio-sensitive counterparts. This heightened expression of miR-181a correlates with decreased sensitivity to radiation treatment, shedding light on

a potential mechanism underlying treatment resistance in CC. Additionally, miR-181a promotes resistance by targeting the pro-apoptotic Protein Kinase C Delta (PRKCD) gene. By binding to the 3′UTR of the PRKCD gene, miR-181a lowers the production of the PRKCD protein, which is implicated in apoptosis (Ke et al., 2013). In 2013 a substantial study revealed the differential expression of twenty miRNAs exhibited a consistent pattern of alteration, with 14 miRNAs overexpressed and 6 suppressed in all three radioresistant CC cell variants compared to controls (Zhang et al., 2013). Notably, a miRNA signature comprising 4 miRNAs (miR-630, miR-1246, miR-1290, and miR-3138) displayed over 5-fold increases in radioresistant cells. Further analysis demonstrated that these four miRNAs could be upregulated in CC cells by radiation treatment in both time-dependent and dose-dependent manners. Ectopic expression of these four miRNAs dramatically increased the survival proportion of irradiated CC cells. Additionally, inhibition of miR-630, one of the specific signature miRNAs, could reverse the radio resistance of CC cells (Zhang et al., 2013). Moreover, a study demonstrated that miR-125a was downregulated in patients with CC who did not respond to standard treatment. Radioresistant CC cell lines (SiHa, CaSki, and HeLa) also exhibited low levels of miR-125a compared to sensitive cell lines. miR-125a regulates the expression of p21 (CDKN1A) in CC cells. The overexpression of miR-125a sensitized CC cells to radiation therapy through the downregulation of p21. The p21 protein was found to be overexpressed in radioresistant cell lines, confirming previous studies (Pedroza-Torres et al., 2018). Furthermore, MiR-15a-3p exhibited downregulation in both CC tissues and cell lines. However, its expression significantly increased upon exposure to radiation. Remarkably, overexpression of miR-15a-3p demonstrated an inhibitory effect on cell proliferation and facilitated apoptosis in radiation-exposed cells. Moreover, tumour Protein D52 (TPD52) emerged as a direct target of miR-15a-3p. Inhibition of TPD52 led to suppressed cell proliferation and induced apoptosis. Notably, tumour xenograft experiments underscored that overexpression of miR-15a-3p heightened sensitivity to radiation therapy by targeting TPD52, highlighting its potential as a therapeutic target for enhancing treatment efficacy in CC (Wu et al., 2018). Moreover, the study investigated the role of miR-4429 in CC cell radio-sensitivity. Initially, the downregulation of miR-4429 in CC cells was validated. Crucially, its association with radio resistance was confirmed by observing its decreased expression in radioresistant CC cells. Gain- and loss-of-function assays demonstrated that miR-4429 sensitized CC cells to irradiation (Sun et al., 2020). Bioinformatics methods were used to identify RAD51 recombinase (RAD51) as a miR-4429 target. RAD51 is essential for DNA damage repair and has been linked to cell radioresistance in a variety of malignancies, including CC. Luciferase reporter tests verified the interaction between miR-4429 and RAD51. Rescue tests showed that miR-4429 increased CC cell radiosensitivity via RAD51. This work indicates miR-4429 as a possible therapeutic target for increasing the radiosensitivity of CC cells by inhibiting RAD51 (Sun et al., 2020).

4.3 Hormonal therapy

MiRNAs can target and regulate the expression of hormone receptors such as estrogen receptor α (ERα) and androgen receptor (AR). By modulating the levels of these receptors, miRNAs can influence the response to hormonal therapy in CC (Klinge, 2009). MiRNAs can target key components of hormone signalling pathways, affecting the sensitivity of cancer cells to hormonal therapy. For example, miR-130a has been shown to promote. CC cell proliferation and invasion by targeting ERα and AR (Deng et al., 2022). Specific miRNAs can serve as potential therapeutic targets in CC treatment. For instance, miR-130a-3p has been identified as a promising candidate target for the treatment of cervical cancer, as it contributes to tumour progression by suppressing ERα and AR. Moreover, miR-107-5p has been shown to promote tumour proliferation and invasion by targeting ERα in endometrial carcinoma. It may also play a role in regulating hormone receptor expression in CC (Bao et al., 2019). Similarly, a study demonstrated that miR-130a-3p promotes cell proliferation and invasion by targeting ERα and AR in CC. It could potentially influence the response to hormonal therapy in CC patients (Bao et al., 2013). Moreover, study shows that regulatory circuitry involving TrkB-STAT3-miR-204-5p has been shown to control the proliferation and invasion of endometrial carcinoma cells. This miRNA may have implications for hormonal therapy in gynaecological cancers (Bao et al., 2013). However, miR-130a In gastric cancer, miR-130a has been reported to promote migration, invasion, and proliferation by targeting Runt-related transcription factor 3 (RUNX3). Its role in regulating hormone receptors and response to hormonal therapy in CC warrants further investigation (Jiang et al., 2015). Altogether, miRNAs have the potential to serve as powerful predictors of response to hormonal therapy also in CC patients.

4.4 Immunotherapy

In recent years, there has been growing interest in understanding the role of miRNAs in the context of immunotherapy for cervical cancer. Immunotherapy aims to harness the body's immune system to target and destroy cancer cells (Kaur and Khatik, 2020). Therefore, some studies have demonstrated the involvement of miRNA in immunotherapy response subjected to cervical cancer. Recently, it has been noted that miR-138, miR-210, and miR-744 have been shown to improve sensitivity to anticancer agents like ACA and CDDP, thereby enhancing the effectiveness of chemotherapy which indirectly enhances the response of the body against CC cells (Phuah et al., 2013). Moreover, by inhibiting Bcl2l2 expression, miR-214 can increase sensitivity to cisplatin. This inhibition accelerates apoptosis and decreases cell growth by upregulating proapoptotic proteins like Bax, caspase-9, caspase-8, and caspase-3 which somewhere triggers the immune response in order to treat CC (Wang et al., 2013). Similarly, Through the AKT-mTOR signalling pathway, miR-218 can enhance sensitivity to CDDP in CC, making the cancer cells more responsive to chemotherapy (Li et al., 2012). Moreover, miRNA-21 has been shown to target immune checkpoint molecules such as Programmed cell Death protein 1 (PD-1) and Programmed Death-Ligand 1 (PD-L1), which are crucial for regulating T cell function and immune response in the tumour environment. Therefore, targeting miRNA-21 in combination with immunotherapies may enhance the efficacy of treatment by reversing immune suppression

and promoting antitumour immunity (Peralta-Zaragoza et al., 2016). Whereas, the miR-200 family members (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) are known to regulate EMT, a process involved in cancer metastasis. Emerging evidence suggests that miR-200 family members may modulate the response to immunotherapy in CC by influencing the tumour microenvironment and immune cell infiltration suggests a promising avenue in enhancing the efficacy of immunotherapy to treat CC (Klicka et al., 2022). Additionally, recent studies have suggested that miR-155 may modulate the response to immunotherapy by regulating the function of immune cells, such as T cells and dendritic cells (Kalkusova et al., 2022). However, Further research is needed to properly understand the roles of miRNAs in regulating the response to immunotherapy and to explore their potential as therapeutic targets in CC treatment.

5 Interplay between miRNAs and signalling pathways in cervical cancer

CC is a complex disease characterized by the dysregulation of various signaling pathways, and the interplay between miRNAs and these pathways has garnered significant attention in cancer research. miRNAs have been implicated in the regulation of various signaling pathways involved in CC, including the PI3K/Akt, Wnt/β-catenin, JAK/STAT, and Notch pathways. miRNA-mediated regulation can influence crucial processes such as cell proliferation, apoptosis, and metastasis (Manzo-Merino et al., 2014). The PI3K/AKT/mTOR pathway is frequently dysregulated in CC and plays a crucial role in cell proliferation, survival, and metastasis. miRNAs have been implicated in modulating this pathway by targeting key components such as PI3K, AKT, and mTOR (Bahrami et al., 2017).

Similarly, aberrant activation of the Wnt/β-catenin pathway has been implicated in CC development and progression. miRNAs, such as members of the miR-200 family, have been found to suppress the Wnt/β-catenin pathway by targeting regulators such as ZEB1 and ZEB2, thereby inhibiting epithelial-mesenchymal transition (EMT) and metastasis (Zhang et al., 2024). Conversely, miRNAs, such as miR-21 and miR-135a, promote Wnt/β-catenin signaling by targeting negative regulators, such as APC and GSK-3β, leading to enhanced tumor growth and invasiveness. The MAPK signaling pathway is similarly dysregulated and critical for cell proliferation, survival, and differentiation (Lei et al., 2020). miRNAs have been shown to regulate MAPK pathway activity by targeting components including MAPK kinases and phosphatases. For example, miR-9 has been demonstrated to target MAPK1, which regulates the proliferation and migration of CC cells. Additionally, the JAK/STAT pathway is involved in CC progression and immune evasion. miRNAs can influence JAK/STAT signaling by targeting cytokines, receptors, and downstream effectors. Dysregulation of JAK/STAT pathway activation contributes to CC development and progression. Overall, the interplay between miRNAs and signaling pathways in CC is complex and multifaceted (Valle-Mendiola et al., 2023). Understanding the specific miRNA-mediated regulatory networks within signaling pathways can provide valuable insights into the molecular mechanisms underlying CC, paving the way for the development of novel therapeutic strategies.

5.1 PI3K/AKT/mTOR signaling pathway

The phosphoinositide-3 kinase (PI3K), AKT, and mammalian target of rapamycin (mTOR) signaling pathway stand as a pivotal player in driving CC progression, its dynamics meticulously influenced by miRNAs. miRNAs are adept at orchestrating gene expression (Yu et al., 2022). The pathway's dysregulation fuels tumour development, bolstering growth, invasiveness, and metastasis in CC. Within this intricate landscape, numerous miRNAs have emerged as potent regulators of PI3K/AKT/mTOR, either directly engaging pathway components or indirectly modulating its activity by targeting upstream regulators or downstream effectors (Bahrami et al., 2017). Notable examples include miR-21, miR-214, and miR-126, implicated in promoting CC advancement through PI3K/AKT/mTOR pathway activation, contrasting with miR-145, miR-133a, and miR-218, which act as TS by stifling pathway activation. Deeper insights into the nuanced interplay between miRNAs and the PI3K/AKT/mTOR pathway offer promising avenues for therapeutic intervention and the identification of biomarkers essential for navigating CC management effectively (Doghish et al., 2023). Lately, miR-99b directly targets and negatively regulates the expression of mTOR in CC cells. This downregulation of mTOR expression is crucial in inhibiting the PI3K/AKT/mTOR pathway, which is known to play a significant role in cell proliferation and tumour growth. miR-99b overexpression leads to reduced levels of key proteins in the PI3K/AKT/mTOR signalling cascade, including PI3K, AKT, mTOR, and ribosomal protein S6 kinase (p70S6K) (Li YJ. et al., 2019). By targeting these components, miR-99b disrupts the signalling pathway at multiple levels, inhibiting downstream signalling responses that promote cell proliferation, migration, and survival. Through its regulatory effects on the PI3K/AKT/mTOR pathway, miR-99b suppresses cell proliferation, invasion, and migration in CC cells. This inhibition of cellular activities is essential in slowing down the progression of CC and reducing the aggressiveness of the disease (Li YJ. et al., 2019). Similarly, a study demonstrated that miRNA-383 suppresses the PI3K-AKT-MTOR signalling pathway by targeting and down-regulating PARP2. that miR-383 can negatively regulate PARP2, which in turn leads to the inhibition of PARP2 gene expression. This downregulation of PARP2 by miR-383 results in decreased expression of key components of the PI3K-AKT-MTOR pathway, including PI3K, AKT, mTOR, and p70S6K. By targeting PARP2, miR-383 effectively disrupts the signaling cascade of the PI3K-AKT-MTOR pathway, thereby inhibiting its activity, and potentially attenuating CC progression (Teng et al., 2018). Moreover, a study investigated the functional role and molecular mechanism of miR-125 in CC. Then qRT-PCR was employed to detect miR-125 and VEGF mRNA expression, and western blot analysis to assess protein levels of various markers including VEGF, E-cadherin, N-cadherin, vimentin, AKT, p-AKT, PI3K, p-PI3K, and MTT. Further, transwell assays were utilized to evaluate CC cell progression, including cell viability, migration, and invasion. Consequently, revealed that miR-125 was downregulated while VEGF was upregulated in both CC tissues and cell lines CaSki and SiHa. MiR-125 was found to inhibit proliferation, invasion, and migration by targeting VEGF in cervical cancer. Additionally, miR-125 negatively regulated VEGF expression in CC tissues.

Furthermore, demonstrated that miR-520d-5p inhibited the activation of the PI3K/AKT signaling pathway (Fu et al., 2020).

5.2 Wnt/b-catenin signaling pathway

The interplay between miRNAs and the Wnt/β-catenin pathway is pivotal in CC progression. miRNAs, regulate gene expression post-transcriptionally, impacting the activity of the Wnt/β-catenin pathway, crucial for cancer development. Various miRNAs directly target Wnt/β-catenin components, exerting dual effects (Peng et al., 2017). For instance, miR-200 family members suppress Wnt/β-catenin signaling, inhibiting EMT and metastasis (Su et al., 2012). Conversely, miR-21 and miR-135a enhance Wnt/β-catenin activation, promoting tumour growth (Gebrie, 2022). Moreover, the pathway reciprocally influences miRNA expression, shaping CC pathogenesis. Wnt ligands are pivotal signaling molecules that initiate the Wnt/β-catenin pathway upon binding to membrane protein receptors (Yang et al., 2018). This interaction triggers a cascade of events, culminating in the accumulation of β-catenin within the cell. Importantly, β-catenin is shielded from phosphorylation by GSK-3β, a crucial step that prevents its degradation by the intracytoplasmic damage complex (Yang et al., 2018). Consequently, non-phosphorylated β-catenin evades ubiquitination and destruction, allowing it to amass in the cytoplasm before translocating into the nucleus (Yang et al., 2018). Once in the nucleus, β-catenin partners with TCF/LEF transcription factors, activating downstream targets such as the cellular myelocytomatosis viral oncogene (c-Myc) (Voronkov and Krauss, 2013). This activation cascade fuels cancer cell proliferation and differentiation, underscoring the pivotal role of the β-catenin-TCF/LEF complex in driving the Wnt/β-catenin signaling pathway (Voronkov and Krauss, 2013). *In vivo*, FAM201A promotes cell survival, migration, and invasion in CC showing that high expression of FAM201A can upregulate FLOT1 expression by sponging of miR-1271-5p. This stimulates the Wnt/β-catenin pathway, promoting CC development and metastasis. AXIN2, a miR-205-5p target gene, has been shown to inhibit Wnt/β-catenin pathway activity. DKK1 and β-catenin are indicators for the Wnt/β-catenin pathway. Low expression of HNRN-PU-AS1 and high expression of miR-205-5p can boost β-catenin production while inhibiting DDK1 expression, activating the Wnt/β-catenin pathway. High expression of AXIN2 suppresses the Wnt/β-catenin pathway, decreasing cell proliferation and inducing apoptosis in CC (Wang et al., 2022).

5.3 MAPK signaling pathway

The Mitogen-Activated Protein Kinase (MAPK) signaling pathway is a critical intracellular signaling cascade involved in regulating various cellular processes, including proliferation, differentiation, and apoptosis (Shao et al., 2018). Dysregulation of the MAPK pathway has been implicated in the pathogenesis of CC, contributing to tumour initiation, progression, and metastasis. In recent years, miRNAs have emerged as key regulators of the MAPK pathway in CC (Asl et al., 2021). These small non-coding RNAs modulate gene expression post-transcriptionally, affecting multiple components of the MAPK pathway. Certain miRNAs have been identified to either promote or suppress MAPK pathway activity by targeting key signalling molecules such as Ras, Raf, MEK, and ERK (Asl et al., 2021). For instance, miR-21 has been shown to enhance MAPK pathway activation by targeting negative regulators, thereby promoting CC cell proliferation and invasion (Gebrie, 2022). Conversely, miR-143 acts as a tumour suppressor by inhibiting the MAPK pathway, thereby suppressing CC progression. Understanding the intricate interplay between miRNAs and the MAPK pathway in CC provides valuable insights into the molecular mechanisms underlying tumorigenesis (Zhao et al., 2017). As a result, a study utilized qRTPCR to analyze the expression of MiR-338-3p and MACC1 in CC and investigated the effects of miR-338-3p and MACC1 on cell growth. A luciferase reporter assay was used to confirm the target gene of miR-338-3p in CC cells. Hence, identified MACC1 as a functional downstream target of miR-338-3p. Overexpressing miR-338-3p lowered MACC1 expression in CC cells, potentially inhibiting CC (Hua et al., 2017). Moreover, researchers investigated the role of miR-329-3p in CC. They found that miR-329-3p expression was reduced in CC tissues and cell lines and correlated with tumour grade, stage, and lymph node metastasis. Upregulation of miR-329-3p inhibited cell proliferation, migration, and invasion (Li et al., 2017). The researchers identified MAPK1 as a direct target gene of miR-329-3p, which was upregulated in CC tissues and inversely correlated with miR-329-3p expression. Silencing MAPK1 mimicked the effects of miR-329-3p overexpression, while restoring MAPK1 expression reversed these effects. These findings suggest that miR-329-3p acts as a tumour suppressor in CC by targeting MAPK1, indicating its potential as a therapeutic target for this disease (Li et al., 2017). Downregulation of miR-99a and miR-125b-2 in CC leads to TRIB2, HOXA1, and mTOR overexpression (Gao et al., 2018). Research indicates that the oncogene TRIB2 is elevated in verities of malignancies and may influence the selectivity of MAPK activation. Similarly, the transcription factor HOXA1 boosts cancer indicators by activating MAPK signalling (Granados-López et al., 2017). Furthermore, an investigation assessed expression levels of HOTAIR and miR-23b in CC samples using real-time PCR. Results indicated elevated HOTAIR and reduced miR-23b expression in cancerous tissues and cell lines (Li et al., 2018). Knockdown of HOTAIR led to apoptosis promotion, as well as inhibition of cell proliferation and invasion both *in vitro* and *in vivo*. Additionally, HOTAIR was found to potentially competitively bind miR-23b, thereby indirectly modulating MAPK1 expression (Li et al., 2018). These findings unveil a novel oncogenic pathway involving HOTAIR in CC, offering insights into its potential prognostic and therapeutic implications.

5.4 JAK/STAT signaling pathway

The JAK/STAT signalling pathway, a crucial player in regulating cell growth, survival, and differentiation, is tragically misconstrued in the context of CC (Luo and Balko, 2019). The Janus kinase (JAK) family initiates a cascade in response to various cytokines and growth factors. This activates STAT proteins, which translocate to the nucleus and orchestrate changes in gene expression. However,

in CC, this complementary movement transforms into a turbulent result (Dutta and Li, 2013). HPV oncoproteins, particularly E6 and E7, disrupt this pathway by directly activating JAKs or by modifying STAT proteins. This leads to persistent activation of STAT3, a key player in promoting cell proliferation, migration, and resistance to apoptosis. Additionally, chronic inflammation, often associated with HPV infection, fuels this pathway via cytokines like interleukin-6 (IL-6) (Gutiérrez-Hoya and Soto-Cruz, 2020). This dysregulated JAK/STAT signaling fosters a conducive environment to tumorigenesis and contributes to aggressive cancer phenotypes. Specific JAK inhibitors and STAT3 antagonists are being explored, aiming to silence oncogenic music and restore cellular symphony. Challenges such as potential off-target effects and complex interactions with other pathways exist; unraveling the JAK/STAT story in CC offers a beacon of hope for novel and effective therapeutic interventions (Valle-Mendiola et al., 2023). However, a study demonstrated the downregulation of miR-9 in cervical adenocarcinoma due to frequent hypermethylation, exerting a tumor suppressor role by targeting various genes, including IL-6. Hypermethylation of miR-9 precursor promoters was observed in cervical adenocarcinoma tissues, and demethylation treatment increased mature miR-9 expression in HeLa cells. Some assays revealed CKAP2, HSPC159, IL-6, and TC10 as novel direct target genes of miR-9, with pathway analysis indicating their involvement in the Jak/STAT3 pathway downstream of IL-6. Ectopic miR-9 expression inhibited Jak/STAT3 signalling activity, which was partially reversed by exogenous IL-6. Overall, miR-9 has the potential to suppress tumors in cervical adenocarcinoma and suggests repression of tumorigenesis through inhibition of the IL-6/Jak/STAT3 pathway (Zhang et al., 2016). Moreover, miR-126 functions as a tumor suppressor in CC cells *in vitro*, which inhibits proliferation, migration, and invasion by suppressing MMP2 and MMP9 expression and inactivating the JAK2/STAT3 signalling pathway by targeting ZEB1, suggesting that miR-126 may be a novel potential target for the diagnosis and treatment of patients with CC (Xu et al., 2019).

6 MicroRNAs as diagnostic and prognostic biomarkers for cervical cancer

miRNAs have become prominent biomarkers for assessing the diagnosis and prognosis of CC, attributable to their aberrant expression profiles in cancerous tissues relative to healthy tissues (Banno et al., 2014). Numerous studies have shown the dysregulation of specific miRNAs in CC tissues and their potential role in disease progression. These small non-coding RNAs can be easily detected in body fluids such as blood and cervical secretions, making them attractive candidates for non-invasive diagnostic tests. Several specific miRNAs have been identified as potential biomarkers for CC. For instance, miR-21, miR-182, miR-183, miR-214, and miR-224 have been consistently upregulated in CC cells and tissues, while miR-150, miR-200b, miR-636, miR-205, and miR-187 are commonly downregulated (Mitra and Elangovan, 2021; Shademan et al., 2023). Detecting dysregulated miRNAs in patient samples shows potential for creating non-invasive CC screening tools, especially in resource-limited settings. Additionally, miRNAs have prognostic value in CC, correlating with clinical parameters like tumor stage, lymph node metastasis, and patient survival (Chen S. et al., 2020). High expression levels of certain miRNAs, such as miR-224 and miR-182, have been associated with advanced tumour stage, lymph node metastasis, and poor prognosis in CC patients. Conversely, low expression levels of tumour-suppressive miRNAs like miR-150, miR-200b, miR-636, miR-205, and miR-187 have been linked to aggressive tumour behaviour, induce cell apoptosis and reduced overall survival rates (Sharma and Gupta, 2020). These findings suggest that miRNA expression profiles could serve as reliable prognostic indicators to guide treatment decisions and improve patient outcomes. In addition to their diagnostic and prognostic value, miRNAs show potential as therapeutic targets for CC. Modulating miRNA expression levels using miRNA mimics has shown potential in preclinical studies to inhibit tumour growth, metastasis, and enhance chemosensitivity in CC models (Di Fiore et al., 2022). Recently, a bioinformatic approach has investigated that that miR-21 may function as a highly sensitive and specific marker for the diagnosis of CC (Deng et al., 2022). Additionally, a study showed miRNA-21 is an oncogenic miRNA molecule playing a key role in the development and progression of cervical malignancy. Whereas it has good diagnostic accuracy as well. In addition, the upregulation of miRNA-21 could predict a worse outcome in terms of prognosis in CC patients (Deng et al., 2022). Moreover, miR-885-5p expression was decreased in CC, and downregulation of miR-885-5p promoted the progression of CC cells has been elucidated via an investigation and concluded that miR-885-5p may be an independent prognostic predictor and therapeutic target for treating CC (Zu et al., 2022). A study examined the expression of certain miRNAs in various samples from women with cervical precancer and cancer to explore their potential as non-invasive biomarkers for diagnosing and prognosing CC. They assessed the levels of three oncomiRs (miR-21, miR-199a, and miR-155-5p) and three tumor suppressor (TS) miRNAs (miR-34a, miR-145, and miR-218) using qRT-PCR and correlated their expression with clinicopathological parameters and survival outcomes. The findings revealed significant overexpression of oncomiRs and downregulation of TS miRNAs (Aftab et al., 2021). A combination of miR-145-5p, miR-218-5p, and miR-34a-5p in urine achieved high sensitivity and specificity in distinguishing precancer and cancer patients from healthy controls, with a correlation to serum and tumor tissue expression. Additionally, miR-34a-5p and miR-218-5p were identified as independent prognostic factors for overall survival in CC patients. The findings suggest that evaluating specific miRNA expression in non-invasive urine samples could serve as a reliable biomarker for early detection and prognosis of CC (Garofalo et al., 2014). However, further research is needed to validate the efficacy and safety of miRNA-based therapies for clinical use in CC patients.

7 Therapeutic interventions of miRNAs in cervical cancer

CC is a major health issue worldwide, necessitating the development of new treatment techniques to enhance patient outcomes. Recently, miRNA-based medicines have emerged as

potential treatments for CC. Nucleic acid-based therapies, such as miRNA mimic therapy, anti-miRNA therapy, and their combination with radiotherapy, hold significant promise in medicine (Garofalo et al., 2014). These approaches exploit the unique regulatory roles of miRNAs in modulating gene expression and cellular pathways. MiRNA mimic therapy involves the use of synthetic miRNA mimics to supplement or enhance the function of specific miRNAs that may be expressed or dysfunctional in certain diseases. Thus, miR-143 is a TS miRNA that is often downregulated in CC. Studies have shown that delivery of synthetic miR-143 mimics can inhibit cell proliferation, induce apoptosis, and suppress tumor growth in CC cell lines (Liu et al., 2018). miR-218 is another TS miRNA that plays a role in CC progression. Studies have demonstrated that the introduction of synthetic miR-218 mimics can inhibit cell migration, invasion, and EMT in CC cells, suggesting a potential therapeutic approach for metastatic CC (Li et al., 2022). Although miR-21 is often associated with oncogenic functions in various cancers, including CC, its role in this context is complex. Some studies have explored the use of synthetic miR-21 mimics to investigate their effects on CC cell proliferation, migration, and invasion, highlighting the potential dual roles of miR-21 in cervical tumorigenesis (Bhattacharjee et al., 2022). Furthermore, miR-181a has been implicated in chemoresistance of cervical squamous cell carcinoma. Research has shown that delivery of synthetic miR-181a mimics can enhance chemosensitivity to cisplatin by targeting specific genes involved in drug resistance pathways, offering a potential strategy to overcome resistance in CC treatment (González-Quintana et al., 2016). Anti-miRNA therapy targets specific miRNAs promoting cancer. In CC, upregulated miR-21 accelerates tumor growth and metastasis. Preclinical studies use anti-miR-21 oligonucleotides to block miR-21, curbing oncogenic pathways, and decreasing cell proliferation and invasion (Toden et al., 2021). miR-155 is another miRNA implicated in CC progression and immune evasion. Research has investigated the use of anti-miR-155 oligonucleotides to block miR-155 function and restore immune responses in the tumor microenvironment, potentially enhancing the antitumor immune response in CC (Bayraktar and Van Roosbroeck, 2018). Furthermore, miR-10b is involved in promoting metastasis and invasiveness of CC. Therefore, studies have explored the use of anti-miR-10b oligonucleotides to inhibit miR-10b expression and to suppress the metastatic properties of CC cells (Menon et al., 2022). miR-34a is a tumor suppressor miRNA that is often downregulated in CC. Interestingly, researchers have investigated the use of anti-miR-34a oligonucleotides to antagonize miR-34a inhibition and restore its tumor-suppressive functions, leading to decreased proliferation and enhanced apoptosis in CC cells (Yin et al., 2023). However, Combining miRNA-based therapies with radiotherapy for the treatment of CC holds promise for enhancing the treatment efficacy and overcoming resistance mechanisms. Consequently, preclinical studies have shown that upregulation of miR-34a in CC cells can sensitize them to radiotherapy by enhancing apoptosis and inhibiting cell proliferation. Combining miR-34a mimics with radiotherapy has demonstrated synergistic effects in reducing tumor growth and improving treatment outcomes in CC models (Yin et al., 2023). Moreover, inhibition of miR-21, which is often upregulated in CC and is associated with radioresistance, has been investigated as a strategy to enhance the efficacy of radiotherapy. Studies have shown that combining anti-miR-21 oligonucleotides with radiotherapy can sensitize CC cells to radiation-induced cell death and overcome radioresistance (Javanmardi et al., 2016). Modulation of miR-155 expression in CC cells has also been explored as a potential approach to enhance the effects of radiotherapy. By using miR-155 mimics or inhibitors in combination with radiotherapy, researchers aim to modulate immune responses, the tumor microenvironment, and radiation sensitivity to improve treatment outcomes in CC (Bayraktar and Van Roosbroeck, 2018). Furthermore, miR-218, a tumor suppressor miRNA that is downregulated in CC, has been studied for its potential role in enhancing the effects of radiotherapy (Banno et al., 2014). These nucleic acid-based therapies represent innovative approaches to precision medicine, offering the potential for targeted and personalized treatment strategies for CC. Researchers are exploring new avenues for improving patient outcomes and advancing the field of therapeutic nucleic acids by harnessing the regulatory functions of miRNAs and combining them with established treatment modalities, such as radiotherapy. However, more detailed investigations are required to validate the efficacy of nucleic acid-based therapies in the management of CC.

8 Conclusion

This comprehensive review delineates the intricate roles of miRNAs in the pathogenesis and treatment response of CC. By exerting influence over a myriad of critical processes in CC progression, such as cancer cell proliferation, evasion of apoptosis, cell cycle regulation, invasion, angiogenesis, and metastasis, miRNAs have emerged as key regulators in this formidable disease. Notably, modulation of pivotal molecular pathways, including the PI3K/AKT/mTOR, Wnt/β-catenin, JAK/STAT, and MAPK pathways, underscores their significance in CC biology. Moreover, their involvement in therapy resistance highlights the pressing need for targeted therapeutic strategies against miRNAs in CC pathogenesis along with their potential as diagnostic and prognostic biomarkers. This review also sheds light on therapeutic interventions involving miRNAs in CC, paving the way for future research endeavors aimed at deciphering their precise mechanisms and exploiting their therapeutic potential to combat CC effectively. However, further detailed investigations are warranted to unravel the intricate mechanisms underlying the role of miRNAs as potent players in CC pathogenesis and therapeutic response.

Author contributions

PC: Conceptualization, Formal Analysis, Writing–original draft, Writing–review and editing. SP: Data curation, Formal Analysis, Supervision, Resources, Validation, Visualization, Writing–review and editing. AH: Data curation, Formal Analysis, Supervision, Resources, Validation, Visualization, Writing–review and editing. DE: Conceptualization, Formal Analysis, Project administration, Writing–original draft, Writing–review and editing. SL: Formal Analysis, Investigation, Validation, Writing–review and editing. RK: Formal Analysis, Investigation, Supervision, Validation, Visualization, Writing–review and editing. MA: Formal Analysis,

Investigation, Validation, Visualization, Writing–review and editing. DI: Validation, Visualization, Writing–review and editing. PP: Conceptualization, Formal Analysis, Validation, Visualization, Writing–review and editing. AO: Conceptualization, Investigation, Validation, Visualization, Writing–review and editing. FK: Conceptualization, Supervision, Validation, Visualization, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The author extends the appreciation to the Deanship of Postgraduate Studies and Scientific Research at Majmaah University for funding this research work through the project number R-2024-1237.

Conflict of interest

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

Publisher's note

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

References

Abbas, M., Mehdi, A., Khan, F. H., Verma, S., Ahmad, A., Khatoon, F., et al. (2021). Role of miRNAs in cervical cancer: a comprehensive novel approach from pathogenesis to therapy. *J. Gynecol. Obstetrics Hum. Reproduction* 50 (9), 102159. doi:10.1016/j.jogoh.2021.102159

Abdelfattah, A. M., Park, C., and Choi, M. Y. (2014). Update on non-canonical microRNAs. *Biomol. concepts* 5 (4), 275–287. doi:10.1515/bmc-2014-0012

Acunzo, M., Romano, G., Wernicke, D., and Croce, C. M. (2015). MicroRNA and cancer–a brief overview. *Adv. Biol. Regul.* 57, 1–9. doi:10.1016/j.jbior.2014.09.013

Aftab, M., Poojary, S. S., Seshan, V., Kumar, S., Agarwal, P., Tandon, S., et al. (2021). Urine miRNA signature as a potential non-invasive diagnostic and prognostic biomarker in cervical cancer. *Sci. Rep.* 11 (1), 10323. doi:10.1038/s41598-021-89388-w

Asl, E. R., Amini, M., Najafi, S., Mansoori, B., Mokhtarzadeh, A., Mohammadi, A., et al. (2021). Interplay between MAPK/ERK signaling pathway and MicroRNAs: a crucial mechanism regulating cancer cell metabolism and tumor progression. *Life Sci.* 278, 119499. doi:10.1016/j.lfs.2021.119499

Aslan, C., Maralbashi, S., Salari, F., Kahroba, H., Sigaroodi, F., Kazemi, T., et al. (2019). Tumor-derived exosomes: Implication in angiogenesis and antiangiogenesis cancer therapy. *J. Cell. Physiol.* 234 (10), 16885–16903. doi:10.1002/jcp.28374

Bahrami, A., Hasanzadeh, M., Hassanian, S. M., ShahidSales, S., Ghayour-Mobarhan, M., Ferns, G. A., et al. (2017). The potential value of the PI3K/Akt/mTOR signaling pathway for assessing prognosis in cervical cancer and as a target for therapy. *J. Cell. Biochem.* 118 (12), 4163–4169. doi:10.1002/jcb.26118

Banno, K., Iida, M., Yanokura, M., Kisu, I., Iwata, T., Tominaga, E., et al. (2014). MicroRNA in cervical cancer: OncomiRs and tumor suppressor miRs in diagnosis and treatment. *Sci. World J.* 2, 1–8. doi:10.1155/2014/178075

Bao, W., Wang, H. H., Tian, F. J., He, X. Y., Qiu, M. T., Wang, J. Y., et al. (2013). A TrkB–STAT3–miR-204-5p regulatory circuitry controls proliferation and invasion of endometrial carcinoma cells. *Mol. cancer* 12, 155–159. doi:10.1186/1476-4598-12-155

Bao, W., Zhang, Y., Li, S., Fan, Q., Qiu, M., Wang, Y., et al. (2019). miR-107-5p promotes tumor proliferation and invasion by targeting estrogen receptor-α in endometrial carcinoma. *Oncol. Rep.* 41 (3), 1575–1585. doi:10.3892/or.2018.6936

Bayraktar, R., and Van Roosbroeck, K. (2018). miR-155 in cancer drug resistance and as target for miRNA-based therapeutics. *Cancer Metastasis Rev.* 37, 33–44. doi:10.1007/s10555-017-9724-7

Bhattacharjee, R., Dey, T., Kumar, L., Kar, S., Sarkar, R., Ghorai, M., et al. (2022). Cellular landscaping of cisplatin resistance in cervical cancer. *Biomed. and Pharmacother.* 153, 113345. doi:10.1016/j.biopha.2022.113345

Bofill-De Ros, X., and Vang Ørom, U. A. (2024). Recent progress in miRNA biogenesis and decay. *RNA Biol.* 21 (1), 1–8. doi:10.1080/15476286.2023.2288741

Causin, R. L., Freitas, A. J., Trovo Hidalgo Filho, C. M., Reis, R. D., Reis, R. M., and Marques, M. M. (2021). A systematic review of microRNAs involved in cervical cancer progression. *Cells* 10 (3), 668. doi:10.3390/cells10030668

Chen, M., Ai, G., Zhou, J., Mao, W., Li, H., and Guo, J. (2019). circMTO1 promotes tumorigenesis and chemoresistance of cervical cancer via regulating miR-6893. *Biomed. and Pharmacother.* 117, 109064. doi:10.1016/j.biopha.2019.109064

Chen, S., Gao, C., Wu, Y., and Huang, Z. (2020b). Identification of prognostic miRNA signature and lymph node metastasis-related key genes in cervical cancer. *Front. Pharmacol.* 11, 544. doi:10.3389/fphar.2020.00544

Chen, X. F., and Liu, Y. (2016). MicroRNA-744 inhibited cervical cancer growth and progression through apoptosis induction by regulating Bcl-2. *Biomed. and Pharmacother.* 81, 379–387. doi:10.1016/j.biopha.2016.04.023

Chen, Y., Song, Y., Mi, Y., Jin, H., Cao, J., Li, H., et al. (2020a). microRNA-499a promotes the progression and chemoresistance of cervical cancer cells by targeting SOX6. *Apoptosis* 25, 205–216. doi:10.1007/s10495-019-01588-y

Chengizkhan, G., Thangavelu, S. K., Muthusami, S., Banerjee, A., Pathak, S., Natarajan, G., et al. (2024). Regulation of cancer stemness, cell signaling, reactive oxygen species, and microRNAs in cancer stem cells. *Cancer Stem Cells Signal. Pathw.*, 243–263. doi:10.1016/b978-0-443-13212-4.00026-x

Chevillet, J. R., Lee, I., Briggs, H. A., He, Y., and Wang, K. (2014). Issues and prospects of microRNA-based biomarkers in blood and other body fluids. *Molecules* 19 (5), 6080–6105. doi:10.3390/molecules19056080

Choi, P. W., Liu, T. L., Wong, C. W., Liu, S. K., Lum, Y. L., and Ming, W. K. (2022). The dysregulation of MicroRNAs in the development of cervical pre-cancer—an update. *Int. J. Mol. Sci.* 23 (13), 7126. doi:10.3390/ijms23137126

Cipolla, G. A. (2014). A non-canonical landscape of the microRNA system. *Front. Genet.* 5, 337. doi:10.3389/fgene.2014.00337

Conti, I., Varano, G., Simioni, C., Laface, I., Milani, D., Rimondi, E., et al. (2020). miRNAs as influencers of cell–cell communication in tumor microenvironment. *Cells* 9 (1), 220. doi:10.3390/cells9010220

Deng, Z. M., Chen, G. H., Dai, F. F., Liu, S. Y., Yang, D. Y., Bao, A. Y., et al. (2022). The clinical value of miRNA-21 in cervical cancer: a comprehensive investigation based on microarray datasets. *PLoS One* 17 (4), e0267108. doi:10.1371/journal.pone.0267108

Di Fiore, R., Suleiman, S., Drago-Ferrante, R., Subbannayya, Y., Pentimalli, F., Giordano, A., et al. (2022). Cancer stem cells and their possible implications in cervical cancer: a short review. *Int. J. Mol. Sci.* 23 (9), 5167. doi:10.3390/ijms23095167

Doghish, A. S., Ali, M. A., Elyan, S. S., Elrebehy, M. A., Mohamed, H. H., Mansour, R. M., et al. (2023). miRNAs role in cervical cancer pathogenesis and targeted therapy: signaling pathways interplay. *Pathology-Research Pract.* 244, 154386. doi:10.1016/j.prp.2023.154386

Dong, R., Qiu, H., Du, G., Wang, Y., Yu, J., and Mao, C. (2014). Restoration of microRNA-218 increases cellular chemosensitivity to cervical cancer by inhibiting cell-cycle progression. *Mol. Med. Rep.* 10 (6), 3289–3295. doi:10.3892/mmr.2014.2622

Du, G., Cao, D., and Meng, L. (2017). miR-21 inhibitor suppresses cell proliferation and colony formation through regulating the PTEN/AKT pathway and improves paclitaxel sensitivity in cervical cancer cells. *Mol. Med. Rep.* 15 (5), 2713–2719. doi:10.3892/mmr.2017.6340

Dutta, P., and Li, W. X. (2013). Role of the JAK-STAT signalling pathway in cancer. *eLS.* doi:10.1002/9780470015902.a0025214

Ergin, K., and Çetinkaya, R. (2022). "Regulation of microRNAs," in *miRNomics: MicroRNA biology and computational analysis*, 1–32.

Fan, Q., Huang, T., Sun, X., Yang, X., Wang, J., Liu, Y., et al. (2021). miR-130a-3p promotes cell proliferation and invasion by targeting estrogen receptor α and androgen receptor in cervical cancer. *Exp. Ther. Med.* 21 (5), 414–420. doi:10.3892/etm.2021.9858

Fan, Z., Cui, H., Xu, X., Lin, Z., Zhang, X., Kang, L., et al. (2015). MiR-125a suppresses tumor growth, invasion and metastasis in cervical cancer by targeting STAT3. *Oncotarget* 6 (28), 25266–25280. doi:10.18632/oncotarget.4457

Feng, C., Ma, F., Hu, C., Ma, J. A., Wang, J., Zhang, Y., et al. (2018). SOX9/miR-130a/CTR1 axis modulates DDP-resistance of cervical cancer cell. *Cell Cycle* 17 (4), 448–458. doi:10.1080/15384101.2017.1395533

Ferreira, H. J., and Esteller, M. (2018). Non-coding RNAs, epigenetics, and cancer: tying it all together. *Cancer Metastasis Rev.* 37, 55–73. doi:10.1007/s10555-017-9715-8

Fu, K., Zhang, L., Liu, R., Shi, Q., Li, X., and Wang, M. (2020). MiR-125 inhibited cervical cancer progression by regulating VEGF and PI3K/AKT signaling pathway. *World J. Surg. Oncol.* 18, 115–120. doi:10.1186/s12957-020-01881-0

Gandellini, P., Profumo, V., Folini, M., and Zaffaroni, N. (2011). MicroRNAs as new therapeutic targets and tools in cancer. *Expert Opin. Ther. targets* 15 (3), 265–279. doi:10.1517/14728222.2011.550878

Gao, C., Zhou, C., Zhuang, J., Liu, L., Liu, C., Li, H., et al. (2018). MicroRNA expression in cervical cancer: novel diagnostic and prognostic biomarkers. *J. Cell. Biochem.* 119 (8), 7080–7090. doi:10.1002/jcb.27029

Garofalo, M., Di Leva, G., and M Croce, C. (2014). MicroRNAs as anti-cancer therapy. *Curr. Pharm. Des.* 20 (33), 5328–5335. doi:10.2174/1381612820666140128211346

Gebrie, A. (2022). Disease progression role as well as the diagnostic and prognostic value of microRNA-21 in patients with cervical cancer: a systematic review and meta-analysis. *Plos one* 17 (7), e0268480. doi:10.1371/journal.pone.0268480

Geretto, M., Pulliero, A., Rosano, C., Zhabayeva, D., Bersimbaev, R., and Izzotti, A. (2017). Resistance to cancer chemotherapeutic drugs is determined by pivotal microRNA regulators. *Am. J. cancer Res.* 7 (6), 1350–1371.

Gong, Z., Yang, J., Li, J., Yang, L., Le, Y., Wang, S., et al. (2014). Novel insights into the role of microRNA in lung cancer resistance to treatment and targeted therapy. *Curr. cancer drug targets* 14 (3), 241–258. doi:10.2174/1568009614666140305104845

González-Quintana, V., Palma-Berré, L., Campos-Parra, A. D., López-Urrutia, E., Peralta-Zaragoza, O., Vazquez-Romo, R., et al. (2016). MicroRNAs are involved in cervical cancer development, progression, clinical outcome and improvement treatment response (Review). *Oncol. Rep.* 35 (1), 3–12. doi:10.3892/or.2015.4369

Granados-López, A. J., Ruiz-Carrillo, J. L., Servín-González, L. S., Martínez-Rodríguez, J. L., Reyes-Estrada, C. A., Gutiérrez-Hernández, R., et al. (2017). Use of mature miRNA strand selection in miRNAs families in cervical cancer development. *Int. J. Mol. Sci.* 18 (2), 407. doi:10.3390/ijms18020407

Gregory, P. A., Bert, A. G., Paterson, E. L., Barry, S. C., Tsykin, A., Farshid, G., et al. (2008). The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat. Cell Biol.* 10 (5), 593–601. doi:10.1038/ncb1722

Gutiérrez-Hoya, A., and Soto-Cruz, I. (2020). Role of the JAK/STAT pathway in cervical cancer: its relationship with HPV E6/E7 oncoproteins. *Cells* 9 (10), 2297. doi:10.3390/cells9102297

Hasan, M. T., Islam, M. R., Islam, M. R., Altahan, B. R., Ahmed, K., Bui, F. M., et al. (2023). Systematic approach to identify therapeutic targets and functional pathways for the cervical cancer. *J. Genet. Eng. Biotechnol.* 21 (1), 10. doi:10.1186/s43141-023-00469-x

Havens, M. A., Reich, A. A., Duelli, D. M., and Hastings, M. L. (2012). Biogenesis of mammalian microRNAs by a non-canonical processing pathway. *Nucleic acids Res.* 40 (10), 4626–4640. doi:10.1093/nar/gks026

He, Y., Lin, J., Ding, Y., Liu, G., Luo, Y., Huang, M., et al. (2016). A systematic study on dysregulated micro RNA s in cervical cancer development. *Int. J. cancer* 138 (6), 1312–1327. doi:10.1002/ijc.29618

Hemmat, N., Mokhtarzadeh, A., Aghazadeh, M., Jadidi-Niaragh, F., Baradaran, B., and Bannazadeh Baghi, H. (2020). Role of microRNAs in epidermal growth factor receptor signaling pathway in cervical cancer. *Mol. Biol. Rep.* 47, 4553–4568. doi:10.1007/s11033-020-05494-4

Herrera-Carrillo, E., and Berkhout, B. (2017). Dicer-independent processing of small RNA duplexes: mechanistic insights and applications. *Nucleic. Acids Res.* 45 (18), 10369–10379. doi:10.1093/nar/gkx779

Holcakova, J., Bartosik, M., Anton, M., Minar, L., Hausnerova, J., Bednarikova, M., et al. (2021). New trends in the detection of gynecological precancerous lesions and early-stage cancers. *Cancers* 13 (24), 6339. doi:10.3390/cancers13246339

Hu, J., Wang, R., Liu, Y., Zhou, J., Shen, K., and Dai, Y. (2021). Baicalein represses cervical cancer cell growth, cell cycle progression and promotes apoptosis via blocking akt/mtor pathway by the regulation of circhiat1/mir-19a-3p axis. *OncoTargets Ther.* 9, 905–916. doi:10.2147/OTT.S282790

Hu, Q. L., Xu, Z. P., Lan, Y. F., and Li, B. (2020). miR-636 represses cell survival by targeting CDK6/Bcl-2 in cervical cancer. *Kaohsiung J. Med. Sci.* 36 (5), 328–335. doi:10.1002/kjm2.12181

Hua, F. F., Liu, S. S., Zhu, L. H., Wang, Y. H., Liang, X., Ma, N., et al. (2017). MiRNA-338-3p regulates cervical cancer cells proliferation by targeting MACC1 through MAPK signaling pathway. *Eur. Rev. Med. and Pharmacol. Sci.* 21 (23), 5342–5352. doi:10.26355/eurrev_201712_13919

Huang, Y., Huang, H., Li, M., Zhang, X., Liu, Y., and Wang, Y. (2017). MicroRNA-374c-5p regulates the invasion and migration of cervical cancer by acting on the Foxc1/snail pathway. *Biomed. and Pharmacother.* 94, 1038–1047. doi:10.1016/j.biopha.2017.07.150

Huang, Y., Li, Y., Wang, F. F., Lv, W., Xie, X., and Cheng, X. (2016). Over-expressed miR-224 promotes the progression of cervical cancer via targeting RASSF8. *PLoS One* 11 (9), e0162378. doi:10.1371/journal.pone.0162378

Iorio, M. V., and Croce, C. M. (2012). MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol. Med.* 4 (3), 143–159. doi:10.1002/emmm.201100209

Iwakawa, H. O., and Tomari, Y. (2022). Life of RISC: formation, action, and degradation of RNA-induced silencing complex. *Mol. Cell* 82 (1), 30–43. doi:10.1016/j.molcel.2021.11.026

Jafri, M. A., Al-Qahtani, M. H., and Shay, J. W. (2017). Role of miRNAs in human cancer metastasis: implications for therapeutic intervention. *InSeminars cancer Biol.* 44, 117–131. doi:10.1016/j.semcancer.2017.02.004

Javanmardi, S., Reza Aghamaali, M., Sadat Abolmaali, S., Mohammadi, S., and Mohammad Tamaddon, A. (2016). miR-21, an oncogenic target miRNA for cancer therapy: molecular mechanisms and recent advancements in chemo and radio-resistance. *Curr. gene Ther.* 16 (6), 375–389. doi:10.2174/1566523217666170102105119

Jiang, H., Yu, W. W., Wang, L. L., and Peng, Y. (2015). miR-130a acts as a potential diagnostic biomarker and promotes gastric cancer migration, invasion and proliferation by targeting RUNX3. *Oncol. Rep.* 34 (3), 1153–1161. doi:10.3892/or.2015.4099

Kakotkin, V. V., Semina, E. V., Zadorkina, T. G., and Agapov, M. A. (2023). Prevention strategies and early diagnosis of cervical cancer: current state and prospects. *Diagnostics* 13 (4), 610. doi:10.3390/diagnostics13040610

Kalkusova, K., Taborska, P., Stakheev, D., and Smrz, D. (2022). The role of miR-155 in antitumor immunity. *Cancers* 14 (21), 5414. doi:10.3390/cancers14215414

Karimi, F., and Mollaei, H. (2021). Potential of miRNAs in cervical cancer chemoresistance. *Gene Rep.* 23, 101109. doi:10.1016/j.genrep.2021.101109

Kaur, K., and Khatik, G. L. (2020). Cancer immunotherapy: an effective tool in cancer control and treatment. *Curr. Cancer Ther. Rev.* 16 (1), 62–69. doi:10.2174/1875630 1mtawanzks4

Ke, G., Liang, L., Yang, J. M., Huang, X., Han, D., Huang, S., et al. (2013). MiR-181a confers resistance of cervical cancer to radiation therapy through targeting the pro-apoptotic PRKCD gene. *Oncogene* 32 (25), 3019–3027. doi:10.1038/onc.2012.323

Khan, A. Q., Ahmed, E. I., Elareer, N. R., Junejo, K., Steinhoff, M., and Uddin, S. (2019). Role of miRNA-regulated cancer stem cells in the pathogenesis of human malignancies. *Cells* 8 (8), 840. doi:10.3390/cells8080840

Klicka, K., Grzywa, T. M., Mielniczuk, A., Klinke, A., and Włodarski, P. K. (2022). The role of miR-200 family in the regulation of hallmarks of cancer. *Front. Oncol.* 12, 965231. doi:10.3389/fonc.2022.965231

Klinge, C. M. (2009). Estrogen regulation of microRNA expression. *Curr. genomics* 10 (3), 169–183. doi:10.2174/138920209788185289

Lei, Y., Chen, L., Zhang, G., Shan, A., Ye, C., Liang, B., et al. (2020). MicroRNAs target the Wnt/β-catenin signaling pathway to regulate epithelial-mesenchymal transition in cancer (Review). *Oncol. Rep.* 44 (4), 1299–1313. doi:10.3892/or.2020.7703

Li, B., Hu, Y., Ye, F., Li, Y., Lv, W., and Xie, X. (2010). Reduced miR-34a expression in normal cervical tissues and cervical lesions with high-risk human papillomavirus infection. *Int. J. Gynecol. Cancer* 20 (4), 597–604. doi:10.1111/IGC.0b013e3181d63170

Li, B. H., Zhou, J. S., Ye, F., Cheng, X. D., Zhou, C. Y., Lu, W. G., et al. (2011b). Reduced miR-100 expression in cervical cancer and precursors and its carcinogenic effect through targeting PLK1 protein. *Eur. J. Cancer* 47 (14), 2166–2174. doi:10.1016/j.ejca.2011.04.037

Li, H., An, X., and Fu, Q. (2022). miR-218 affects the invasion and metastasis of cervical cancer cells by inhibiting the expression of SFMBT1 and DCUNIDI. *Cell. Mol. Biol.* 68 (2), 81–86. doi:10.14715/cmb/2022.68.2.12

Li, J., Hu, L., Tian, C., Lu, F., Wu, J., and Liu, L. (2015). microRNA-150 promotes cervical cancer cell growth and survival by targeting FOXO4. *BMC Mol. Biol.* 16, 24–29. doi:10.1186/s12867-015-0052-6

Li, J., Ping, Z., and Ning, H. (2012). MiR-218 impairs tumor growth and increases chemo-sensitivity to cisplatin in cervical cancer. *Int. J. Mol. Sci.* 13 (12), 16053–16064. doi:10.3390/ijms131216053

Li, J. H., Xiao, X., Zhang, Y. N., Wang, Y. M., Feng, L. M., Wu, Y. M., et al. (2011a). MicroRNA miR-886-5p inhibits apoptosis by down-regulating Bax expression in human cervical carcinoma cells. *Gynecol. Oncol.* 120 (1), 145–151. doi:10.1016/j.ygyno.2010.09.009

Li, Q., Feng, Y., Chao, X., Shi, S., Liang, M., Qiao, Y., et al. (2018). HOTAIR contributes to cell proliferation and metastasis of cervical cancer via targetting miR-23b/MAPK1 axis. *Biosci. Rep.* 38 (1), BSR20171563. doi:10.1042/BSR20171563

Li, T., Li, M., Xu, C., Xu, X., Ding, J., Cheng, L., et al. (2019a). miR-146a regulates the function of Th17 cell differentiation to modulate cervical cancer cell growth and

apoptosis through NF-κB signaling by targeting TRAF6. *Oncol. Rep.* 41 (5), 2897–2908. doi:10.3892/or.2019.7046

Li, W., Liang, J., Zhang, Z., Lou, H., Zhao, L., Xu, Y., et al. (2017). MicroRNA-329-3p targets MAPK1 to suppress cell proliferation, migration and invasion in cervical cancer. *Oncol. Rep.* 37 (5), 2743–2750. doi:10.3892/or.2017.5555

Li, Y. J., Wang, Y., and Wang, Y. Y. (2019b). *Retracted: microRNA-99b suppresses human cervical cancer cell activity by inhibiting the PI3K/AKT/mTOR signaling pathway*.

Liang, H., Luo, R., Chen, X., Zhao, Y., and Tan, A. (2023). [Retracted] miR-187 inhibits the growth of cervical cancer cells by targeting FGF9. *Oncol. Rep.* 50 (5), 195. doi:10.3892/or.2023.8632

Liu, M., Jia, J., Wang, X., Liu, Y., Wang, C., and Fan, R. (2018). Long non-coding RNA HOTAIR promotes cervical cancer progression through regulating BCL2 via targeting miR-143-3p. *Cancer Biol. and Ther.* 19 (5), 391–399. doi:10.1080/15384047.2018.1423921

Liu, S., Zhang, P., Chen, Z., Liu, M., Li, X., and Tang, H. (2013). MicroRNA-7 downregulates XIAP expression to suppress cell growth and promote apoptosis in cervical cancer cells. *FEBS Lett.* 587 (14), 2247–2253. doi:10.1016/j.febslet.2013.05.054

Liu, Y., Li, H., Pi, R., Yang, Y., Zhao, X., and Qi, X. (2019). Current strategies against persistent human papillomavirus infection (Review). *Int. J. Oncol.* 55 (3), 570–584. doi:10.3892/ijo.2019.4847

Lizano, M., Carrillo-García, A., La Cruz-Hernández, D., Castro-Muñoz, L. J., and Contreras-Paredes, A. (2024). Promising predictive molecular biomarkers for cervical cancer. *Int. J. Mol. Med.* 53 (6), 1–8. doi:10.3892/ijmm.2024.5374

Luo, N., and Balko, J. M. (2019). Role of JAK-STAT pathway in cancer signaling. Predictive biomarkers in oncology: applications in precision. *Medicine*, 311–319. doi:10.1007/978-3-319-95228-4_26

Ma, J., Zhang, F., and Sun, P. (2020). miR-140-3p impedes the proliferation of human cervical cancer cells by targeting RRM2 to induce cell-cycle arrest and early apoptosis. *Bioorg. and Med. Chem.* 28 (3), 115283. doi:10.1016/j.bmc.2019.115283

MacFarlane, L. A., and R Murphy, P. (2010). MicroRNA: biogenesis, function and role in cancer. *Curr. genomics.* 11 (7), 537–561. doi:10.2174/138920210793175895

Manzo-Merino, J., Contreras-Paredes, A., Vázquez-Ulloa, E., Rocha-Zavaleta, L., Fuentes-Gonzalez, A. M., and Lizano, M. (2014). The role of signaling pathways in cervical cancer and molecular therapeutic targets. *Archives Med. Res.* 45 (7), 525–539. doi:10.1016/j.arcmed.2014.10.008

Masadah, R., Rauf, S., Pratama, M. Y., Tiribelli, C., and Pascut, D. (2021). The role of microRNAs in the cisplatin-and radio-resistance of cervical cancer. *Cancers* 13 (5), 1168. doi:10.3390/cancers13051168

Medina, P. P., and Slack, F. J. (2008). microRNAs and cancer: an overview. *Cell cycle* 7 (16), 2485–2492. doi:10.4161/cc.7.16.6453

Melo, S. A., and Esteller, M. (2011). Dysregulation of microRNAs in cancer: playing with fire. *FEBS Lett.* 585 (13), 2087–2099. doi:10.1016/j.febslet.2010.08.009

Menon, A., Abd-Aziz, N., Khalid, K., Poh, C. L., and Naidu, R. (2022). miRNA: a promising therapeutic target in cancer. *Int. J. Mol. Sci.* 23 (19), 11502. doi:10.3390/ijms231911502

Miao, J., Regenstein, J. M., Xu, D., Zhou, D., Li, H., Zhang, H., et al. (2020). The roles of microRNA in human cervical cancer. *Archives Biochem. biophysics* 690, 108480. doi:10.1016/j.abb.2020.108480

Mitra, T., and Elangovan, S. (2021). Cervical cancer development, chemoresistance, and therapy: a snapshot of involvement of microRNA. *Mol. Cell. Biochem.* 476 (12), 4363–4385. doi:10.1007/s11010-021-04249-4

Ortiz, G. G., Mohammadi, Y., Nazari, A., Ataeinaeini, M., Kazemi, P., Yasamineh, S., et al. (2023). A state-of-the-art review on the MicroRNAs roles in hematopoietic stem cell aging and longevity. *Cell Commun. Signal.* 21 (1), 85. doi:10.1186/s12964-023-01117-0

Ortiz-Quintero, B. (2020). Extracellular MicroRNAs as intercellular mediators and noninvasive biomarkers of cancer. *Cancers* 12 (11), 3455. doi:10.3390/cancers12113455

Oyervides-Muñoz, M. A., Pérez-Maya, A. A., Rodríguez-Gutiérrez, H. F., Gómez-Macias, G. S., Fajardo-Ramírez, O. R., Treviño, V., et al. (2018). Understanding the HPV integration and its progression to cervical cancer. *Infect. Genet. Evol.* 61, 134–144. doi:10.1016/j.meegid.2018.03.003

Pal, A., and Kundu, R. (2020). Human papillomavirus E6 and E7: the cervical cancer hallmarks and targets for therapy. *Front. Microbiol.* 10, 3116. doi:10.3389/fmicb.2019.03116

Panda, M., and Prajapati, V. K. (2024). Paradigms in miRNA biogenesis pathways. *InMicroRNA Hum. Infect. Dis.* 1, 3–21. Academic Press. doi:10.1016/b978-0-323-99661-7.00003-5

Pardini, B., De Maria, D., Francavilla, A., Di Gaetano, C., Ronco, G., and Naccarati, A. (2018). MicroRNAs as markers of progression in cervical cancer: a systematic review. *BMC cancer* 18, 696–697. doi:10.1186/s12885-018-4590-4

Pedroza-Torres, A., Campos-Parra, A. D., Millan-Catalan, O., Loissell-Baltazar, Y. A., Zamudio-Meza, H., Cantú de León, D., et al. (2018). MicroRNA-125 modulates radioresistance through targeting p21 in cervical cancer. *Oncol. Rep.* 39 (3), 1532–1540. doi:10.3892/or.2018.6219

Peng, Y., and Croce, C. M. (2016). The role of MicroRNAs in human cancer. *Signal Transduct. Target. Ther.* 1 (1), 15004–15009. doi:10.1038/sigtrans.2015.4

Peng, Y., Zhang, X., Feng, X., Fan, X., and Jin, Z. (2017). The crosstalk between microRNAs and the Wnt/β-catenin signaling pathway in cancer. *Oncotarget* 8 (8), 14089–14106. doi:10.18632/oncotarget.12923

Peralta-Zaragoza, O., Deas, J., Meneses-Acosta, A., De la O-Gómez, F., Fernández-Tilapa, G., Gómez-Cerón, C., et al. (2016). Relevance of miR-21 in regulation of tumor suppressor gene PTEN in human cervical cancer cells. *BMC cancer* 16, 215–216. doi:10.1186/s12885-016-2231-3

Phuah, N. H., In, L. L., Azmi, M. N., Ibrahim, H., Awang, K., and Nagoor, N. H. (2013). Alterations of microRNA expression patterns in human cervical carcinoma cells (Ca Ski) toward 1′ S-1′-acetoxychavicol acetate and cisplatin. *Reprod. Sci.* 20 (5), 567–578. doi:10.1177/1933719111459220

Qin, W., Dong, P., Ma, C., Mitchelson, K., Deng, T., Zhang, L., et al. (2012). MicroRNA-133b is a key promoter of cervical carcinoma development through the activation of the ERK and AKT1 pathways. *Oncogene* 31 (36), 4067–4075. doi:10.1038/onc.2011.561

Rani, V., and Sengar, R. S. (2022). Biogenesis and mechanisms of microRNA-mediated gene regulation. *Biotechnol. Bioeng.* 119 (3), 685–692. doi:10.1002/bit.28029

Robaina, M. C., Mazzoccoli, L., Arruda, V. O., de Souza Reis, F. R., Apa, A. G., de Rezende, L. M., et al. (2015). Deregulation of DNMT1, DNMT3B and miR-29s in Burkitt lymphoma suggests novel contribution for disease pathogenesis. *Exp. Mol. pathology* 98 (2), 200–207. doi:10.1016/j.yexmp.2015.03.006

Santovito, D., and Weber, C. (2022). Non-canonical features of microRNAs: paradigms emerging from cardiovascular disease. *Nat. Rev. Cardiol.* 19 (9), 620–638. doi:10.1038/s41569-022-00680-2

Saslow, D., Solomon, D., Lawson, H. W., Killackey, M., Kulasingam, S. L., Cain, J., et al. (2012). American cancer society, American society for colposcopy and cervical pathology, and American society for clinical pathology screening guidelines for the prevention and early detection of cervical cancer. *Am. J. Clin. pathology* 137 (4), 516–542. doi:10.1309/AJCPTGD94EVRSJCG

Sen, P., Ganguly, P., and Ganguly, N. (2018). Modulation of DNA methylation by human papillomavirus E6 and E7 oncoproteins in cervical cancer. *Oncol. Lett.* 15 (1), 11–22. doi:10.3892/ol.2017.7292

Shademan, B., Karamad, V., Nourazarian, A., Masjedi, S., Isazadeh, A., Sogutlu, F., et al. (2023). MicroRNAs as targets for cancer diagnosis: interests and limitations. *Adv. Pharm. Bull.* 13 (3), 435–445. doi:10.34172/apb.2023.047

Shao, J., Wang, C., Li, L., Liang, H., Dai, J., Ling, X., et al. (2018). Luteoloside inhibits proliferation and promotes intrinsic and extrinsic pathway-mediated apoptosis involving MAPK and mTOR signaling pathways in human cervical cancer cells. *Int. J. Mol. Sci.* 19 (6), 1664. doi:10.3390/ijms19061664

Sharma, G., Dua, P., and Mohan Agarwal, S. (2014). A comprehensive review of dysregulated miRNAs involved in cervical cancer. *Curr. genomics.* 15 (4), 310–323. doi:10.2174/1389202915666140528003249

Sharma, P. C., and Gupta, A. (2020). MicroRNAs: potential biomarkers for diagnosis and prognosis of different cancers. *Transl. cancer Res.* 9 (9), 5798–5818. doi:10.21037/tcr-20-1294

Shen, S., Zhang, S., Liu, P., Wang, J., and Du, H. (2020). Potential role of microRNAs in the treatment and diagnosis of cervical cancer. *Cancer Genet.* 248, 25–30. doi:10.1016/j.cancergen.2020.09.003

Shen, S. N., Wang, L. F., Jia, Y. F., Hao, Y. Q., Zhang, L., and Wang, H. (2013). Upregulation of microRNA-224 is associated with aggressive progression and poor prognosis in human cervical cancer. *Diagn. Pathol.* 8, 69–77. doi:10.1186/1746-1596-8-69

Shen, Y., Zhou, J., Li, Y., Ye, F., Wan, X., Lu, W., et al. (2014). miR-375 mediated acquired chemo-resistance in cervical cancer by facilitating EMT. *PloS one* 9 (10), e109299. doi:10.1371/journal.pone.0109299

Shirley, K., Reichard, K., and Grover, N. (2022). "Small noncoding RNA, microRNA in gene regulation," in *InFundamentals of RNA structure and function* (Cham: Springer International Publishing), 167–190.

Si, W., Shen, J., Zheng, H., and Fan, W. (2019). The role and mechanisms of action of microRNAs in cancer drug resistance. *Clin. epigenetics* 11, 25–24. doi:10.1186/s13148-018-0587-8

Song, J., and Li, Y. (2017). miR-25-3p reverses epithelial-mesenchymal transition via targeting Sema4C in cisplatin-resistance cervical cancer cells. *Cancer Sci.* 108 (1), 23–31. doi:10.1111/cas.13104

Su, J., Zhang, A., Shi, Z., Ma, F., Pu, P., Wang, T., et al. (2012). MicroRNA-200a suppresses the Wnt/β-catenin signaling pathway by interacting with β-catenin. *Int. J. Oncol.* 40 (4), 1162–1170. doi:10.3892/ijo.2011.1322

Sun, H., Fan, G., Deng, C., and Wu, L. (2020). miR-4429 sensitized cervical cancer cells to irradiation by targeting RAD51. *J. Cell. physiology* 235 (1), 185–193. doi:10.1002/jcp.28957

Taghizadeh, E., Jahangiri, S., Rostami, D., Taheri, F., Renani, P. G., Taghizadeh, H., et al. (2019). Roles of E6 and E7 human papillomavirus proteins in molecular

pathogenesis of cervical cancer. *Curr. Protein Peptide Sci.* 20 (9), 926–934. doi:10.2174/1389203720666190618101441

Tang, T., Wong, H. K., Gu, W., Yu, M. Y., To, K. F., Wang, C. C., et al. (2013). MicroRNA-182 plays an onco-miRNA role in cervical cancer. *Gynecol. Oncol.* 129 (1), 199–208. doi:10.1016/j.ygyno.2012.12.043

Teng, P., Jiao, Y., Hao, M., and Tang, X. (2018). RETRACTED: microRNA-383 suppresses the PI3K-AKT-MTOR signaling pathway to inhibit development of cervical cancer via down-regulating PARP2. *J. Cell. Biochem.* 119 (7), 5243–5252. doi:10.1002/jcb.26585

Tian, R. Q., Wang, X. H., Hou, L. J., Jia, W. H., Yang, Q., Li, Y. X., et al. (2011). MicroRNA-372 is down-regulated and targets cyclin-dependent kinase 2 (CDK2) and cyclin A1 in human cervical cancer, which may contribute to tumorigenesis. *J. Biol. Chem.* 286 (29), 25556–25563. doi:10.1074/jbc.M111.221564

Toden, S., Zumwalt, T. J., and Goel, A. (2021). Non-coding RNAs and potential therapeutic targeting in cancer. *Biochimica Biophysica Acta (BBA)-Reviews Cancer* 1875 (1), 188491. doi:10.1016/j.bbcan.2020.188491

Treiber, T., Treiber, N., and Meister, G. (2019). Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. *Nat. Rev. Mol. Cell Biol.* 20 (1), 5–20. doi:10.1038/s41580-018-0059-1

Valle-Mendiola, A., Gutiérrez-Hoya, A., and Soto-Cruz, I. (2023). JAK/STAT signaling and Cervical Cancer: from the cell surface to the Nucleus. *Genes* 14 (6), 1141. doi:10.3390/genes14061141

Vasan, N., Baselga, J., and Hyman, D. M. (2019). A view on drug resistance in cancer. *Nature* 575 (7782), 299–309. doi:10.1038/s41586-019-1730-1

Voronkov, A., and Krauss, S. (2013). Wnt/beta-catenin signaling and small molecule inhibitors. *Curr. Pharm. Des.* 19 (4), 634–664. doi:10.2174/138161213804581837

Wang, F., Li, B., and Xie, X. (2016). *The roles and clinical significance of microRNAs in cervical cancer*.

Wang, F., Liu, M., Li, X., and Tang, H. (2013). MiR-214 reduces cell survival and enhances cisplatin-induced cytotoxicity via down-regulation of Bcl2l2 in cervical cancer cells. *FEBS Lett.* 587 (5), 488–495. doi:10.1016/j.febslet.2013.01.016

Wang, J. Y., and Chen, L. J. (2019). The role of miRNAs in the invasion and metastasis of cervical cancer. *Biosci. Rep.* 39 (3), BSR20181377. doi:10.1042/BSR20181377

Wang, Q., Qin, J., Chen, A., Zhou, J., Liu, J., Cheng, J., et al. (2015). Downregulation of microRNA-145 is associated with aggressive progression and poor prognosis in human cervical cancer. *Tumor Biol.* 36, 3703–3708. doi:10.1007/s13277-014-3009-3

Wang, X., Xie, Y., and Wang, J. (2018a). [ARTICLE WITHDRAWN] overexpression of MicroRNA-34a-5p inhibits proliferation and promotes apoptosis of human cervical cancer cells by downregulation of bcl-2. *Oncol. Res.* 26 (6), 977–985. doi:10.3727/096504017X15037506066252

Wang, X., Zhang, H., and Chen, X. (2019). Drug resistance and combating drug resistance in cancer. *Cancer drug Resist.* 2 (2), 141. doi:10.20517/cdr.2019.10

Wang, Y., Wang, Z., Cheng, K., and Hao, Q. (2022). FAM201A promotes cervical cancer progression and metastasis through miR-1271-5p/Flotillin-1 axis targeting-induced Wnt/β-catenin pathway. *J. Oncol.* 2022, 1–25. doi:10.1155/2022/1123839

Wang, Y. F., Yang, H. Y., Shi, X. Q., and Wang, Y. (2018b). Upregulation of microRNA-129-5p inhibits cell invasion, migration and tumor angiogenesis by inhibiting ZIC2 via downregulation of the Hedgehog signaling pathway in cervical cancer. *Cancer Biol. and Ther.* 19 (12), 1162–1173. doi:10.1080/15384047.2018.1491497

Wen, C. X., Tian, H. L., Chen, E., Liu, J. F., and Liu, X. X. (2021). MiRNA-873-5p acts as a potential novel biomarker and promotes cervical cancer progression by regulating ZEB1 via notch signaling pathway. *Dose-Response* 19 (1), 15593258211001255. doi:10.1177/15593258211001255

Wu, X. G., Zhou, C. F., Zhang, Y. M., Yan, R. M., Wei, W. F., Chen, X. J., et al. (2019). Cancer-derived exosomal miR-221-3p promotes angiogenesis by targeting THBS2 in cervical squamous cell carcinoma. *Angiogenesis* 22, 397–410. doi:10.1007/s10456-019-09665-1

Wu, Y., Huang, J., Xu, H., and Gong, Z. (2018). Over-expression of miR-15a-3p enhances the radiosensitivity of cervical cancer by targeting tumor protein D52. *Biomed. and Pharmacother.* 105, 1325–1334. doi:10.1016/j.biopha.2018.06.033

Würdinger, T., Tannous, B. A., Saydam, O., Skog, J., Grau, S., Soutschek, J., et al. (2008). miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. *Cancer Cell* 14 (5), 382–393. doi:10.1016/j.ccr.2008.10.005

Xiong, Y., Sun, F., Dong, P., Watari, H., Yue, J., Yu, M. F., et al. (2017). iASPP induces EMT and cisplatin resistance in human cervical cancer through miR-20a-FBXL5/BTG3 signaling. *J. Exp. and Clin. Cancer Res.* 36, 48–50. doi:10.1186/s13046-017-0520-6

Xu, H., Zhu, J., Hu, C., Song, H., and Li, Y. (2016). Inhibition of microRNA-181a may suppress proliferation and invasion and promote apoptosis of cervical cancer cells through the PTEN/Akt/FOXO1 pathway. *J. physiology Biochem.* 72, 721–732. doi:10.1007/s13105-016-0511-7

Xu, J., Wang, H., Wang, H., Chen, Q., Zhang, L., Song, C., et al. (2019). The inhibition of miR-126 in cell migration and invasion of cervical cancer through regulating ZEB1. *Hereditas* 156, 11–20. doi:10.1186/s41065-019-0087-7

Xu, S., Li, W., Wu, J., Lu, Y., Xie, M., Li, Y., et al. (2022). The role of miR-129-5p in cancer: a novel therapeutic target. *Curr. Mol. Pharmacol.* 15 (4), 647–657. doi:10.2174/1874467214666210914122010

Xu, Y., Sun, Y., Song, X., and Ren, J. (2023). The mechanisms and diagnostic potential of lncRNAs, miRNAs, and their related signaling pathways in cervical cancer. *Front. Cell Dev. Biol.* 11, 1170059. doi:10.3389/fcell.2023.1170059

Yamamoto, N., Kinoshita, T., Nohata, N., Itesako, T., Yoshino, H., Enokida, H., et al. (2013). Tumor suppressive microRNA-218 inhibits cancer cell migration and invasion by targeting focal adhesion pathways in cervical squamous cell carcinoma. *Int. J. Oncol.* 42 (5), 1523–1532. doi:10.3892/ijo.2013.1851

Yang, J. S., and Lai, E. C. (2011). Alternative miRNA biogenesis pathways and the interpretation of core miRNA pathway mutants. *Mol. Cell* 43 (6), 892–903. doi:10.1016/j.molcel.2011.07.024

Yang, M., Wang, M., Li, X., Xie, Y., Xia, X., Tian, J., et al. (2018). Wnt signaling in cervical cancer? *J. Cancer* 9 (7), 1277–1286. doi:10.7150/jca.22005

Yang, Y., Liu, Y., Liu, W., Li, C., Liu, Y., Hu, W., et al. (2022). miR-122 inhibits the cervical cancer development by targeting the oncogene RAD21. *Biochem. Genet.* 60 (1), 303–314. doi:10.1007/s10528-021-10098-z

Yin, M., Zhang, Z., and Wang, Y. (2023). Anti-tumor effects of miR-34a by regulating immune cells in the tumor microenvironment. *Cancer Med.* 12 (10), 11602–11610. doi:10.1002/cam4.5826

Yu, L., Wei, J., and Liu, P. (2022). Attacking the PI3K/Akt/mTOR signaling pathway for targeted therapeutic treatment in human cancer. *InSeminars cancer Biol.* 85, 69–94. Academic Press. doi:10.1016/j.semcancer.2021.06.019

Zaporozhchenko, I. A., Rykova, E. Y., and Laktionov, P. P. (2020). The fundamentals of miRNA biology: structure, biogenesis, and regulatory functions. *Russ. J. Bioorg. Chem.* 46, 1–13. doi:10.1134/s106816202001015x

Zeng, F., Xue, M., Xiao, T., Li, Y., Xiao, S., Jiang, B., et al. (2016). MiR-200b promotes the cell proliferation and metastasis of cervical cancer by inhibiting FOXG1. *Biomed. and Pharmacother.* 79, 294–301. doi:10.1016/j.biopha.2016.02.033

Zeng, K., Zheng, W., Mo, X., Liu, F., Li, M., Liu, Z., et al. (2015). Dysregulated microRNAs involved in the progression of cervical neoplasm. *Archives Gynecol. obstetrics* 292, 905–913. doi:10.1007/s00404-015-3702-5

Zhang, B., Chen, J., Ren, Z., Chen, Y., Li, J., Miao, X., et al. (2013). A specific miRNA signature promotes radioresistance of human cervical cancer cells. *Cancer Cell Int.* 13, 118–8. doi:10.1186/1475-2867-13-118

Zhang, F., Liu, J., and Xie, B. B. (2019a). Downregulation of microRNA-205 inhibits cell invasion and angiogenesis of cervical cancer through TSLC1-mediated Akt signaling pathway. *J. Cell. physiology* 234 (10), 18626–18638. doi:10.1002/jcp.28501

Zhang, H., Yan, T., Liu, Z., Wang, J., Lu, Y., Li, D., et al. (2018). MicroRNA-137 is negatively associated with clinical outcome and regulates tumor development through EZH2 in cervical cancer. *J. Cell. Biochem.* 119 (1), 938–947. doi:10.1002/jcb.26259

Zhang, J., He, H., Wang, K., Xie, Y., Yang, Z., Qie, M., et al. (2020). miR-326 inhibits the cell proliferation and cancer stem cell-like property of cervical cancer *in vitro* and oncogenesis *in vivo* via targeting TCF4. *Ann. Transl. Med.* 8 (24), 1638. doi:10.21037/atm-20-6830

Zhang, J., Jia, J., Zhao, L., Li, X., Xie, Q., Chen, X., et al. (2016). Down-regulation of microRNA-9 leads to activation of IL-6/Jak/STAT3 pathway through directly targeting IL-6 in HeLa cell. *Mol. Carcinog.* 55 (5), 732–742. doi:10.1002/mc.22317

Zhang, L., Li, H., Yuan, M., Li, M., and Zhang, S. (2019c). Cervical cancer cells-secreted exosomal microRNA-221-3p promotes invasion, migration and angiogenesis of microvascular endothelial cells in cervical cancer by down-regulating MAPK10 expression. *Cancer Manag. Res.* 9, 10307–10319. doi:10.2147/CMAR.S221527

Zhang, Y., Sun, B., Zhao, L., Liu, Z., Xu, Z., Tian, Y., et al. (2019b). Up-regulation of miRNA-148a inhibits proliferation, invasion, and migration while promoting apoptosis of cervical cancer cells by down-regulating RRS1. *Biosci. Rep.* 39 (5), BSR20181815. doi:10.1042/BSR20181815

Zhang, Y., Sun, X., Li, Z., Han, X., Wang, W., Xu, P., et al. (2024). Interactions between miRNAs and the Wnt/β-catenin signaling pathway in endometriosis. *Biomed. and Pharmacother.* 171, 116182. doi:10.1016/j.biopha.2024.116182

Zhao, S., Yao, D., Chen, J., Ding, N., and Ren, F. (2015). MiR-20a promotes cervical cancer proliferation and metastasis *in vitro* and *in vivo*. *PloS one* 10 (3), e0120905. doi:10.1371/journal.pone.0120905

Zhao, Y., Liu, X., and Lu, Y. X. (2017). MicroRNA-143 regulates the proliferation and apoptosis of cervical cancer cells by targeting HIF-1α. *Eur. Rev. Med. and Pharmacol. Sci.* 21 (24), 5580–5586. doi:10.26355/eurrev_201712_13997

Zu, Y., Wang, Q., and Wang, H. (2022). Identification of miR-885-5p as a tumor biomarker: regulation of cellular function in cervical cancer. *Gynecol. Obstetric Investigation* 86 (6), 525–532. doi:10.1159/000520980

frontiers | Frontiers in Cell and Developmental Biology

TYPE Review
PUBLISHED 08 October 2024
DOI 10.3389/fcell.2024.1455572

OPEN ACCESS

EDITED BY
Lucio Miele,
Louisiana State University, United States

REVIEWED BY
Narendra Bharathy,
Eurofins Lancaster Laboratories, United States
Zhiming Li,
Columbia University, United States

*CORRESPONDENCE
Rajesh Kumar Yadav,
rkyadav@nii.ac.in

†These authors have contributed equally to this work

RECEIVED 27 June 2024
ACCEPTED 10 September 2024
PUBLISHED 08 October 2024

CITATION
Yadav P, Jain R and Yadav RK (2024) Emerging roles of cancer-associated histone mutations in genomic instabilities.
Front. Cell Dev. Biol. 12:1455572.
doi: 10.3389/fcell.2024.1455572

Emerging roles of cancer-associated histone mutations in genomic instabilities

Priyanka Yadav †, Ronit Jain † and Rajesh Kumar Yadav *

Cancer Epigenomics Laboratory, National Institute of Immunology, New Delhi, India

Epigenetic mechanisms often fuel the quick evolution of cancer cells from normal cells. Mutations or aberrant expressions in the enzymes of DNA methylation, histone post-translational modifications, and chromatin remodellers have been extensively investigated in cancer pathogenesis; however, cancer-associated histone mutants have gained momentum in recent decades. Next-generation sequencing of cancer cells has identified somatic recurrent mutations in all the histones (H3, H4, H2A, H2B, and H1) with different frequencies for various tumour types. Importantly, the well-characterised H3K27M, H3G34R/V, and H3K36M mutations are termed as oncohistone mutants because of their wide roles, from defects in cellular differentiation, transcriptional dysregulation, and perturbed epigenomic profiles to genomic instabilities. Mechanistically, these histone mutants impart their effects on histone modifications and/or on irregular distributions of chromatin complexes. Recent studies have identified the crucial roles of the H3K27M and H3G34R/V mutants in the DNA damage response pathway, but their impacts on chemotherapy and tumour progression remain elusive. In this review, we summarise the recent developments in their functions toward genomic instabilities and tumour progression. Finally, we discuss how such a mechanistic understanding can be harnessed toward the potential treatment of tumours harbouring the H3K27M, H3G34R/V, and H3K36M mutations.

KEYWORDS

chromatin, genomic instability, oncohistone, epigenetic mechanism, histone mutation, central nervous system, oncology

1 Introduction

Cells of the same kind have the same genetic information and genes, but only a small subset of them are transcribed at any given time. Among the many regulatory pathways, it is possible that epigenetic mechanisms can cause heritable changes in gene expressions without altering the genetic sequence, thereby transforming transient signalling events into long-term changes in organism performance. The DNA encodes genetic information within nucleosomal arrays to form chromatin (Talbert and Henikoff, 2010; Allis and Jenuwein, 2016). In eukaryotic cells, the DNA is wrapped around a histone octamer containing two copies of each core histone, namely H2A, H2B, H3, and H4, to form nucleosomes. In the nucleosomes, unstructured N-terminal tails extend outward and are subjected to post-translational modifications (PTMs). Each amino acid and PTM on the small tails of the histones uniquely determine the nucleosome structure and impact the functions of the proteins that add the PTMs (writers), recognise the PTMs (readers), and remove the PTMs (erasers). Additionally, histone variants such as the H3.3 add further

complexities to the eukaryotic epigenome by regulating the chromatin structure functions (Kallappagoudar et al., 2015; Maze et al., 2014; Cohen and Meshorer, 2024). Within the nucleus, the organisation of the chromatin into higher-order structures enables formation of chromatin domains that carry out the diverse cellular signalling functions, including gene expression regulation and providing a conducive chromatin environment. The dynamic PTMs of histones, such as acetylation, methylation, and phosphorylation, serve as key mediators of signalling events by controlling DNA accessibility for the assembly of writers, readers, erasers, and chromatin remodeller proteins. Furthermore, PTMs are tightly regulated by the complex interplay of transcription factors, chromatin-modifying complexes, and signalling pathways, which in turn integrate precise control of the gene expression upon developmental cues with environmental stimuli. To develop stable and reversible epigenomic phenotypes, epigenetic signals from the developmental and environmental cues must be centrally integrated at the nucleus (Flavahan et al., 2017). Perturbations of the chromatin structure have profound effects on cells through alteration of gene expressions or initiation of genomic instabilities (Jones and Baylin, 2007).

Chromatin is not only the packaging material of the DNA but also the orchestrator of signalling events whenever cells experience intrinsic or extrinsic DNA damage (Dabin et al., 2023; Yao and Dai, 2014; Jackson and Bartek, 2009). Therefore, in response to DNA lesions, multiple cellular pathways regulate the DNA damage response (DDR) network to sense, signal, and repair the DNA lesions in the context of chromatin (Dabin et al., 2023; Jackson and Bartek, 2009; Arnould et al., 2023; Pinto et al., 2021; Ferrand et al., 2020). Furthermore, chromatin-modifying complexes, chromatin remodellers, and histone PTMs bridge the DNA lesions with the DDR pathways by including DNA damage and mitotic checkpoint proteins. Their dysregulation can cause genome instabilities and mutations that drive cancer cell development, allowing cell clones and cell-to-cell variations both inside tumours and between the tumour and its metastasis (Shen and Laird, 2013). Inactivating mutations in the epigenomic components can disrupt gene expression and genomic stability through DNA methylation–demethylation reactions, PTMs, and alteration of the positioned nucleosomes (You and Jones, 2012).

In this review, we summarise the most recent data on how oncohistones contribute to tumour development mechanistically through their strong impacts on the chromatin states, gene expressions, and genomic stability. Recent studies have reported how various histone mutations impair the chromatin structure–function relationships, resulting in genomic instabilities, dysregulated DDR pathway activation, and defects in DNA repair (Giacomini et al., 2024; Rominiyi and Collis, 2022). However, more precise studies are required to establish the mechanisms and identify the mutation-specific functions of oncohistones in genome instability and the DDR pathways. Finally, we also discuss how combinatorial inhibitors in the chromatin modulator and DDR network could provide opportunities for treating cancers involving H3K27M, H3G34R/V, and H3K36M mutations. By compiling findings from various studies, this review aims to provide a comprehensive understanding that would enable new therapeutic approaches.

2 Methodology

Histone mutations in cancer were discovered in 2012 through genome sequencing events. We searched for cancer-associated histone mutations from 2012 to January 2024 on PubMed, Google search, and the cancer genome database using the following phrases: 'histone mutation in cancer', 'histone H3 in cancer', 'H3K27M', 'H3G34R', 'H3G34V', 'H3G34W', 'H3K36M', 'oncohistone', 'histone mutation in glioma', 'histone mutation and brain tumour', 'histone mutation in bone tumour', 'oncohistone and glioblastoma', 'cancer-associated histone mutants in genomic instability or DNA damage', 'H3K27M or H3G34R/V', and 'genomic instability or DNA damage'. All resulting articles and reviews were critically analysed to summarise the data without using specific inclusion/exclusion criteria. However, the main focus of this review was to identify the emerging roles of cancer-associated histone mutations in genomic instabilities. To ensure simplicity and a mechanistic understanding, the specific focus of this review is on cancer-associated histone H3 mutations, while other histone mutants are excluded. For simplicity, we also include a mechanistic analysis of the H3.3G34R, H3.3G34V, and H3.3K27M mutants in tumorigenesis as a consequence of gene expression changes or the impacts of genomic instabilities. Readers are also referred to another review on histone localisation and nomenclatures regarding chromosomes (Amatori et al., 2021).

3 How do histone mutations contribute to cancer?

Following the discovery of the human genome sequence, numerous studies have found genomic aberrations in cancer cells through next-generation sequencing. Before 2012, aberrant gene expressions or mutations were identified in the writers, erasers, readers, and chromatin remodeller proteins of various epithelial, haematological, and other cancers (Shen and Laird, 2013), but mutations in the histones themselves are now emerging as a common feature of many cancers (Bonner et al., 2023; Sahu and Lu, 2022; Hanahan, 2022).

3.1 Histone H3 is mutated in various cancers

Among the solid tumours, those developing in the brain and central nervous system (CNS) (approximately 100 types) have caused the most cancer-related deaths in children (Capper et al., 2018; Downing et al., 2012; Louis et al., 2021). The major difference between adult and paediatric brain and solid tumours is the tissue development and organogenesis determining the molecular characteristics of tumours (Downing et al., 2012). Country-wise surveys indicate that tumours occurring in the CNS vary from 1.12 to 5.14 cases per 100,000 persons per country. Among these, paediatric high-grade gliomas (pHGGs) constitute approximately 10% of the tumours, and about 40% of the deaths occur due to pHGGs including glioblastomas and diffuse intrinsic pontine gliomas (DIPGs) (Louis et al., 2021; Johnson et al., 2014; Moudgil-Joshi et al., 2021; Louis et al., 2016). Standard care for adult HGGs include resection, radiotherapy, and temozolomide

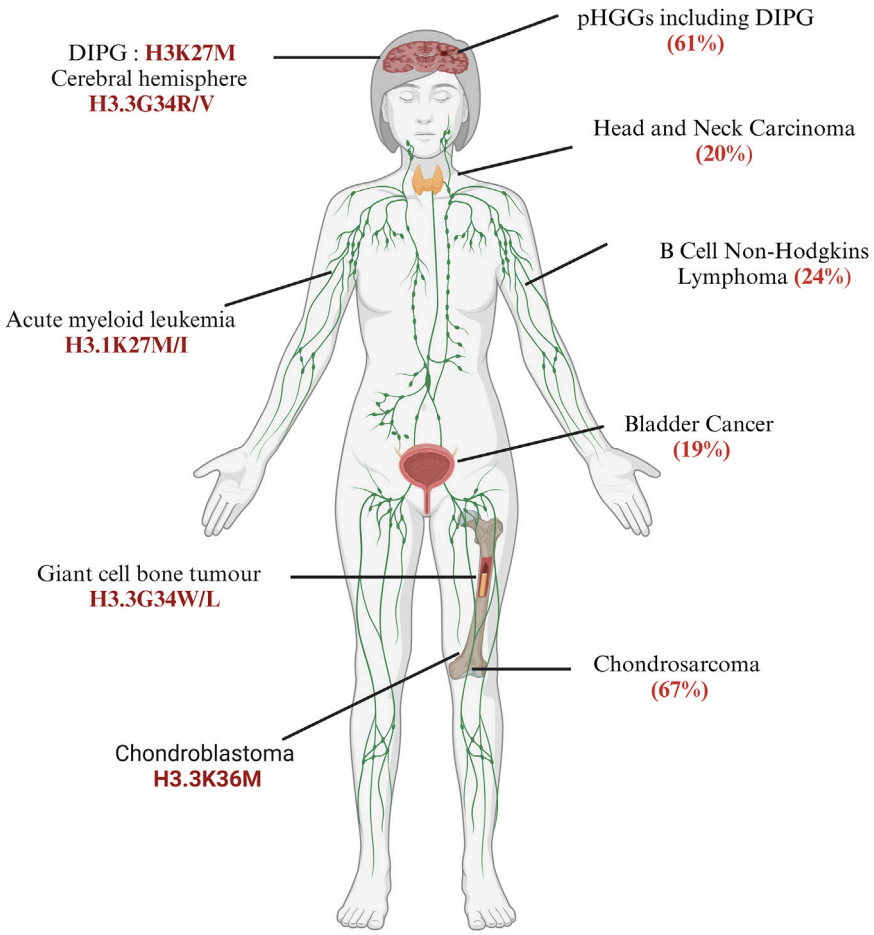


FIGURE 1
Frequency and distribution of somatic histone mutations across different cancer types: the left panel indicates specific histone H3 mutants discussed in this review, while the right panel mentions the total frequency of core histones in patients, as discussed by Bonner et al. (2023). Diffuse intrinsic pontine gliomas (DIPGs) (created with BioRender.com, accessed on 9 June 2024).

(TMZ) administration; however, this course of cancer management does not work properly with pHGGs as it is clear that the molecular basis of pHGG is different from that of adult HGG. Even though the histology of paediatric glioma is similar to that of adult glioma, the advent of next-generation sequencing, proteomics, and epigenetic profile analyses have led researchers to classify pHGGs into many subtypes (Louis et al., 2021). pHGGs are aggressive cancers with poor survival rates (Ocasio et al., 2023); pHGGs and their adult counterparts can be distinguished by molecular subtyping for histone mutational status in neuro-oncology (Louis et al., 2021; Moudgil-Joshi et al., 2021; Faury et al., 2007; Mackay et al., 2017). For example, diffuse midline glioma (DMG) with H3K27 mutation is a novel type of CNS tumour declared by the World Health Organization (Louis et al., 2016). Herein, we discuss the prevalence of histone mutations in various cancers.

3.1.1 Puzzling piece of histone mutations: tissue-specific or multiple tissue prevalence?

pHGGs consist of 80% of DMGs, which include both DIPGs and other diffuse gliomas harbouring missense mutations of lysine-27 to methionine (H3.3K27M); however, the cerebral hemispheres of older adolescents and young adults harbour approximately 30% of diffuse gliomas with glycine-34 to arginine or valine (H3.3G34R/V) mutations encoded mainly by the *H3F3A* gene (Ocasio et al., 2023; Khuong-Quang et al., 2012; Wu et al., 2012; Schwartzentruber et al., 2012; Taylor et al., 2014; Fontebasso et al., 2014). Notably, the H3.3G34R and H3.3K27M mutations are mutually exclusive, and their localisation and expressions of region-specific neurodevelopmental signatures are quite different in the brain (Mackay et al., 2017; Khuong-Quang et al., 2012; Bressan et al., 2021; Sturm et al., 2012). H3.3G34 tryptophan (W) and H3.3G34 leucine (L) are found in the giant cell tumours of the bone (GCTB) (Behjati et al., 2013) but not in CNS tumours; furthermore, 11% of paediatric primitive neuroectodermal tumours of the CNS (CNS-PNETs) have the H3.3G34R mutation (Gessi et al., 2013). Paediatric patients with glioblastomas and CNS-PNETs should be diagnosed carefully. Interestingly, in adults, H3.3K27M was found only in the *H3F3A* gene but not in other histone H3 genes (Schulte et al., 2020). The histone H3 variant H3.3 protein also encoded by *H3F3B* has a lysine-36 to methionine (H3.3K36M) mutation in 90% of chondroblastomas (Behjati et al., 2013; Fang et al., 2016) and glycine-34 to tryptophan/leucine (H3.3G34W/L) in GCTBs. These oncohistones broadly contribute to tumour development by impacting gene expressions and the

regulatory attributes of cellular differentiation (Sahu and Lu, 2022; Mohammad and Helin, 2017). Further cataloguing of cancers has enabled the identification of an expanding landscape of 'oncohistone' mutations in various human cancers (11% of tumours having somatic histone mutations) (Bonner et al., 2023; Nacev et al., 2019). Overall, the estimation of all cancers including those in children, young adults, and adults show that the highest prevalence rates were found in 67% of chondrosarcomas, more than 60% of pHGGs, and 30% of lymphomas (Bonner et al., 2023) (Figure 1). Identification of H3K27M in medulloblastoma has serious implications for the diagnostic value of cancer subtyping (Dottermusch et al., 2022). In posterior fossa A (PFA) ependymomas, one subtype of ependymomas (EPN) is rarely mutated for H3.3K27M but these tumours have increased expressions of the enhancer of zeste homologue inhibitory protein (EZHIP), which causes reduced H3K27 methylation like in H3K27M, suggesting the importance of H3K27 residues as hotspot mutations in brain tumorigenesis (Jenseit et al., 2022; Hubner et al., 2019; Ryall et al., 2017). Outside the CNS, H3.1K27M or H3.1K27I mutations are frequently found in acute myeloid leukaemia (AML) but not in H3.3 proteins (Lehnertz et al., 2017). It is clearly noted that DMGs harbouring H3.1K27M and H3.3K27M contribute differently to tumorigenesis based on the epigenomic profile and transcriptional status (Zhang et al., 2024; Liu et al., 2022; Jessa et al., 2022; Castel et al., 2015). These studies suggest that histone H3 mutations are both tissue-specific and tissue-independent, meaning that the same mutants may be found in other tissues as well (Figure 1). This fact will be further discussed in the next section through the mechanistic aspects of histone mutations.

3.2 H3K27M, H3G34R/V, and H3K36M mutations alter the chromatin states in several cell types

To understand the impacts of histone mutations on the epigenome of cancer cells, several model systems have been utilised for *in vitro* studies to *in vivo* characterisations using tissue cultures, patient-derived samples, mouse models, and *Drosophila* to eukaryotic yeast systems, for which the readers are referred to other works (Zhang et al., 2023; Chaouch and Lasko, 2021). Herein, we summarise the data obtained in the context of tissue cultures, mouse models, and xenograft models to establish whether histone mutants contribute to tumorigenesis alone or in combination with other factors. We also correlate these findings with those from other eukaryotic model systems to understand the biology of the histone mutant phenotypes independent of the heterogeneity of cancer cells wherever required.

3.2.1 H3K27M alters PTMs at the H3K27 residue

The context of the epigenome in the developmental pathways is essentially a dynamic manifestation of the signalling molecules, growth factors, cellular memory, and differentiation cues to establish a faithful cellular context in organogenesis. Histones are just one component of a complex chromatin environment. In the evolutionary context, multiple alleles of histones are present in the cells to provide redundancy for cell survival. Ideally, deletions or a few mutations in the histones should not have dramatic impacts on cell survival or tumorigenesis. Interestingly, histone alterations in cancers occur in just one allele, and they are somatic heterozygous mutations. Therefore, the foremost thing is to determine whether the histone mutant acts dominantly in the presence of a wild-type (WT) copy of the histone. It is crucial to determine the contributions of histone mutations in tumour development by understanding the altered landscape of the epigenome in cancer cells.

The histone H3K27 undergoes methylation and acetylation depending on the context of the developmental signals through a complex interplay of chromatin modifiers, such as methyltransferases, demethylases, acetyltransferases, and deacetylases. H3K27 methylation is regulated by the polycomb repressive complex 2 (PRC2) and recruits PRC1 for monoubiquitination of histone H2A at lysine-119 (H2AK119ub) (Blackledge and Klose, 2021). Their interplay regulates the development of mammalian cells through the regulation of chromatin structure–function relationships (Blackledge and Klose, 2021). Only one H3K27M mutant histone H3 is present along with a WT copy of histone H3; moreover, H3.3K27M inhibits the catalytic subunit of PRC2, namely lysine methyltransferase EZH2 (enhancer of zeste 2, KMT6), and causes global reduction of repressive histone H3K27 trimethylation accompanied by enrichment of H3K27 trimethylation and EZH2 itself at a certain genomic locus (Lewis et al., 2013; Venneti et al., 2013; Chan et al., 2013; Harutyunyan et al., 2019) (Figure 2). The H3K27M mutant binds tightly with EZH2 and blocks its methyltransferase activities as well as genome-wide deposition of H3K27me2 and H3K27me3 (Lewis et al., 2013; Harutyunyan et al., 2019; Justin et al., 2016). However, the EZH2 expression remains unaltered (Venneti et al., 2013). Another model proposes that H3K27M disrupts the auto-methylation of the PRC2 subunits (EZH2 and SUZ12) (Lee et al., 2019). H3.3K27M patient cells show reduced dimethylation and trimethylation of H3K27 globally; however, EZH2 with these PTMs are localised on the cancer-associated genes, suggesting additional mechanisms of action of the H3K27M mutant histone (Chan et al., 2013; Bender et al., 2013). Interestingly, analysis of live-cell single-molecular dynamics of PRC2 suggests that H3.3K27M delays the chromatin residence time and target search time of EZH2 (Tatavosian et al., 2018).

H3.3 in cooperation with acetyltransferase (p300) is required for H3K27 acetylation (H3K27Ac), which is a mark of an active promoter and enhancer (Choi et al., 2024). H3K27Ac co-localises with H3.3K27M and bromodomain proteins at the actively transcribed genes, which excludes PRC2 from the H3K27M-occupied regions (Piunti et al., 2017). H3.3K27M mutants mainly inhibit the spread the of H3K27me3 marks, provided that PRC2 deposition and propagation remain the same (Harutyunyan et al., 2019; Chen et al., 2020a) (Figure 2). This results in dysregulation of the super-enhancers of specific gene clusters, such as the NOTCH pathway genes (Chen et al., 2020a).

3.2.2 H3K27M and H3G34R mutants both impact DNA methylation

The biological context and dynamic interactions between H3K27me3 and DNA methylation regulate the chromatin structure for transcription and other DNA-templated processes such as DNA repair. H3.3K27M and H3.3G34R both reduce the

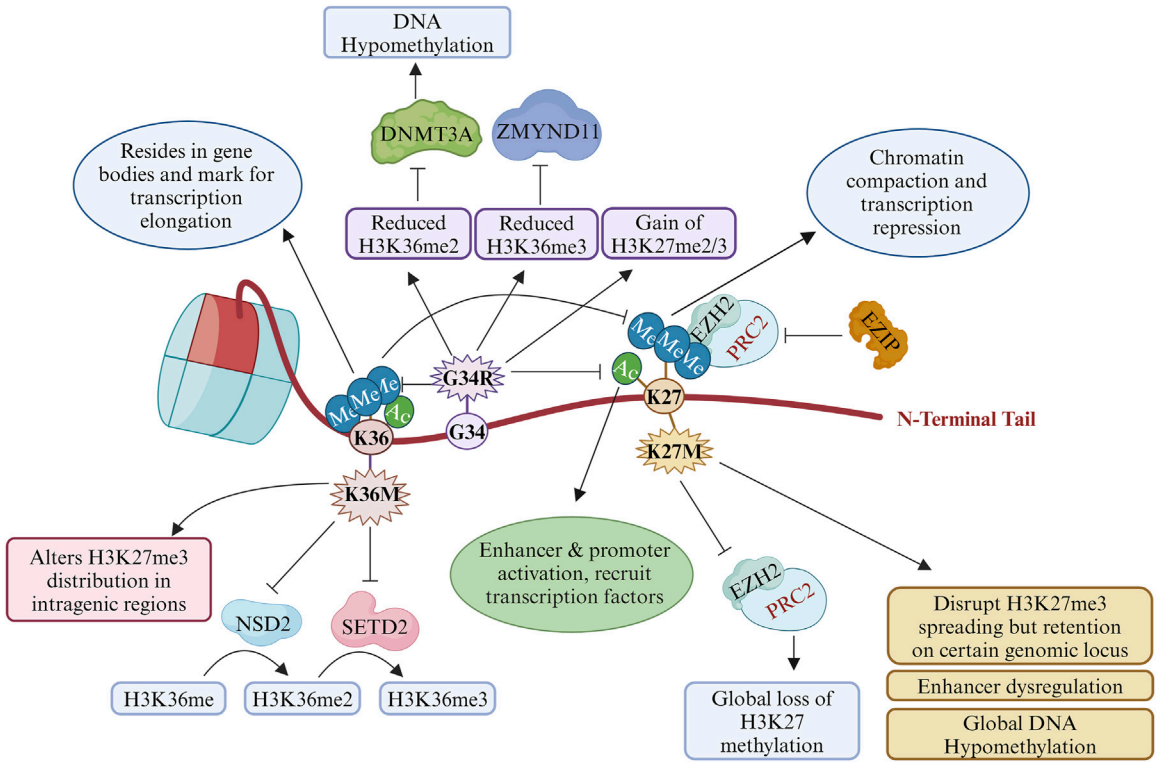


FIGURE 2
Roles and transcriptional impacts of oncohistones in cancer: post-translational modifications (PTMs) at K27 and K36 by key oncohistone mutations H3K27M, H3G34R, and H3K36M. This illustration describes how these oncohistones alter the bindings of reader, writer, and eraser proteins to promote transcriptional regulation and genomic instability. ZMYND11, zinc finger MYND-type containing 11; NSD2, nuclear receptor-binding SET domain protein 2; DNMT3A, DNA (cytosine-5)-methyltransferase 3 alpha; SETD2, SET domain containing 2; EZH2, enhancer of zeste homologue 2; PRC2, polycomb repressive complex 2; EZIP, EZH2 interaction protein (created with BioRender.com, accessed on 9 June 2024).

global DNA methylation levels in K27M in pHGGs (Sturm et al., 2012; Bender et al., 2013; Kfoury-Beaumont et al., 2022) (Figure 2). G34 and K27 appear as separate entities in a DNA-methylation-based classification of CNS tumours (Capper et al., 2018; Louis et al., 2021). Since DNA methylation at specific genes regulates stem cell proliferation and stem cell properties, it is not surprising that aberrant expressions of gene patterns are involved in stem cell regulation, differentiation, and tumorigenesis owing to the dominant negative activity of H3K27M expression (Kfoury-Beaumont et al., 2022). To identify the epigenomic factors and other proteins in an unbiased manner, Siddaway et al. (2022) identified the transcription factors, H3K9 methyltransferases, and DNA repair proteins along with many published chromatin modifiers, such as the PRC2 component. Thus, H3K27M alters the chromatin states and transcriptional outputs by changing the epigenomic factor recruitment to profoundly impact tumorigenesis.

3.2.3 H3G34R/V and H3K36M mutations alter the PTMs at H3K36 methylation

H3K36 methylation is crucial for controlling gene transcriptions, and its perturbation will contribute to cancer development. Adult secondary glioblastoma multiforme (GBM) harbours isocitrate dehydrogenase 1/2 (IDH1/2) mutations (about 98%), which are rarely found in childhood GBM, and also regulate methylation at the H3K27 and H3K36 residues (Sturm et al., 2012). This section highlights how H3K36 methylation is perturbed in paediatric CNS tumours as well as soft-tissue bone tumours.

H3G34 lies close to the H3K36 residue that can undergo methylation as well as acetylation and is implicated in several DNA-templated processes such as transcription, dosage compensation, DNA replication, and DNA damage repair (Kallappagoudar et al., 2015; Carpenter, 2012). Histone methylations occur at the arginine or lysine residues, with H3K36 being methylated in three forms. The yeast enzyme SET2, which is a homologue of the human SETD2, generates H3K36 me1/me2/me3 methylation, but separate enzymes such as MMSET or NSD1 or NSD2 (Wolf–Hirschhorn syndrome candidate 1, WHSC1) are responsible for monomethylation and dimethylation in humans while SETD2 undergoes trimethylation (Kallappagoudar et al., 2015; Carpenter, 2012; Sharda and Humphrey, 2022) (Figure 2). H3.3G34R/V blocks the activity of SETD2, which is itself mutated in 15% of pHGGs (Kallappagoudar et al., 2015; Lewis et al., 2013; Huang et al., 2020; Yadav et al., 2017; Fontebasso et al., 2013). Accordingly, each G34 mutant has a differential impact on H3.3K36me2/3 *in cis* (Lewis et al., 2013; Yadav et al., 2017; Khazaei et al., 2023; Lowe et al., 2021; Shi et al., 2018) (Figure 2). Fang et al. (2018) identified a H3 'G33-G34' motif that serves as a docking site for the SETD2 enzyme. Furthermore, in GCTBs, H3.3G34L/W mutants inhibit SETD2 enzymatic activity like the H3.3G34R/V mutants (Shi et al., 2018). The level of H3K36ac also depends on the

amino acid substitution at the H3G34 residue (Yadav et al., 2017; Lowe et al., 2021). Therefore, characteristics of amino acid substitution at H3G34 regulate the PTMs at the H3K36 residue.

H3K36me3 is a highly enriched mark on the gene bodies of actively transcribed genes, which are involved in transcriptional elongation and DNA repair in the transcriptionally active regions (Bannister et al., 2005). Reduced H3K36 methylation causes an aberrant gain of H3K27me2/3 and loss of H3K27ac on a gene with SETD2 activity (Jain et al., 2020). G34R cause hypo DNA methylation like H3K27M through reduced recruitment of the decreased DNA methyltransferase DNMT3A by impaired binding with H3K36me2 (Khazaei et al., 2023). H3.3G34R and H3.3K27M mutations modulate the recruitment of H3.3 at the transcriptional active sites (Newhart et al., 2013). On the other hand, the transcriptional repressor ZMYND11 interacts with H3K36 methylation to regulate gene expression while the H3.3G34R mutant abrogates the binding of ZMYND11 (Bressan et al., 2021) (Figure 2). Furthermore, the H3.3G34V mutation prevents ZMYND11 binding to the H3.3K36me3 peptide (Wen et al., 2014). H3.3G34R reduces H3K9me3 and H3K36me3 by inhibiting the enzymatic activities of the KDM4 family of K9/K36 demethylase-like adult counterparts, where the IDH1/2 mutants inhibit KDM4 (Voon et al., 2018). H3K9 methylation and heterochromatin are intact in the H3G34R mutant fission yeast cells (Yadav et al., 2017). These studies implicate the effects of G34 substitution on H3K27 methylation, DNA methylation, H3K36 methylation, and enhancers of the cancer-associated or developmental controlling genes.

H3.3K36M mutant proteins cause global reductions of H3K36 methylation in human chondroblastoma by inhibiting two H3K36 methyltransferases, i.e., NSD2 and SETD2 (Fang et al., 2016). Similar to the H3.3K27M mutants, the H3K36M mutant nucleosomes inhibit H3K36 methyltransferase enzymatic activities, resulting in the loss of H3K36 trimethylation (Lu et al., 2016; Yang et al., 2016) (Figure 2). Furthermore, H3K36M inhibits NSD1, NSD2, and H3K36me2 status phenocopy of the genetic deletions of these methyltransferases (Rajagopalan et al., 2021). However, the GCTB G34 mutations (H3.3G34L/W) reduce H3K36 methylation *in cis* (Shi et al., 2018; Yang et al., 2016). H3.3G34W mutation increases the splicing alterations in GCTBs by interacting with several splicing factors (significant interactor-trans-acting splicing factor hnRNPA1L2) (Lee et al., 2024).

3.2.4 Does the H3K27M mutant alone cause tumorigenesis?

In DIPGs, the H3.3K27M mutation occurs concurrently with p53 mutations and platelet-derived growth factor receptor α polypeptide (PDGFRA) amplification (Khuong-Quang et al., 2012; Sturm et al., 2012; Jones and Baker, 2014; Funato et al., 2014). Expression of H3.3K27M in the neural progenitor cells (NPCs) derived from human embryonic stem cells increases its cellular proliferation (Funato et al., 2014). Furthermore, H3.3K27M with overexpression of the constitutively active PDGFRA mutant and knockdown of p53 has increased cell proliferation, transformation, and tumorigenicity for NPCs (Funato et al., 2014). Single-cell sequencing of the H3.3K27M gliomas suggests that their cell origins resemble oligodendrocyte precursor cells and lack differentiated malignant cells (Filbin et al., 2018). Thus, the development of preclinical glioma models of H3K27M requires an additional mutation in p53 as well as others like PDGF and ACVR1, as reviewed here (Sahu and Lu, 2022). Knockdown of H3.3K27M in DIPG xenografts restores H3K27me3 and inhibits tumour growth. It was found that the loss of H3K27me3 reduces the differentiation of NPCs by regulating the poised promoter status of the cancer-associated genes (Silveira et al., 2019). In *C. elegans,* modelling of H3.3K27M resulted in alteration of H3K27me3 that produces ectopic DNA replication and cell cycle progression (Delaney et al., 2019). Local inhibition of the pre-existing H3K27me3 by H3.3K27M upregulated the Jun amino-terminal kinase (JNK) in germ cells, which may be used as targets for the tumour-derived H3.3K27M cells (Delaney et al., 2019). The yeast system lacks PRC2 and DNA methylation, so *C. elegans* and *Drosophila* may be used as model systems to study H3K27M mutations (Zhang et al., 2023; Chaouch and Lasko, 2021).

3.2.5 Do H3G34R/V and H3K36M mutations cause tumorigenesis?

Engineering the H3.3G34R mutation in human astrocytes showed increased proliferation compared to the WT astrocytes (Chen et al., 2020a). H3.3G34R/V/W knock-in mice show distinct developmental defects, and modelling these mutations in a fission yeast system causes differential genomic instabilities, suggesting that each substitution of the G34 residue produces unique phenotypes (Khazaei et al., 2023; Lowe et al., 2021). This is further illustrated by the role of H3.3K36M in chondrocytes, which exhibit increased colony formation, blocked apoptosis, and differentiation (Fang et al., 2016), such as the differentiation of the mesenchymal progenitor cells. H3.3K36M impacts chondrocyte differentiation and limb development but no tumour development has been recorded in this knock-in mouse model, suggesting additional requirements for tumour development (Abe et al., 2021). However, undifferentiated sarcomas are produced by the H3K36M mutant, suggesting tissue-specific functions of the oncohistone (Lu et al., 2016). H3K36 methylation also alters the genome-wide gain in H3K27 methylation in the H3.3K36M mutant, which redistributes PRC1 and hence de-represses the genes responsible for mesenchymal differentiation in the H3.3K36M mutant cells (Lu et al., 2016). Thus, it is crucial to delineate the individual roles of H3K36me2 and H3K36me3 in H3K36M-driven oncogenesis (Rajagopalan et al., 2021). H3.3K36M alters H3K27me3 distribution through the global loss of H3K36me2 (Abe et al., 2021).

In pHGGs, H3.3G34R/V mutations are accompanied by tumour protein p53 (TP53) loss and PDGFRA amplification (Mackay et al., 2017; Chen et al., 2020b). Furthermore, H3.3G34R/V HGGs are similar to their adult counterparts because both tumours have mutations in the chromatin remodelling protein (ATRX) and TP53 (Schwartzentruber et al., 2012; Chen et al., 2020b; Liu et al., 2012; Korshunov et al., 2016). However, H3.3G34W mutants are found only in mesenchymal tissues (Khazaei et al., 2023; Jain et al., 2020; Chen et al., 2020b).

H3.3G34R/V inhibits neuronal differentiation and contributes to tumorigenesis through altered gene expressions (Chen et al., 2020b). In glioblastomas, G34 mutations upregulate the oncogene MYCN (Bjerke et al., 2013). These studies highlight the direct impacts of histone mutations on PTMs, which regulate the

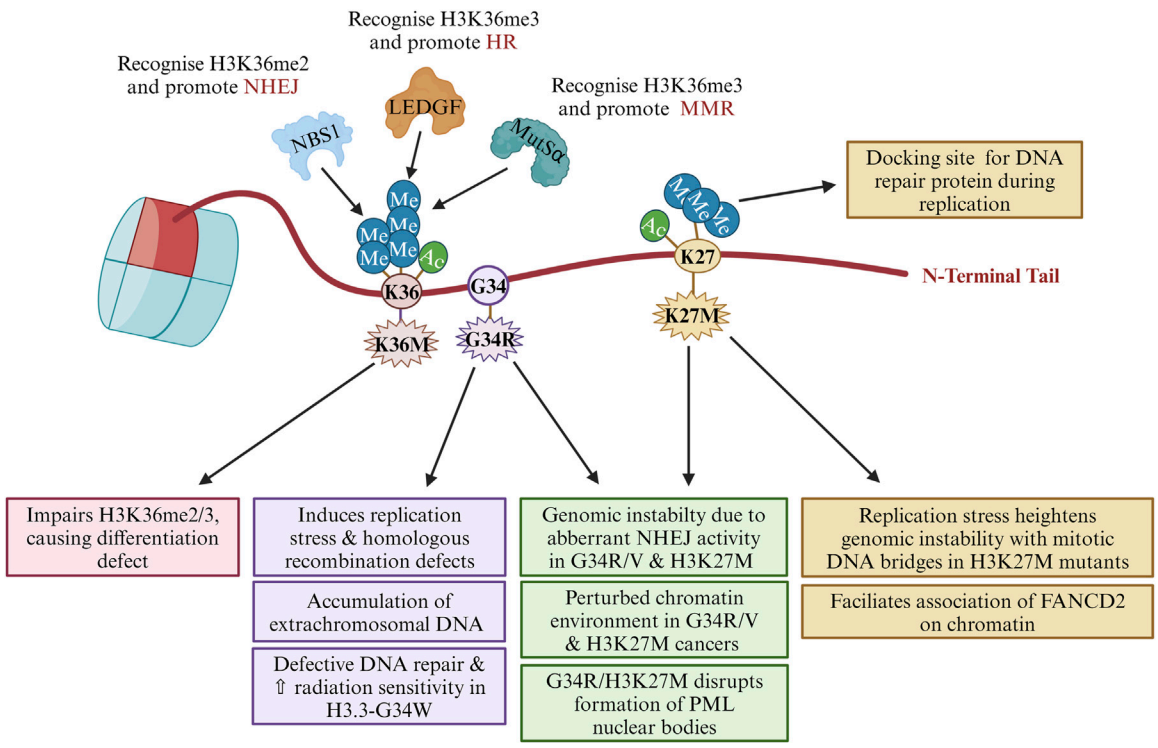


FIGURE 3
Oncohistone descriptions in genomic instability: contributions of histone PTMs at H3K27, H3G34, and H3K36 to DNA repair pathway choice. This figure illustrates the roles of the PTMs at histone H3 lysine-27 (H3K27) and lysine-36 (H3K36) in promoting genomic instabilities. Specific PTMs at these residues are depicted, highlighting their influences on the chromatin structure and functions, particularly in the context of DNA damage response (DDR) and replication stress. The schematic representation illustrates how these oncohistone mutations disrupt normal chromatin dynamics and DDR processes, increasing replication stress and genomic instability. FANCD2, Fanconi anaemia complementation group D2; LEDGF, lens epithelium-derived growth factor; NBS1, Nijmegen breakage syndrome 1; MutSα, MutS homologue alpha (MSH2-MSH6 heterodimer) (created with BioRender.com, accessed on 9 June 2024).

transcriptional profiles of tumour cells. However, these PTMs also regulate the DDR pathways in cells, so we discuss how histone mutations play crucial roles in genomic instabilities by modulating the epigenome through altered PTM profiles in the tumour cells.

4 Histone H3.3 and its PTMs at K27 and K36 contribute to DDR in several cell types

Histones can be classified as canonical (H3.1 and H3.2) or replication-dependent histones and non-canonical (H3.3) or replication-independent histone variants depending on their requirements during DNA replication (Talbert and Henikoff, 2010). A countable number of amino acid differences between these variants make them distinguishable in terms of chaperone binding, chromatin localisations, and other functions (Kallappagoudar et al., 2015; Ferrand et al., 2020; Szenker et al., 2011). The protein H3.3 plays an extensive role in regulating chromatin structures and cellular differentiation (Cohen and Meshorer, 2024; Shi et al., 2017). During DNA damage signalling, chromatin remodelling occurs through deposition of H3.3 and is critically regulated by ATRX and the switch/sucrose non-fermentable (SWI/SNF) family chromatin remodeller protein CHD1 to regulate genomic stability during transcription as well as replication (Choi et al., 2024). For additional details, readers are referred to reviews on chaperones and other complexes (Choi et al., 2024; Szenker et al., 2011). Extensive deposition of the histone variant H3.3 across the chromosomal domain has been reviewed through different chaperone binding activities that regulate the maintenance of chromosomal integrity and DDR, including the chromatin dynamics during DNA damage (Ferrand et al., 2020). Mouse models suggest that during mammalian development, the H3.3 null mutant causes defects in the heterochromatic structures and genome integrity (Jang et al., 2015).

When DNA damage occurs during transcription, evidence suggests that the locus must be silenced to avoid conflicts of the transcription machinery with the DNA repair complexes (Campbell et al., 2013). Upon DNA damage and repair, transcription recovery at the locus requires H3.3 deposition (Frey et al., 2014; Adam et al., 2013). H3.3 deposition is also required during the S phase only when cells encounter UV irradiation, suggesting the importance of the histone H3.3 during transcription and replication-associated DNA damage (Frey et al., 2014; Adam et al., 2013). Furthermore, the repair depends upon the DNA damage-associated poly (ADP-ribose) polymerase 1 (PARP1) activity, attracting the chromatin remodelling complex and other gene silencing proteins to shut off nearby gene transcriptions to facilitate DNA repair (Chou et al., 2010; O'Hagan et al., 2011). Alternative to these mechanisms, PARP1 also recruits CHD2 that triggers chromatin relaxation

and H3.3 deposition at the DNA damage locus (Dabin et al., 2023; Luijsterburg et al., 2016). Furthermore, EZH2-dependent H3K27me3 marks may act as molecular 'timers' of DDR pathways when DNA damage occurs during replication (Ito et al., 2018) (Figure 3). The G34 small residue lies between H3K27 and H3K36 and may control the binding of epigenetic factors for DDR through its impact on the PTMs of K267 and K36.

DNA lesions occur in the context of chromatin, which controls the choice of pathways by which these lesions are repaired (Figure 3). CNS tumours are mostly managed clinically through radiation, which produces DNA double-strand breaks (DSBs). The main repair pathways are DNA non-homologous end-joining (NHEJ) and homologous recombination (HR). Several factors determine the fate of DSB repairs, such as cell cycle stages, chromatin environment surrounding the DNA lesions, availability of repair proteins, compartmentalisation within the nucleus, and transcription status around the lesions (Arnould et al., 2023; Campbell et al., 2013). Furthermore, selective utilisation of NHEJ *versus* HR is evident in nervous system development (Jackson and Bartek, 2009; Orii et al., 2006).

H3K36me3 resides on the gene body and marks the elongation of transcription (Barski et al., 2007). Interestingly, its role in genomic instability and other cellular functions is found to increase each day (Carpenter, 2012). H3K36me3 modulates DNA repairs at the transcriptionally active regions as well as DNA lesions through mismatch repair (MMR) (Sharda and Humphrey, 2022; Fang et al., 2018). H3K36me2 promotes NHEJ, whereas H3K36me3 promotes HR as the PTMs are recognised by Nijmegen breakage syndrome 1(NBS1) and lens epithelium-derived growth factor (LEDGF) proteins, respectively (Sharda and Humphrey, 2022; Fnu et al., 2011; Pfister et al., 2014) (Figure 3). Depletion of H3K36me3 leads to decreased ataxia-telangiectasia mutated (ATM) and p53 phosphorylations, defective DNA end-resection, impaired damage recruitment of RPA and RAD51, and low HR efficiency; it provides binding sites for the PWWP methyl reader domain of LEDGF, promoting HR repair through interactions with the C-terminal binding protein 1 (CtBP1) interacting protein (CtIP) (Aymard et al., 2014). Overexpression of H3K36me3 demethylase KDM4A reduces HR efficiency in cells. Dimethylation of H3K36 is induced by ionising radiation and accumulates around the DSBs, leading to increased accumulation of NHEJ factors (Pfister et al., 2014). Klein et al. (2018) found that the common cancer-related substitution of H3K36 to methionine disrupts the H3K36me-writing enzymes and H3K36me-specific readers, potentially leading to oncogenic effects.

5 H3K27M, H3G34R/V, and H3K36M roles in genomic instability or DDR

Loss of heterozygosity of the DNA repair pathway genes and a slight gain in PARP1 expression were found in low samples of DIPGs (Zarghooni et al., 2010). Receptor tyrosine kinase (RTK) inhibitors are often targeted in CNS tumours and other cancers. However, recent studies suggest that signalling events can modulate, stimulate, or inhibit HR or the NHEJ repair pathways (Chabot et al., 2021; Liu et al., 2023). Investigators have found that DDR mechanisms within tumour cells are dysregulated, which can cause tumorigenesis or can be exploited as chemotherapeutic agents in cancer treatment (Rominiyi and Collis, 2022). Therefore, it is essential to develop a protocol to measure the efficiency of the DNA repair pathways in cancer therapy. For example, the recombination proficiency score (RPS) is calculated based on the expressions of the DNA repair pathway genes (Rif1, PARI, RAD51, and Ku80) to determine the fate of chemotherapy (Pitroda et al., 2014). In the next section, we examine the roles of H3K27M, H3G34R/V, and H3K36M in literature for genomic instability or DDR; this helps identify many combined strategies to target both the DDR and RTK signalling pathways (Liu et al., 2023).

5.1 Chromosomal abnormalities in CNS tumours

Genomic instability plays a pivotal role in tumorigenesis, contributing to the development and progression of cancer through various mechanisms. Genomic instability essentially refers to the fact that cancer cells exhibit higher rates of genetic alterations, such as copy number alterations (CNAs), chromosomal rearrangements, and mutations, than normal cells, as discussed for CNS tumours in the previous section. This instability results from various causes, including defects in the DNA repair mechanisms, replication stress, and abnormal telomere maintenance pathways; it is a hallmark of cancer and a major cause of tumorigenesis (Hanahan, 2022).

Comparisons of paediatric and adult glioblastomas show that (a) frequent gains of chromosome 1q were 30% and 9%, respectively; (b) chromosome seven gains were 13% and 74%, respectively; (c) chromosome 10q losses were 35% and 80%, respectively (Paugh et al., 2010). Radio resistance is commonly observed in DMGs (Liu et al., 2023). On the contrary, radiation-induced tumours show significant increases in PDGFRA amplification and 1q gains in childhood gliomagenesis (Paugh et al., 2010). Adult CNS tumours have high CDK6 amplification, 10q loss, and 17q gain, whereas paediatric cases have a high frequency and high specificity of 3q and 4q losses across MYC/MYCN oncogene amplification, suggesting variations in the chromosomal abnormalities between adult and paediatric CNS tumours (Korshunov et al., 2016; Liu et al., 2023; Korshunov et al., 2010).

5.2 Contributions of H3K27M, H3G34R/V, and H3K36M mutations to DDR pathways

During tumour development and ongoing treatment, several processes regulate DNA repair and cell cycle gene expressions. High CNAs are reported in H3.3 mutant gliomas, and mitotic abnormalities such as mitotic bulky and ultrafine DNA bridges were observed in an inducible H3.3K27M cell culture model (Bockaj et al., 2021). This is similar to the replicative stress mechanism reported in a fission yeast model for H3G34R mutation (Yadav et al., 2017). Accumulation of extrachromosomal DNA was observed in the H3.3G34R model mouse and H3.3G34R-harbouring human pHGG cells because of downregulation of the DNA repair pathway genes (Haase et al., 2022). Consistent with this, the H3.3G34R fission yeast cells show lagging chromosomes and are sensitive to replication-stress-specific DNA-damaging drugs (Yadav et al., 2017; Lowe et al., 2021). Thus, it is evident that H3.3 mutant cells display increased genomic instability phenotypes owing to

compromised DDR responses. It may also be speculated that H3.3G34R and IDH1/2 mutations co-operate with ATRX-mutated glioblastomas, leading to alternative lengthening of the telomeres, which is a process that is sometimes regulated through the HR pathways in the absence of or compromised functions of telomerase genes (Udugama et al., 2021). These studies suggest that DNA damage occurs through replication in the H3G34R/H3K27M cells.

H3.3K27M/G34R mutations disrupt the formation of promyelocytic leukaemia (PML) nuclear bodies that are the main drivers of leukaemia in the blood (Voon et al., 2023) (Figure 3). This is crucial as the PML nuclear bodies are important regulators of genome maintenance, and their disruption sensitises the H3.3-mutated glioma cells (Voon et al., 2023; Chang et al., 2018). In adult CNS tumours, the IDH1-R132H mutant epigenetically upregulates DDR and also disrupts the formation of PML bodies (Nunez et al., 2019).

H3.1K27M-engineered human dermal fibroblast cells show reduced foci for 53BP1, an NHEJ protein (Zhang et al., 2018). Consistent with this, increased rates of genomic insertions or deletions and copy number variations occur through p53-dependent apoptosis in these cells (Zhang et al., 2018). Furthermore, hypo-methylation on H3K27 decreases the NHEJ efficiency and facilitates association of Fanconi anaemia complementation group D2 (FANCD2) on the chromatin, which is a central player in the choice of DNA repair pathway (Zhang et al., 2018; Cohn and D'Andrea, 2008) (Figure 3).

Interestingly, H3.3G34W shows DSB repair defects in bone tumours that are sensitive to ionising radiation (IR). It was reported that the enhanced interactions of H3.3G34W with damaged nucleosomes led to dysregulated interactions with the NHEJ key effectors, such as KU70/80 (Mancarella et al., 2024).

H3.3G34R/V/D mutants have shown reduced interactions with the MMR protein MutS homologue α (MutSα; an MSH2-MSH6 heterodimer) and a mutator phenotype similar to that of MMR-defective cells (Fang et al., 2018) (Figure 3). This is due to the reduced recruitment of MutSα by the reduction of H3K36me3. However, further studies are required to highlight the importance of MMR pathways in the context of tumorigenesis (Dabin et al., 2023).

H3K36M oncohistone mutation inhibits SETD2 methyltransferase activity, with S-adenosylmethionine (SAM) indirectly affecting the interactions and maintaining the proper fold state in the SETD2-H3K36M-SAM complex structure (Zhang et al., 2017). Although H3K27M and H3G34R/V mutations have been extensively studied for their roles in paediatric gliomas and other cancers, H3K36M is less explored yet equally critical. Unlike H3K27M, which is known for its repressive effects on the polycomb group proteins, H3K36M primarily affects the methylation landscape associated with actively transcribed genes.

6 Can genomic instabilities and additional genetic requirements of oncohistone-containing cancer cells be exploited as a therapeutic regimen?

pHGG histone mutants are a double-edged sword as these cancer cells are proliferative as well as have genomic instability phenotypes, as discussed in Sections 3 and 5. Therefore, finding synthetic lethal interactions between histone mutants with DDR pathway genes or co-occurring mutational genes may offer promising therapeutic approaches for cancers with histone mutations (Figure 4). Screening has been used to identify vulnerabilities in the DDR-deficient cells, which can be targeted with specific inhibitors or combination therapies to selectively eliminate cancer cells while sparing normal cells. Blum (1950) hypothesised that successive doses of UV radiation increases the rate of cellular proliferation with unclear mechanisms. Genomic instability contributes to cancer progression; however, increased proliferation may pose challenges to accurately repair the error rates in replication due to various oncogenic replication stresses. Activated oncogenes produce DNA DSBs due to stalling and collapse of the DNA replication forks during continuous cell proliferation, and TP53 acts as the guardian of genomic stability (Halazonetis et al., 2008). However, mutant p53 is oncogenic through inactivation of the DNA damage sensor protein ATM activation or promotion of the chromatin association and nuclear activity of PARP1 for alternative means to counter genomic instabilities in cancer cells (Song et al., 2007; Polotskaia et al., 2015). Since the H3.3K27M mutation occurs concurrently with a mutation in p53, the APR-246 drug (target mutant p53) produces oxidative stress in H3.3K27M DIPGs (Nikolaev et al., 2020) (Figure 4). Therefore, the context of activated mutant oncogenes or mutant tumour-suppressor proteins as targets is important.

Although DNA repair deficiencies can cause cancer evolution, PARP1 inhibitors can be used as treatment modalities in DNA-damage-compromised cells (Rominiyi and Collis, 2022; Volkova et al., 2020). DNA damage contributes to tumorigenesis by providing mutational clonality so that the normal cells may promote cancer cells; however, excessive DNA damage in cancer cells can be exploited for chemotherapy. Lin et al. (2019) proposed a mechanism-based drug design strategy for targeting drug-resistant gliomas; this strategy is promising for overcoming drug resistance and improving glioma treatment outcomes.

Giacomini et al. (2024) reported a novel mechanism driving pHGGs and highlighted aberrant DNA repair as a key contributor to tumour development. Mutations in the histone H3.3, particularly at K27M and G34R, promote genome instabilities by enhancing the NHEJ repair of replication-associated damage. They suggested that polynucleotide kinase 3′-phosphatase (PNKP) is a critical mediator of this aberrant repair process, showing increased association with mutant H3.3; hence, targeting PNKP is a promising therapeutic strategy for gliomas and other cancers with these mutations (Figure 4). Although H3K27 methylation is impaired, PRC2 is necessary for the proliferation of H3.3K27M-expressing tumours (Mohammad et al., 2017). EZH2 inhibitors abrogate the cell growths of these tumours through upregulation of the tumour suppressor protein p16INK4A (Mohammad et al., 2017). Bromodomain protein inhibitors (JQ1) can reduce DIPG cell proliferations by enhancing their terminal neuronal differentiation (Piunti et al., 2017) (Figure 4).

Components of the chromatin remodelling SWI/SNF complex proteins are upregulated in H3.3K27M gliomas, and their degradation leads to reduced viability of H3.3K27M cells (Mota

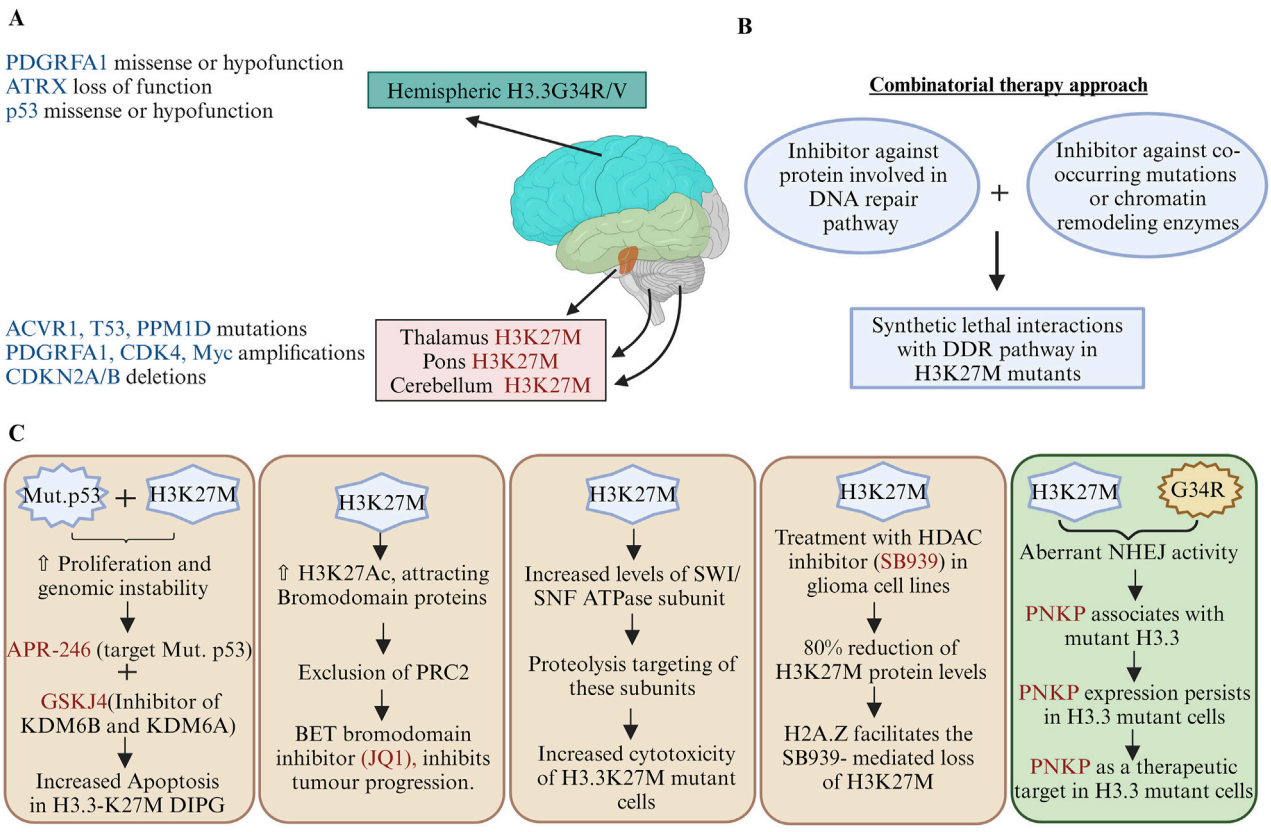


FIGURE 4
Therapeutic strategies targeting oncohistone mutations: **(A)** Anatomical distribution and genetic landscape of oncohistone mutations; the diagram illustrates the distribution of histone mutations H3K27M and G34R/V across different brain regions, highlighting their associations with various genetic alterations. **(B)** Synthetic lethal interactions with the DDR pathway; a combination of inhibitors against DNA repair pathway proteins and chromatin remodelling enzymes may be exploited to target cancer cells with histone mutations. **(C)** Therapeutic strategies targeting oncohistone mutations; mechanistic impacts of H3K27M and G34R/V mutations on cancer cell survival and their genetic requirements for other cancer-associated genes. Few illustrations depict how a potential therapeutic target can be designed to reduce the growth of H3.3 mutant cancer cells. For example, the combination of APR-246 and GSKJ4 enhances apoptosis in H3.3K27M DIPGs, addressing how co-mutations of p53 and H3K27M-associated dysregulations in the epigenomic profile may be exploited to identify novel and precise cancer treatments. PDGFRA1, platelet-derived growth factor receptor alpha 1; ATRX, alpha thalassemia retardation X-linked; ACVR1, activin A receptor type 1; p53, tumour protein 53; PPMID, protein phosphatase Mg^{2+}/Mn^{2+}-dependent 1D; CDK4, cyclin-dependent kinase 4; CDKN2A/B, cyclin-dependent kinase inhibitor 2A/2B; SWI/SNF, switch/sucrose non-fermentable; PNKP, polynucleotide kinase 3′-phosphatase (created with BioRender.com, accessed on 9 June 2024).

et al., 2023). GSK-J4 is a Jumonji family histone demethylase (JMJD3) inhibitor. H3K27 methylation increases in H3.3K27M cells upon treatment with GSK-J4 and has antitumour activities for cells harbouring H3.3K27M *in vitro* as well as *in vivo* (Hashizume et al., 2014). The mutant-p53-targeting drug APR-246 produces oxidative stress, and combining it with GSKJ4 increases apoptosis in H3.3K27M DIPGs (Nikolaev et al., 2020) (Figure 4). Histone deacetylase (HDAC) inhibitors like pracinostat/SB939, panobinostat, vorinostat, and entinostat destabilise the H3.3K27M protein in multiple glioma cell lines (Leszczynska et al., 2024). Mechanistically, co-occurrence of the H2A.Z histone with H3.3K27M nucleosome facilitates loss of H3.3K27M but is inhibited by chloroquine, which is an inhibitor of autophagosome-lysosomal degradation and a DNA-intercalating agent (Leszczynska et al., 2024).

In cancer cells, DNA repair and/or DDR factors functionally interact with chromatin to orchestrate the DNA repair processes by endogenous or exogenous DNA lesions generated through tumorigenic progression or DNA damaging agents used during chemotherapy. Combination therapies are often dependent upon the interactions of the genes with the drugs (Lin et al., 2019). Synthetic lethal interactions with DDR pathways may also be used as a therapeutic approach. A combination of radiotherapy and DDR inhibitors (DDRi) has been shown to reduce tumour growth in H3.3G34R pHGG-bearing mice (Haase et al., 2022) (Figure 4).

The H3K27M and H3G34R/V mutations disrupt the chromatin structure and functions, resulting in defects in the recruitment of DNA repair proteins, thereby impairing the DNA repair efficiency kinetics and increasing genomic instability. All these events promote tumour progression with distinct molecular mechanisms. However, further investigations into the specific molecular mechanisms underlying these dysregulations are required to identify potential therapeutic targets. Consequently, understanding the DNA repair mechanisms underlying these mutations as well as their implications in targeted therapeutic strategies are critical for advancing precision medicine approaches to glioma treatment.

7 Histones H4, H2A, H2B, and H1 are mutated in various cancers

Although this review primarily addresses histone H3 mutations and their implications to the DDR pathways and genomic instabilities, mutations in other canonical core histones, such as H2A, H2B, and linker histone H1, are significantly prevalent across various cancers. Existing literature provides limited insights into how the mutations in these histone variants contribute to the DDR pathways and genomic instabilities. Nevertheless, such mutations are known to disrupt the nucleosomal structures and lead to deregulation of gene expressions.

One such mutation is the H2BE76K mutation found in bladder and head and neck cancers, which tends to form stable dimers with H2A but disrupt H2B-H4 interactions and prevent stable histone octamer formation (Espinoza Pereira et al., 2023; Arimura et al., 2018). Genes upregulated in the H2BE76K mutant cells are involved in cell adhesion and proliferation. In the breast cancer cell line, the transcription of ADAM19 (a disintegrin and metalloproteinase-domain-containing protein 19), a cancer-associated gene, increases by H2BE76K mutation through facilitation of transcription elongation processes (Kang et al., 2021). Unlike other histone mutants, the H2BE76K variant does not impact the global levels of histone PTMs but has been observed to increase chromatin accessibility at the promoters and enhancers via nucleosomal dysfunctions (Kang et al., 2021).

Other examples include the H2BG52D and H2BP102L oncohistones, which affect HR repair by impairing histone eviction and RAD51 recruitment; the heterozygotes for these mutants also exhibit increased genotoxic sensitivity and concomitant reductions in H2B ubiquitination (H2Bub) *in cis* (Qin et al., 2024). The H2BG52D mutation identified in pancreatic cancer and other malignancies impairs DNA–histone interactions, leading to decreased nucleosome stability and disrupted gene regulation. Although H2BG52D does not affect cell proliferation, it significantly enhances cell migration and accelerates wound closure in assays, indicating its role in promoting cancer progression (Wan et al., 2020). Missense mutations in the histone H2A gene account for approximately 20% of all histones missense mutations observed in cancers. The most common H2A mutated residue E121 decreases nucleosome sliding, and another commonly mutated residue R29 increases nucleosome sliding while decreasing stability and enhancing dimer exchange (Bagert et al., 2021).

Other H2A (sH2A) histone variants, which are predominantly expressed in the testes during spermatogenesis in placental mammals, are essential for normal testicular functions (Molaro et al., 2018). These variants are known to destabilise nucleosomes and modulate alternative splicing, as evidenced by studies in germline mutant mice. Additionally, research has demonstrated that peptides derived from the sH2A.B variant can interact with human leucocyte antigen (HLA) molecules, suggesting a potential role for sH2A.B in immune evasion (Lundegaard et al., 2008). Aberrant expressions of the sH2A variants have been observed in several cancers, including diffuse large B-cell lymphoma and Hodgkin's lymphoma. These dysregulated expressions of the sH2A variants contribute to the oncogenic phenotypes seen in these malignancies (Chew et al., 2021).

The linker histone H1 that is essential for chromatin condensation and gene repression is often mutated in mature B-cell neoplasms and is recognised in literature for its tumour suppressor role. Depletion of H1 cause reduction of H3K27 methylation but increase in H3K36 methylation, altered chromatin compartmentalisation, and enhanced chromatin interactions (Willcockson et al., 2021). Among the cancer-associated mutations, S101F in H1 results in a loss-of-function phenotype, disrupting the interaction between H1 and DNA. Pan-cancer analyses also show mutations at G102 and S103 that may impair DNA binding. Mutant H1 demonstrates accelerated dissociation and diminished chromatin association, compromising chromatin binding and compaction (Yusufova et al., 2021).

Several histone H4 mutations such as R3C, L49F, S1C, and K79N have been reported in various cancers; although the precise roles of these mutations remain unclear, their high frequency of occurrence in cancers suggests their potential roles in oncogenic processes and chromatin dysregulation (Nacev et al., 2019).

The H4R3C mutation is the most mutated histone H4 residue in cancers and disrupts H4R3me2, which is catalysed by Protein arginine methyltransferase 5 and linked to transcriptional repression at specific genomic loci (Chen et al., 2017). H4R3me2s also serves as a binding site for DNMT3A, promoting DNA methylation (Zhao et al., 2009). Loss of H4R3me2 due to H4R3C mutation leads to reduced DNMT3A binding, decreased DNA methylation, gene activation, and may contribute to oncogenesis.

The H4 H75E mutation in the LRS domain, which engages with H2B, impedes global genomic nucleotide excision repair by disrupting the recruitment of RAD4 to the chromatin. This reduction in DNA repair efficiency occurs without affecting the chromatin structure or accessibility, thereby diminishing the effectiveness of damage recognition (Selvam et al., 2019). Additionally, the residues D68 and R92, which are commonly mutated in cancers, are critical for hydrogen bonding with H2B. Alterations in these residues can likely impair H4-H2B interactions, leading to nucleosomal instabilities (Nacev et al., 2019).

8 Conclusion

The contributions of epigenetic mechanisms are crucial for identifying the tumour stages as well as heterogeneities. Vulnerabilities of the chromatin structure can be exploited to target cancer. pHGGs harbour missense mutation of H3.3K27M and H3.3G34R/V, whereas bone tumours have H3.3K36M mutations. Substantial studies have reported that these mutations promote tumorigenesis (known as oncohistones). Selection of tissue-specific cell lines may play significant roles in determining the oncogenic potentials of histone mutations identified upon genome sequencing of cancer cells. Many studies show that the oncogenic potential of a histone mutation depends on co-occurring mutations in other proteins. DDR network perturbation in the H3.3K27M and H3.3G34R/V mutant cancer cells needs further investigation so that we can identify the synthetic lethality between the DNA repair genes and oncohistones. Additionally, histone mutants may perturb chromatin in a context-dependent manner by dysregulating the chromatin modifying complexes. Therefore, one may consider targeting epigenetic complexes to

achieve synthetic lethality in tumours harbouring histone mutants. Histones H3K27M and H3K36M are *trans*-acting whereas the H3.3G34 mutants are *cis*-acting, suggesting that cancer-associated histone mutations may differentially modulate PTMs on the histone tails. Furthermore, enrichment of the histone PTMs on the chromatin domains may vary with the nature of the histone mutations, which could regulate gene expression patterns or other DNA-templated processes, such as DNA repairs in oncohistone-containing cancer cells.

The following are some of the major questions that remain unanswered or have partial data to support them:

- Oncogenic histone mutations cause genome instabilities in cancer cells. How do specific repair pathways contribute to the survival of cancer cells in oncohistones?
- Are there any common mechanisms of genome instabilities due to H3.3K27M and H3.3G34R/V histone mutations?
- H3.3K27M and H3.3G34R/V mutant cancer cells have perturbed chromatin environments. Can epigenetic drugs be combined with conventional chemotherapeutics for the treatment of these cancers?

Although we have mainly discussed H3 mutations in this review, other canonical core histones, such as H2A, H2B, and linker histone H1, are also mutated in cancers, and their roles in tumorigenesis constitute active research areas. It is crucial to dissect the functionality of histone mutations to design combination therapies, immunotherapies, or precision medicines in cancer management.

Author contributions

PY: investigation, methodology, validation, visualization, writing–original draft, and writing–review and editing. RJ: investigation, methodology, validation, visualization, writing–original draft, and writing–review and editing. RY: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing–original draft, and writing–review and editing.

Funding

The authors declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Ramalingaswami Re-entry Fellowship (BT/RLF/Reentry/15/2017) of the Department of Biotechnology, Government of India, and a core grant from the National Institute of Immunology (NII), New Delhi.

Acknowledgments

The authors would like to apologise to all the researchers whose works could not be cited because of space limitations.

Conflict of interest

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

Publisher's note

References

Abe, S., Nagatomo, H., Sasaki, H., and Ishiuchi, T. (2021). A histone H3.3K36M mutation in mice causes an imbalance of histone modifications and defects in chondrocyte differentiation. *Epigenetics* 16 (10), 1123–1134. doi:10.1080/15592294.2020.1841873

Adam, S., Polo, S. E., and Almouzni, G. (2013). Transcription recovery after DNA damage requires chromatin priming by the H3.3 histone chaperone HIRA. *Cell* 155 (1), 94–106. doi:10.1016/j.cell.2013.08.029

Allis, C. D., and Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. *Nat. Rev. Genet.* 17 (8), 487–500. doi:10.1038/nrg.2016.59

Amatori, S., Tavolaro, S., Gambardella, S., and Fanelli, M. (2021). The dark side of histones: genomic organization and role of oncohistones in cancer. *Clin. Epigenetics* 13 (1), 71. doi:10.1186/s13148-021-01057-x

Arimura, Y., Ikura, M., Fujita, R., Noda, M., Kobayashi, W., Horikoshi, N., et al. (2018). Cancer-associated mutations of histones H2B, H3.1 and H2A.Z.1 affect the structure and stability of the nucleosome. *Nucleic Acids Res.* 46 (19), 10007–18.

Arnould, C., Rocher, V., Saur, F., Bader, A. S., Muzzopappa, F., Collins, S., et al. (2023). Chromatin compartmentalization regulates the response to DNA damage. *Nature* 623 (7985), 183–192. doi:10.1038/s41586-023-06635-y

Aymard, F., Bugler, B., Schmidt, C. K., Guillou, E., Caron, P., Briois, S., et al. (2014). Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks. *Nat. Struct. Mol. Biol.* 21 (4), 366–374. doi:10.1038/nsmb.2796

Bagert, J. D., Mitchener, M. M., Patriotis, A. L., Dul, B. E., Wojcik, F., Nacev, B. A., et al. (2021). Oncohistone mutations enhance chromatin remodeling and alter cell fates. *Nat. Chem. Biol.* 17 (4), 403–411. doi:10.1038/s41589-021-00738-1

Bannister, A. J., Schneider, R., Myers, F. A., Thorne, A. W., Crane-Robinson, C., and Kouzarides, T. (2005). Spatial distribution of di- and tri-methyl lysine 36 of histone H3 at active genes. *J. Biol. Chem.* 280 (18), 17732–17736. doi:10.1074/jbc.M500796200

Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Schones, D. E., Wang, Z., et al. (2007). High-resolution profiling of histone methylations in the human genome. *Cell* 129 (4), 823–837. doi:10.1016/j.cell.2007.05.009

Behjati, S., Tarpey, P. S., Presneau, N., Scheipl, S., Pillay, N., Van Loo, P., et al. (2013). Distinct H3F3A and H3F3B driver mutations define chondroblastoma and giant cell tumor of bone. *Nat. Genet.* 45 (12), 1479–1482. doi:10.1038/ng.2814

Bender, S., Tang, Y., Lindroth, A. M., Hovestadt, V., Jones, D. T., Kool, M., et al. (2013). Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. *Cancer Cell* 24 (5), 660–672. doi:10.1016/j.ccr.2013.10.006

Bjerke, L., Mackay, A., Nandhabalan, M., Burford, A., Jury, A., Popov, S., et al. (2013). Histone H3.3. mutations drive pediatric glioblastoma through upregulation of MYCN. *Cancer Discov.* 3 (5), 512–519. doi:10.1158/2159-8290.CD-12-0426

Blackledge, N. P., and Klose, R. J. (2021). The molecular principles of gene regulation by Polycomb repressive complexes. *Nat. Rev. Mol. Cell Biol.* 22 (12), 815–833. doi:10.1038/s41580-021-00398-y

Blum, H. F. (1950). On the mechanism of cancer induction by ultraviolet radiation. *J. Natl. Cancer Inst.* 11 (3), 463–495.

Bressan, R.B., Southgate, B., Ferguson, K.M., Blin, C., Grant, V., Alfazema, N., et al. (2021). Regional identity of human neural stem cells determines oncogenic responses to histone H3.3 mutants. *Cell Stem Cell.* 28 (5), 877–93.

Bockaj, I., Martini, T. E. I., de Camargo Magalhaes, E. S., Bakker, P. L., Meeuwsen-de Boer, T. G. J., Armandari, I., et al. (2021). The H3.3K27M oncohistone affects replication stress outcome and provokes genomic instability in pediatric glioma. *PLoS Genet.* 17 (11), e1009868. doi:10.1371/journal.pgen.1009868

Bonner, E. R., Dawood, A., Gordish-Dressman, H., Eze, A., Bhattacharya, S., Yadavilli, S., et al. (2023). Pan-cancer atlas of somatic core and linker histone mutations. *NPJ Genom Med.* 8 (1), 23. doi:10.1038/s41525-023-00367-8

Campbell, S., Ismail, I. H., Young, L. C., Poirier, G. G., and Hendzel, M. J. (2013). Polycomb repressive complex 2 contributes to DNA double-strand break repair. *Cell Cycle* 12 (16), 2675–2683. doi:10.4161/cc.25795

Capper, D., Jones, D. T. W., Sill, M., Hovestadt, V., Schrimpf, D., Sturm, D., et al. (2018). DNA methylation-based classification of central nervous system tumours. *Nature* 555 (7697), 469–474. doi:10.1038/nature26000

Carpenter, E. J. W. P. B. (2012) "Understanding the language of Lys36 methylation at histone H3," in *Nature REVIEWS|MOLECULAR CELL BIOLOGY*.

Castel, D., Philippe, C., Calmon, R., Le Dret, L., Truffaux, N., Boddaert, N., et al. (2015). Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathol.* 130 (6), 815–827. doi:10.1007/s00401-015-1478-0

Chabot, T., Cheraud, Y., and Fleury, F. (2021). Relationships between DNA repair and RTK-mediated signaling pathways. *Biochim. Biophys. Acta Rev. Cancer* 1875 (1), 188495. doi:10.1016/j.bbcan.2020.188495

Chan, K. M., Fang, D., Gan, H., Hashizume, R., Yu, C., Schroeder, M., et al. (2013). The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev.* 27 (9), 985–990. doi:10.1101/gad.217778.113

Chang, H. R., Munkhjargal, A., Kim, M. J., Park, S. Y., Jung, E., Ryu, J. H., et al. (2018). The functional roles of PML nuclear bodies in genome maintenance. *Mutat. Res.* 809, 99–107. doi:10.1016/j.mrfmmm.2017.05.002

Chaouch, A., and Lasko, P. (2021). Drosophila melanogaster: a fruitful model for oncohistones. *Fly. (Austin).* 15 (1), 28–37. doi:10.1080/19336934.2020.1863124

Chen, C. C. L., Deshmukh, S., Jessa, S., Hadjadj, D., Lisi, V., Andrade, A. F., et al. (2020b). Histone H3.3G34-mutant Interneuron progenitors Co-opt PDGFRA for gliomagenesis. *Cell* 183 (6), 1617–1633. doi:10.1016/j.cell.2020.11.012

Chen, H., Lorton, B., Gupta, V., and Shechter, D. (2017). A TGFβ-PRMT5-MEP50 axis regulates cancer cell invasion through histone H3 and H4 arginine methylation coupled transcriptional activation and repression. *Oncogene* 36 (3), 373–386. doi:10.1038/onc.2016.205

Chen, K. Y., Bush, K., Klein, R. H., Cervantes, V., Lewis, N., Naqvi, A., et al. (2020a). Reciprocal H3.3 gene editing identifies K27M and G34R mechanisms in pediatric glioma including NOTCH signaling. *Commun. Biol.* 3 (1), 363. doi:10.1038/s42003-020-1076-0

Chew, G. L., Bleakley, M., Bradley, R. K., Malik, H. S., Henikoff, S., Molaro, A., et al. (2021). Short H2A histone variants are expressed in cancer. *Nat. Commun.* 12 (1), 490. doi:10.1038/s41467-020-20707-x

Choi, J., Kim, T., and Cho, E. J. (2024). HIRA vs. DAXX: the two axes shaping the histone H3.3 landscape. *Exp. Mol. Med.* 56 (2), 251–263. doi:10.1038/s12276-023-01145-3

Chou, D. M., Adamson, B., Dephoure, N. E., Tan, X., Nottke, A. C., Hurov, K. E., et al. (2010). A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. *Proc. Natl. Acad. Sci. U S A.* 107 (43), 18475–18480. doi:10.1073/pnas.1012946107

Cohen, L. R. Z., and Meshorer, E. (2024). The many faces of H3.3 in regulating chromatin in embryonic stem cells and beyond. *Trends Cell Biol.* doi:10.1016/j.tcb.2024.03.003

Cohn, M. A., and D'Andrea, A. D. (2008). Chromatin recruitment of DNA repair proteins: lessons from the fanconi anemia and double-strand break repair pathways. *Mol. Cell* 32 (3), 306–312. doi:10.1016/j.molcel.2008.10.009

Dabin, J., Mori, M., and Polo, S. E. (2023). The DNA damage response in the chromatin context: a coordinated process. *Curr. Opin. Cell Biol.* 82, 102176. doi:10.1016/j.ceb.2023.102176

Delaney, K., Strobino, M., Wenda, J. M., Pankowski, A., and Steiner, F. A. (2019). H3.3K27M-induced chromatin changes drive ectopic replication through misregulation of the JNK pathway in C. elegans. *Nat. Commun.* 10 (1), 2529. doi:10.1038/s41467-019-10404-9

Dottermusch, M., Uksul, N., Knappe, U. J., Erdlenbruch, B., and Wefers, A. K. (2022). An H3F3A K27M-mutation in a sonic hedgehog medulloblastoma. *Brain Pathol.* 32 (3), e13024. doi:10.1111/bpa.13024

Downing, J. R., Wilson, R. K., Zhang, J., Mardis, E. R., Pui, C. H., Ding, L., et al. (2012). The pediatric cancer genome Project. *Nat. Genet.* 44 (6), 619–622. doi:10.1038/ng.2287

Espinoza Pereira, K. N., Shan, J, Licht, J. D., and Bennett, R. L. (2023). Histone mutations in cancer. *Biochem Soc Trans.* 51 (5), 1749–63.

Fang, D., Gan, H., Lee, J. H., Han, J., Wang, Z., Riester, S. M., et al. (2016). The histone H3.3K36M mutation reprograms the epigenome of chondroblastomas. *Science* 352 (6291), 1344–1348. doi:10.1126/science.aae0065

Fang, J., Huang, Y., Mao, G., Yang, S., Rennert, G., Gu, L., et al. (2018). Cancer-driving H3G34V/R/D mutations block H3K36 methylation and H3K36me3-MutSα interaction. *Proc. Natl. Acad. Sci. U S A.* 115 (38), 9598–9603. doi:10.1073/pnas.1806355115

Faury, D., Nantel, A., Dunn, S. E., Guiot, M. C., Haque, T., Hauser, P., et al. (2007). Molecular profiling identifies prognostic subgroups of pediatric glioblastoma and shows increased YB-1 expression in tumors. *J. Clin. Oncol.* 25 (10), 1196–1208. doi:10.1200/JCO.2006.07.8626

Ferrand, J., Rondinelli, B., and Polo, S. E. (2020). Histone variants: Guardians of genome integrity. *Cells* 9 (11), 2424. doi:10.3390/cells9112424

Filbin, M. G., Tirosh, I., Hovestadt, V., Shaw, M. L., Escalante, L. E., Mathewson, N. D., et al. (2018). Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. *Science* 360 (6386), 331–335. doi:10.1126/science.aao4750

Flavahan, W. A., Gaskell, E., and Bernstein, B. E. (2017). Epigenetic plasticity and the hallmarks of cancer. *Science* 357 (6348), eaal2380. doi:10.1126/science.aal2380

Fnu, S., Williamson, E. A., De Haro, L. P., Brenneman, M., Wray, J., Shaheen, M., et al. (2011). Methylation of histone H3 lysine 36 enhances DNA repair by nonhomologous end-joining. *Proc. Natl. Acad. Sci. U S A.* 108 (2), 540–545. doi:10.1073/pnas.1013571108

Fontebasso, A. M., Papillon-Cavanagh, S., Schwartzentruber, J., Nikbakht, H., Gerges, N., Fiset, P. O., et al. (2014). Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. *Nat. Genet.* 46 (5), 462–466. doi:10.1038/ng.2950

Fontebasso, A. M., Schwartzentruber, J., Khuong-Quang, D. A., Liu, X. Y., Sturm, D., Korshunov, A., et al. (2013). Mutations in SETD2 and genes affecting histone H3K36 methylation target hemispheric high-grade gliomas. *Acta Neuropathol.* 125 (5), 659–669. doi:10.1007/s00401-013-1095-8

Frey, A., Listovsky, T., Guilbaud, G., Sarkies, P., and Sale, J. E. (2014). Histone H3.3 is required to maintain replication fork progression after UV damage. *Curr. Biol.* 24 (18), 2195–2201. doi:10.1016/j.cub.2014.07.077

Funato, K., Major, T., Lewis, P. W., Allis, C. D., and Tabar, V. (2014). Use of human embryonic stem cells to model pediatric gliomas with H3.3K27M histone mutation. *Science* 346 (6216), 1529–1533. doi:10.1126/science.1253799

Gessi, M., Gielen, G. H., Hammes, J., Dorner, E., Muhlen, A. Z., Waha, A., et al. (2013). H3.3 G34R mutations in pediatric primitive neuroectodermal tumors of central nervous system (CNS-PNET) and pediatric glioblastomas: possible diagnostic and therapeutic implications? *J. Neurooncol* 112 (1), 67–72. doi:10.1007/s11060-012-1040-z

Giacomini, G., Piquet, S., Chevallier, O., Dabin, J., Bai, S. K., Kim, B., et al. (2024). Aberrant DNA repair reveals a vulnerability in histone H3.3-mutant brain tumors. *Nucleic Acids Res.* 52, 2372–2388. doi:10.1093/nar/gkad1257

Haase, S., Banerjee, K., Mujeeb, A. A., Hartlage, C. S., Nunez, F. M., Nunez, F. J., et al. (2022). H3.3-G34 mutations impair DNA repair and promote cGAS/STING-mediated immune responses in pediatric high-grade glioma models. *J. Clin. Invest* 132 (22), e154229. doi:10.1172/JCI154229

Halazonetis, T. D., Gorgoulis, V. G., and Bartek, J. (2008). An oncogene-induced DNA damage model for cancer development. *Science* 319 (5868), 1352–1355. doi:10.1126/science.1140735

Hanahan, D. (2022). Hallmarks of cancer: new Dimensions. *Cancer Discov.* 12 (1), 31–46. doi:10.1158/2159-8290.CD-21-1059

Harutyunyan, A. S., Krug, B., Chen, H., Papillon-Cavanagh, S., Zeinieh, M., De Jay, N., et al. (2019). H3K27M induces defective chromatin spread of PRC2-mediated repressive H3K27me2/me3 and is essential for glioma tumorigenesis. *Nat. Commun.* 10 (1), 1262. doi:10.1038/s41467-019-09140-x

Hashizume, R., Andor, N., Ihara, Y., Lerner, R., Gan, H., Chen, X., et al. (2014). Pharmacologic inhibition of histone demethylation as a therapy for pediatric brainstem glioma. *Nat. Med.* 20 (12), 1394–1396. doi:10.1038/nm.3716

Huang, T. Y., Piunti, A., Qi, J., Morgan, M., Bartom, E., Shilatifard, A., et al. (2020). Effects of H3.3G34V mutation on genomic H3K36 and H3K27 methylation patterns in isogenic pediatric glioma cells. *Acta Neuropathol. Commun.* 8 (1), 219. doi:10.1186/s40478-020-01092-4

Hubner, J. M., Muller, T., Papageorgiou, D. N., Mauermann, M., Krijgsveld, J., Russell, R. B., et al. (2019). EZHIP/CXorf67 mimics K27M mutated oncohistones and functions as an intrinsic inhibitor of PRC2 function in aggressive posterior fossa ependymoma. *Neuro Oncol.* 21 (7), 878–889. doi:10.1093/neuonc/noz058

Ito, T., Teo, Y. V., Evans, S. A., Neretti, N., and Sedivy, J. M. (2018). Regulation of cellular Senescence by polycomb chromatin modifiers through distinct DNA damage- and histone methylation-dependent pathways. *Cell Rep.* 22 (13), 3480–3492. doi:10.1016/j.celrep.2018.03.002

Jackson, S. P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. *Nature* 461 (7267), 1071–1078. doi:10.1038/nature08467

Jain, S. U., Khazaei, S., Marchione, D. M., Lundgren, S. M., Wang, X., Weinberg, D. N., et al. (2020). Histone H3.3 G34 mutations promote aberrant PRC2 activity and drive tumor progression. *Proc. Natl. Acad. Sci. U S A* 117 (44), 27354–27364. doi:10.1073/pnas.2006076117

Jang, C. W., Shibata, Y., Starmer, J., Yee, D., and Magnuson, T. (2015). Histone H3.3 maintains genome integrity during mammalian development. *Genes Dev.* 29 (13), 1377–1392. doi:10.1101/gad.264150.115

Jenseit, A., Camgoz, A., Pfister, S. M., and Kool, M. (2022). EZHIP: a new piece of the puzzle towards understanding pediatric posterior fossa ependymoma. *Acta Neuropathol.* 143 (1), 1–13. doi:10.1007/s00401-021-02382-4

Jessa, S., Mohammadnia, A., Harutyunyan, A. S., Hulswit, M., Varadharajan, S., Lakkis, H., et al. (2022). K27M in canonical and noncanonical H3 variants occurs in distinct oligodendroglial cell lineages in brain midline gliomas. *Nat. Genet.* 54 (12), 1865–1880. doi:10.1038/s41588-022-01205-w

Johnson, K. J., Cullen, J., Barnholtz-Sloan, J. S., Ostrom, Q. T., Langer, C. E., Turner, M. C., et al. (2014). Childhood brain tumor epidemiology: a brain tumor epidemiology consortium review. *Cancer Epidemiol. Biomarkers Prev.* 23 (12), 2716–2736. doi:10.1158/1055-9965.EPI-14-0207

Jones, C., and Baker, S. J. (2014). Unique genetic and epigenetic mechanisms driving paediatric diffuse high-grade glioma. *Nat. Rev. Cancer* 14 (10), 651–661. doi:10.1038/nrc3811

Jones, P. A., and Baylin, S. B. (2007). The epigenomics of cancer. *Cell* 128 (4), 683–692. doi:10.1016/j.cell.2007.01.029

Justin, N., Zhang, Y., Tarricone, C., Martin, S. R., Chen, S., Underwood, E., et al. (2016). Structural basis of oncogenic histone H3K27M inhibition of human polycomb repressive complex 2. *Nat. Commun.* 7, 11316. doi:10.1038/ncomms11316

Kallappagoudar, S., Yadav, R. K., Lowe, B. R., and Partridge, J. F. (2015). Histone H3 mutations--a special role for H3.3 in tumorigenesis? *Chromosoma* 124 (2), 177–189. doi:10.1007/s00412-015-0510-4

Kang, T. Z. E., Zhu, L., Yang, D., Ding, D., Zhu, X., Wan, Y. C. E., et al. (2021). The elevated transcription of ADAM19 by the oncohistone H2BE76K contributes to oncogenic properties in breast cancer. *J. Biol. Chem.* 296, 100374. doi:10.1016/j.jbc.2021.100374

Kfoury-Beaumont, N., Prakasam, R., Pondugula, S., Lagas, J. S., Matkovich, S., Gontarz, P., et al. (2022). The H3K27M mutation alters stem cell growth, epigenetic regulation, and differentiation potential. *BMC Biol.* 20 (1), 124. doi:10.1186/s12915-022-01324-0

Khazaei, S., Chen, C. C. L., Andrade, A. F., Kabir, N., Azarafshar, P., Morcos, S. M., et al. (2023). Single substitution in H3.3G34 alters DNMT3A recruitment to cause progressive neurodegeneration. *Cell* 186 (6), 1162–1178.e20. doi:10.1016/j.cell.2023.02.023

Khuong-Quang, D. A., Buczkowicz, P., Rakopoulos, P., Liu, X. Y., Fontebasso, A. M., Bouffet, E., et al. (2012). K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol.* 124 (3), 439–447. doi:10.1007/s00401-012-0998-0

Klein, B. J., Krajewski, K., Restrepo, S., Lewis, P. W., Strahl, B. D., and Kutateladze, T. G. (2018). Recognition of cancer mutations in histone H3K36 by epigenetic writers and readers. *Epigenetics* 13 (7), 683–692. doi:10.1080/15592294.2018.1503491

Korshunov, A., Capper, D., Reuss, D., Schrimpf, D., Ryzhova, M., Hovestadt, V., et al. (2016). Histologically distinct neuroepithelial tumors with histone 3 G34 mutation are molecularly similar and comprise a single nosologic entity. *Acta Neuropathol.* 131 (1), 137–146. doi:10.1007/s00401-015-1493-1

Korshunov, A., Remke, M., Werft, W., Benner, A., Ryzhova, M., Witt, H., et al. (2010). Adult and pediatric medulloblastomas are genetically distinct and require different algorithms for molecular risk stratification. *J. Clin. Oncol.* 28 (18), 3054–3060. doi:10.1200/JCO.2009.25.7121

Lee, C. H., Yu, J. R., Granat, J., Saldana-Meyer, R., Andrade, J., LeRoy, G., et al. (2019). Automethylation of PRC2 promotes H3K27 methylation and is impaired in H3K27M pediatric glioma. *Genes Dev.* 33 (19-20), 1428–1440. doi:10.1101/gad.328773.119

Lee, E., Park, Y. J., and Lindroth, A. M. (2024). H3.3-G34W in giant cell tumor of bone functionally aligns with the exon choice repressor hnRNPA1L2. *Cancer Gene Ther.* 31, 1177–1185. doi:10.1038/s41417-024-00776-6

Lehnertz, B., Zhang, Y. W., Boivin, I., Mayotte, N., Tomellini, E., Chagraoui, J., et al. (2017). H3(K27M/I) mutations promote context-dependent transformation in acute myeloid leukemia with RUNX1 alterations. *Blood* 130 (20), 2204–2214. doi:10.1182/blood-2017-03-774653

Leszczynska, K. B., Freitas-Huhtamaki, A., Jayaprakash, C., Dzwigonska, M., Vitorino, F. N. L., Horth, C., et al. (2024). H2A.Z histone variants facilitate HDACi-dependent removal of H3.3K27M mutant protein in pediatric high-grade glioma cells. *Cell Rep.* 43 (2), 113707. doi:10.1016/j.celrep.2024.113707

Lewis, P. W., Muller, M. M., Koletsky, M. S., Cordero, F., Lin, S., Banaszynski, L. A., et al. (2013). Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science* 340 (6134), 857–861. doi:10.1126/science.1232245

Lin, A., Giuliano, C. J., Palladino, A., John, K. M., Abramowicz, C., Yuan, M. L., et al. (2019). Off-target toxicity is a common mechanism of action of cancer drugs undergoing clinical trials. *Sci Transl Med.* 11 (509).

Liu, C., Kuang, S., Wu, L., Cheng, Q., Gong, X., Wu, J., et al. (2023). Radiotherapy and radio-sensitization in H3(K27M) -mutated diffuse midline gliomas. *CNS Neurosci. Ther.* 29 (7), 1721–1737. doi:10.1111/cns.14225

Liu, I., Jiang, L., Samuelsson, E. R., Marco Salas, S., Beck, A., Hack, O. A., et al. (2022). The landscape of tumor cell states and spatial organization in H3-K27M mutant diffuse midline glioma across age and location. *Nat. Genet.* 54 (12), 1881–1894. doi:10.1038/s41588-022-01236-3

Liu, X. Y., Gerges, N., Korshunov, A., Sabha, N., Khuong-Quang, D. A., Fontebasso, A. M., et al. (2012). Frequent ATRX mutations and loss of expression in adult diffuse astrocytic tumors carrying IDH1/IDH2 and TP53 mutations. *Acta Neuropathol.* 124 (5), 615–625. doi:10.1007/s00401-012-1031-3

Louis, D. N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., et al. (2016). The 2016 World Health organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol.* 131 (6), 803–820. doi:10.1007/s00401-016-1545-1

Louis, D. N., Perry, A., Wesseling, P., Brat, D. J., Cree, I. A., Figarella-Branger, D., et al. (2021). The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol.* 23 (8), 1231–1251. doi:10.1093/neuonc/noab106

Lowe, B. R., Yadav, R. K., Henry, R. A., Schreiner, P., Matsuda, A., Fernandez, A. G., et al. (2021). Surprising phenotypic diversity of cancer-associated mutations of Gly 34 in the histone H3 tail. *Elife* 10, e65369. doi:10.7554/eLife.65369

Lu, C., Jain, S. U., Hoelper, D., Bechet, D., Molden, R. C., Ran, L., et al. (2016). Histone H3K36 mutations promote sarcomagenesis through altered histone methylation landscape. *Science* 352 (6287), 844–849. doi:10.1126/science.aac7272

Luijsterburg, M. S., de Krijger, I., Wiegant, W. W., Shah, R. G., Smeenk, G., de Groot, A. J. L., et al. (2016). PARP1 links CHD2-mediated chromatin Expansion and H3.3 deposition to DNA repair by non-homologous end-joining. *Mol. Cell* 61 (4), 547–562. doi:10.1016/j.molcel.2016.01.019

Lundegaard, C., Lamberth, K., Harndahl, M., Buus, S., Lund, O., and Nielsen, M. (2008). NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11. *Nucleic Acids Res.* 36 (Web Server issue), W509–W512. doi:10.1093/nar/gkn202

Mackay, A., Burford, A., Carvalho, D., Izquierdo, E., Fazal-Salom, J., Taylor, K. R., et al. (2017). Integrated molecular Meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. *Cancer Cell* 32 (4), 520–537. doi:10.1016/j.ccell.2017.08.017

Mancarella, D., Ellinghaus, H., Sigismondo, G., Veselinov, O., Kuhn, A., Goyal, A., et al. (2024). Deposition of onco-histone H3.3-G34W leads to DNA repair deficiency and activates cGAS/STING-mediated immune responses. *Int. J. Cancer* 154 (12), 2106–2120. doi:10.1002/ijc.34883

Maze, I., Noh, K. M., Soshnev, A. A., and Allis, C. D. (2014). Every amino acid matters: essential contributions of histone variants to mammalian development and disease. *Nat. Rev. Genet.* 15 (4), 259–271. doi:10.1038/nrg3673

Mohammad, F., and Helin, K. (2017). Oncohistones: drivers of pediatric cancers. *Genes Dev.* 31 (23-24), 2313–2324. doi:10.1101/gad.309013.117

Mohammad, F., Weissmann, S., Leblanc, B., Pandey, D. P., Hojfeldt, J. W., Comet, I., et al. (2017). EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas. *Nat. Med.* 23 (4), 483–492. doi:10.1038/nm.4293

Molaro, A., Young, J. M., and Malik, H. S. (2018). Evolutionary origins and diversification of testis-specific short histone H2A variants in mammals. *Genome Res.* 28 (4), 460–473. doi:10.1101/gr.229799.117

Mota, M., Sweha, S. R., Pun, M., Natarajan, S. K., Ding, Y., Chung, C., et al. (2023). Targeting SWI/SNF ATPases in H3.3K27M diffuse intrinsic pontine gliomas. *Proc. Natl. Acad. Sci. U S A.* 120 (18), e2221175120. doi:10.1073/pnas.2221175120

Moudgil-Joshi, J., and Kaliaperumal, C. (2021). Letter regarding Louis et al: The 2021 WHO Classification of Tumors of the Central Nervous System: A summary. *Neuro Oncol.* 23 (12), 2120–2121. doi:10.1093/neuonc/noab190

Nacev, B. A., Feng, L., Bagert, J. D., Lemiesz, A. E., Gao, J., Soshnev, A. A., et al. (2019). The expanding landscape of 'oncohistone' mutations in human cancers. *Nature* 567 (7749), 473–478. doi:10.1038/s41586-019-1038-1

Newhart, A., Rafalska-Metcalf, I. U., Yang, T., Joo, L. M., Powers, S. L., Kossenkov, A. V., et al. (2013). Single cell analysis of RNA-mediated histone H3.3 recruitment to a cytomegalovirus promoter-regulated transcription site. *J. Biol. Chem.* 288 (27), 19882–19899. doi:10.1074/jbc.M113.473181

Nikolaev, A., Fiveash, J. B., and Yang, E. S. (2020). Combined targeting of mutant p53 and Jumonji family histone demethylase Augments therapeutic Efficacy of radiation in H3K27M DIPG. *Int. J. Mol. Sci.* 21 (2), 490. doi:10.3390/ijms21020490

Nunez, F. J., Mendez, F. M., Kadiyala, P., Alghamri, M. S., Savelieff, M. G., Garcia-Fabiani, M. B., et al. (2019). IDH1-R132H acts as a tumor suppressor in glioma via epigenetic up-regulation of the DNA damage response. *Sci. Transl. Med.* 11 (479), eaaq1427. doi:10.1126/scitranslmed.aaq1427

Ocasio, J. K., Budd, K. M., Roach, J. T., Andrews, J. M., and Baker, S. J. (2023). Oncohistones and disrupted development in pediatric-type diffuse high-grade glioma. *Cancer Metastasis Rev.* 42 (2), 367–388. doi:10.1007/s10555-023-10105-2

O'Hagan, H. M., Wang, W., Sen, S., Destefano Shields, C., Lee, S. S., Zhang, Y. W., et al. (2011). Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. *Cancer Cell* 20 (5), 606–619. doi:10.1016/j.ccr.2011.09.012

Orii, K. E., Lee, Y., Kondo, N., and McKinnon, P. J. (2006). Selective utilization of nonhomologous end-joining and homologous recombination DNA repair pathways during nervous system development. *Proc. Natl. Acad. Sci. U S A.* 103 (26), 10017–10022. doi:10.1073/pnas.0602436103

Paugh, B. S., Qu, C., Jones, C., Liu, Z., Adamowicz-Brice, M., Zhang, J., et al. (2010). Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. *J. Clin. Oncol.* 28 (18), 3061–3068. doi:10.1200/JCO.2009.26.7252

Pfister, S. X., Ahrabi, S., Zalmas, L. P., Sarkar, S., Aymard, F., Bachrati, C. Z., et al. (2014). SETD2-dependent histone H3K36 trimethylation is required for homologous recombination repair and genome stability. *Cell Rep.* 7 (6), 2006–2018. doi:10.1016/j.celrep.2014.05.026

Pinto, L., Baidarjad, H., Entz-Werle, N., and Van Dyck, E. (2021). Impact of chromatin dynamics and DNA repair on genomic stability and treatment resistance in pediatric high-grade gliomas. *Cancers (Basel)* 13 (22), 5678. doi:10.3390/cancers13225678

Pitroda, S. P., Pashtan, I. M., Logan, H. L., Budke, B., Darga, T. E., Weichselbaum, R. R., et al. (2014). DNA repair pathway gene expression score correlates with repair proficiency and tumor sensitivity to chemotherapy. *Sci. Transl. Med.* 6 (229), 229ra42. doi:10.1126/scitranslmed.3008291

Piunti, A., Hashizume, R., Morgan, M. A., Bartom, E. T., Horbinski, C. M., Marshall, S. A., et al. (2017). Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nat. Med.* 23 (4), 493–500. doi:10.1038/nm.4296

Polotskaia, A., Xiao, G., Reynoso, K., Martin, C., Qiu, W. G., Hendrickson, R. C., et al. (2015). Proteome-wide analysis of mutant p53 targets in breast cancer identifies new levels of gain-of-function that influence PARP, PCNA, and MCM4. *Proc. Natl. Acad. Sci. U S A.* 112 (11), E1220–E1229. doi:10.1073/pnas.1416318112

Qin, B., Lu, G., Chen, X., Zheng, C., Lin, H., Liu, Q., et al. (2024). H2B oncohistones cause homologous recombination defect and genomic instability through reducing H2B monoubiquitination in Schizosaccharomyces pombe. *J. Biol. Chem.* 300, 107345. doi:10.1016/j.jbc.2024.107345

Rajagopalan, K. N., Chen, X., Weinberg, D. N., Chen, H., Majewski, J., Allis, C. D., et al. (2021). Depletion of H3K36me2 recapitulates epigenomic and phenotypic changes induced by the H3.3K36M oncohistone mutation. *Proc. Natl. Acad. Sci. U S A.* 118 (9), e2021795118. doi:10.1073/pnas.2021795118

Rominiyi, O., and Collis, S. J. (2022). DDRugging glioblastoma: understanding and targeting the DNA damage response to improve future therapies. *Mol. Oncol.* 16 (1), 11–41. doi:10.1002/1878-0261.13020

Ryall, S., Guzman, M., Elbabaa, S. K., Luu, B., Mack, S. C., Zapotocky, M., et al. (2017). H3 K27M mutations are extremely rare in posterior fossa group A ependymoma. *Childs Nerv. Syst.* 33 (7), 1047–1051. doi:10.1007/s00381-017-3481-3

Sahu, V., and Lu, C. (2022). Oncohistones: Hijacking the histone code. *Annu. Rev. Cancer Biol.* 6, 293–312. doi:10.1146/annurev-cancerbio-070120-102521

Schulte, J. D., Buerki, R. A., Lapointe, S., Molinaro, A. M., Zhang, Y., Villanueva-Meyer, J. E., et al. (2020). Clinical, radiologic, and genetic characteristics of histone H3 K27M-mutant diffuse midline gliomas in adults. *Neurooncol Adv.* 2 (1), vdaa142. doi:10.1093/noajnl/vdaa142

Schwartzentruber, J., Korshunov, A., Liu, X. Y., Jones, D. T., Pfaff, E., Jacob, K., et al. (2012). Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* 482 (7384), 226–231. doi:10.1038/nature10833

Selvam, K., Rahman, S. A., and Li, S. (2019). Histone H4 H75E mutation attenuates global genomic and Rad26-independent transcription-coupled nucleotide excision repair. *Nucleic Acids Res.* 47 (14), 7392–7401. doi:10.1093/nar/gkz453

Sharda, A., and Humphrey, T. C. (2022). The role of histone H3K36me3 writers, readers and erasers in maintaining genome stability. *DNA Repair (Amst)* 119, 103407. doi:10.1016/j.dnarep.2022.103407

Shen, H., and Laird, P. W. (2013). Interplay between the cancer genome and epigenome. *Cell* 153 (1), 38–55. doi:10.1016/j.cell.2013.03.008

Shi, L., Shi, J., Shi, X., Li, W., and Wen, H. (2018). Histone H3.3 G34 mutations alter histone H3K36 and H3K27 methylation in Cis. *J. Mol. Biol.* 430 (11), 1562–1565. doi:10.1016/j.jmb.2018.04.014

Shi, L., Wen, H., and Shi, X. (2017). The histone variant H3.3 in transcriptional regulation and human disease. *J. Mol. Biol.* 429 (13), 1934–1945. doi:10.1016/j.jmb.2016.11.019

Siddaway, R., Canty, L., Pajovic, S., Milos, S., Coyaud, E., Sbergio, S. G., et al. (2022). Oncohistone interactome profiling uncovers contrasting oncogenic mechanisms and identifies potential therapeutic targets in high grade glioma. *Acta Neuropathol.* 144 (5), 1027–1048. doi:10.1007/s00401-022-02489-2

Silveira, A. B., Kasper, L. H., Fan, Y., Jin, H., Wu, G., Shaw, T. I., et al. (2019). H3.3 K27M depletion increases differentiation and extends latency of diffuse intrinsic pontine glioma growth *in vivo*. *Acta Neuropathol.* 137 (4), 637–655. doi:10.1007/s00401-019-01975-4

Song, H., Hollstein, M., and Xu, Y. (2007). p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. *Nat. Cell Biol.* 9 (5), 573–580. doi:10.1038/ncb1571

Sturm, D., Witt, H., Hovestadt, V., Khuong-Quang, D. A., Jones, D. T., Konermann, C., et al. (2012). Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell* 22 (4), 425–437. doi:10.1016/j.ccr.2012.08.024

Szenker, E., Ray-Gallet, D., and Almouzni, G. (2011). The double face of the histone variant H3.3. *Cell Res.* 21 (3), 421–434. doi:10.1038/cr.2011.14

Talbert, P. B., and Henikoff, S. (2010). Histone variants--ancient wrap artists of the epigenome. *Nat. Rev. Mol. Cell Biol.* 11 (4), 264–275. doi:10.1038/nrm2861

Tatavosian, R., Duc, H. N., Huynh, T. N., Fang, D., Schmitt, B., Shi, X., et al. (2018). Live-cell single-molecule dynamics of PcG proteins imposed by the DIPG H3.3K27M mutation. *Nat. Commun.* 9 (1), 2080. doi:10.1038/s41467-018-04455-7

Taylor, K. R., Mackay, A., Truffaux, N., Butterfield, Y., Morozova, O., Philippe, C., et al. (2014). Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. *Nat. Genet.* 46 (5), 457–461. doi:10.1038/ng.2925

Timp, W., and Feinberg, A. P. (2013). Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. *Nat. Rev. Cancer* 13 (7), 497–510. doi:10.1038/nrc3486

Udugama, M., Hii, L., Garvie, A., Cervini, M., Vinod, B., Chan, F. L., et al. (2021). Mutations inhibiting KDM4B drive ALT activation in ATRX-mutated glioblastomas. *Nat. Commun.* 12 (1), 2584. doi:10.1038/s41467-021-22543-z

Venneti, S., Garimella, M. T., Sullivan, L. M., Martinez, D., Huse, J. T., Heguy, A., et al. (2013). Evaluation of histone 3 lysine 27 trimethylation (H3K27me3) and enhancer of Zest 2 (EZH2) in pediatric glial and glioneuronal tumors shows decreased H3K27me3 in H3F3A K27M mutant glioblastomas. *Brain Pathol.* 23 (5), 558–564. doi:10.1111/bpa.12042

Volkova, N. V., Meier, B., Gonzalez-Huici, V., Bertolini, S., Gonzalez, S., Vohringer, H., et al. (2020). Mutational signatures are jointly shaped by DNA damage and repair. *Nat. Commun.* 11 (1), 2169. doi:10.1038/s41467-020-15912-7

Voon, H. P. J., Hii, L., Garvie, A., Udugama, M., Krug, B., Russo, C., et al. (2023). Pediatric glioma histone H3.3 K27M/G34R mutations drive abnormalities in PML nuclear bodies. *Genome Biol.* 24 (1), 284. doi:10.1186/s13059-023-03122-5

Voon, H. P. J., Udugama, M., Lin, W., Hii, L., Law, R. H. P., Steer, D. L., et al. (2018). Inhibition of a K9/K36 demethylase by an H3.3 point mutation found in paediatric glioblastoma. *Nat. Commun.* 9 (1), 3142. doi:10.1038/s41467-018-05607-5

Wan, Y. C. E., Leung, T. C. S., Ding, D., Sun, X., Liu, J., Zhu, L., et al. (2020). Cancer-associated histone mutation H2BG53D disrupts DNA-histone octamer interaction and promotes oncogenic phenotypes. *Signal Transduct. Target Ther.* 5 (1), 27. doi:10.1038/s41392-020-0131-0

Wen, H., Li, Y., Xi, Y., Jiang, S., Stratton, S., Peng, D., et al. (2014). ZMYND11 links histone H3.3K36me3 to transcription elongation and tumour suppression. *Nature* 508 (7495), 263–268. doi:10.1038/nature13045

Willcockson, M. A., Healton, S. E., Weiss, C. N., Bartholdy, B. A., Botbol, Y., Mishra, L. N., et al. (2021). H1 histones control the epigenetic landscape by local chromatin compaction. *Nature* 589 (7841), 293–298. doi:10.1038/s41586-020-3032-z

Wu, G., Broniscer, A., McEachron, T. A., Lu, C., Paugh, B. S., Becksfort, J., et al. (2012). Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat. Genet.* 44 (3), 251–253. doi:10.1038/ng.1102

Yadav, R. K., Jablonowski, C. M., Fernandez, A. G., Lowe, B. R., Henry, R. A., Finkelstein, D., et al. (2017). Histone H3G34R mutation causes replication stress, homologous recombination defects and genomic instability in S. pombe. *Elife* 6, e27406. doi:10.7554/eLife.27406

Yang, S., Zheng, X., Lu, C., Li, G. M., Allis, C. D., and Li, H. (2016). Molecular basis for oncohistone H3 recognition by SETD2 methyltransferase. *Genes Dev.* 30 (14), 1611–1616. doi:10.1101/gad.284323.116

Yao, Y., and Dai, W. (2014). Genomic instability and cancer. *J. Carcinog. Mutagen* 5, 1000165. doi:10.4172/2157-2518.1000165

You, J. S., and Jones, P. A. (2012). Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell* 22 (1), 9–20. doi:10.1016/j.ccr.2012.06.008

Yusufova, N., Kloetgen, A., Teater, M., Osunsade, A., Camarillo, J. M., Chin, C. R., et al. (2021). Histone H1 loss drives lymphoma by disrupting 3D chromatin architecture. *Nature* 589 (7841), 299–305. doi:10.1038/s41586-020-3017-y

Zarghooni, M., Bartels, U., Lee, E., Buczkowicz, P., Morrison, A., Huang, A., et al. (2010). Whole-genome profiling of pediatric diffuse intrinsic pontine gliomas highlights platelet-derived growth factor receptor alpha and poly (ADP-ribose) polymerase as potential therapeutic targets. *J. Clin. Oncol.* 28 (8), 1337–1344. doi:10.1200/JCO.2009.25.5463

Zhang, X., Duan, S., Apostolou, P. E., Wu, X., Watanabe, J., Gallitto, M., et al. (2024). CHD2 regulates Neuron-glioma interactions in pediatric glioma. *Cancer Discov.* 14, 1732–1754. doi:10.1158/2159-8290.CD-23-0012

Zhang, X., Fawwal, D. V., Spangle, J. M., Corbett, A. H., and Jones, C. Y. (2023). Exploring the molecular Underpinnings of cancer-Causing oncohistone mutants using yeast as a model. *J. Fungi (Basel)* 9 (12), 1187. doi:10.3390/jof9121187

Zhang, Y., Chang, J. F., Sun, J., Chen, L., Yang, X. M., Tang, H. Y., et al. (2018). Histone H3K27 methylation modulates the dynamics of FANCD2 on chromatin to facilitate NHEJ and genome stability. *J. Cell Sci.* 131 (12), jcs215525. doi:10.1242/jcs.215525

Zhang, Y., Shan, C. M., Wang, J., Bao, K., Tong, L., and Jia, S. (2017). Molecular basis for the role of oncogenic histone mutations in modulating H3K36 methylation. *Sci. Rep.* 7, 43906. doi:10.1038/srep43906

Zhao, Q., Rank, G., Tan, Y. T., Li, H., Moritz, R. L., Simpson, R. J., et al. (2009). PRMT5-mediated methylation of histone H4R3 recruits DNMT3A, coupling histone and DNA methylation in gene silencing. *Nat. Struct. Mol. Biol.* 16 (3), 304–311. doi:10.1038/nsmb.1568

frontiers | Frontiers in Cell and Developmental Biology

TYPE Review
PUBLISHED 05 November 2024
DOI 10.3389/fcell.2024.1413708

OPEN ACCESS

EDITED BY
Lucio Miele,
Louisiana State University, United States

REVIEWED BY
Abhishek Bhardwaj,
New York University, United States
Yunfeng Zhao,
Ochsner LSU Health, United States

*CORRESPONDENCE
Yawen Pan,
meiqu1998@163.com

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

RECEIVED 07 April 2024
ACCEPTED 18 October 2024
PUBLISHED 05 November 2024

CITATION
Tang B, Kang W, Dong Q, Qin Z, Duan L, Zhao X, Yuan G and Pan Y (2024) Research progress on S-palmitoylation modification mediated by the ZDHHC family in glioblastoma.
Front. Cell Dev. Biol. 12:1413708.
doi: 10.3389/fcell.2024.1413708

Research progress on S-palmitoylation modification mediated by the ZDHHC family in glioblastoma

Beiyan Tang[1†], Wei Kang[2†], Qiang Dong[2], Zhenwei Qin[2], Lei Duan[2], Xianjun Zhao[2], Guoqiang Yuan[3,4] and Yawen Pan[2,3,4*]

[1]The Second Medical College of Lanzhou University, Lanzhou, Gansu, China, [2]Department of Neurosurgery, Second Hospital of Lanzhou University, Lanzhou, Gansu, China, [3]Key Laboratory of Neurology of Gansu Province, Lanzhou University, Lanzhou, Gansu, China, [4]Academician Workstation, The Second Hospital of Lanzhou University, Lanzhou, Gansu, China

S-Palmitoylation has been widely noticed and studied in a variety of diseases. Increasing evidence suggests that S-palmitoylation modification also plays a key role in Glioblastoma (GBM). The zDHHC family, as an important member of S-palmitoyltransferases, has received extensive attention for its function and mechanism in GBM which is one of the most common primary malignant tumors of the brain and has an adverse prognosis. This review focuses on the zDHHC family, essential S-palmitoyltransferases, and their involvement in GBM. By summarizing recent studies on zDHHC molecules in GBM, we highlight their significance in regulating critical processes such as cell proliferation, invasion, and apoptosis. Specifically, members of zDHHC3, zDHHC4, zDHHC5 and others affect key processes such as signal transduction and phenotypic transformation in GBM cells through different pathways, which in turn influence tumorigenesis and progression. This review systematically outlines the mechanism of zDHHC family-mediated S-palmitoylation modification in GBM, emphasizes its importance in the development of this disease, and provides potential targets and strategies for the treatment of GBM. It also offers theoretical foundations and insights for future research and clinical applications.

KEYWORDS

glioblastoma, zDHHC family, S-palmitoylation, molecular mechanism, treatment strategy

1 Introduction

S-palmitoylation is a highly conserved post-translational lipid modification widely present in eukaryotes. It plays crucial roles in various physiological processes by influencing protein structure, localization, transport, and function (Tabaczar et al., 2017). Based on the different acyl acceptors, protein palmitoylation can be classified into S-palmitoylation, N-palmitoylation, and O-palmitoylation (Zhang Y. et al., 2021). Among these modifications, S-palmitoylation involves the attachment of palmitic acid (PA) to target proteins via thioester bonds (Sobocińska et al., 2018). Due to the specificity of thioester bonds, this modification is a valuable reversible modification (Qu et al., 2021). S-palmitoylated proteins are predominantly membrane proteins, especially transmembrane and peripheral membrane proteins (Charollais and Van Der Goot, 2009). Upon modification, target proteins undergo changes in hydrophobicity and

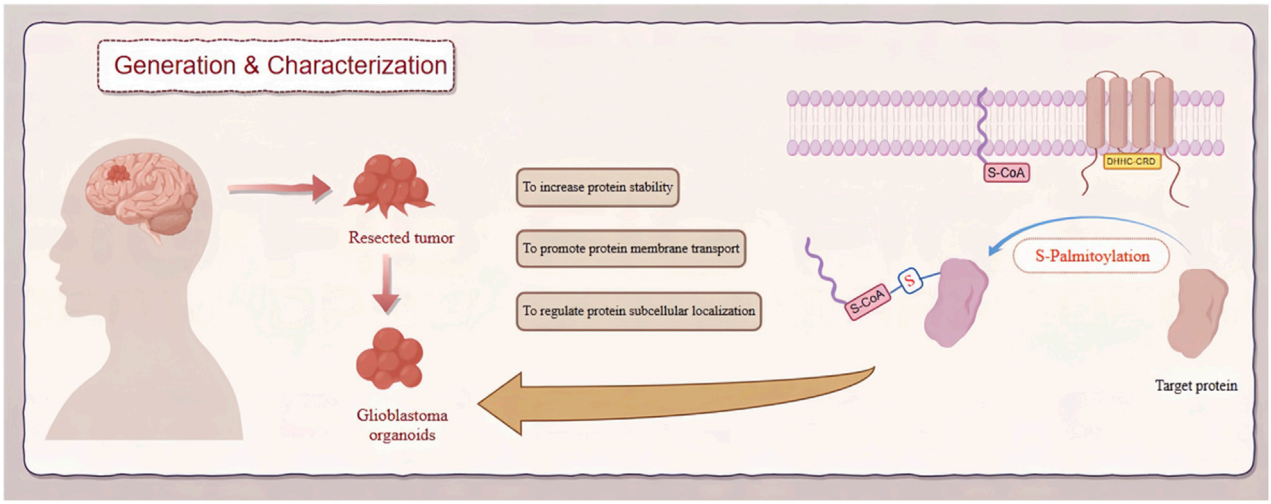


FIGURE 1
Role of zDHHC-mediated S-palmitoylation in GBM. This diagram depicts the involvement of zDHHC enzymes in S-palmitoylation, a post-translational modification, and its effects on the pathogenesis of GBM. zDHHC enzymes facilitate the addition of palmitoyl groups to specific substrate proteins, altering their localization, stability, and function. These modifications influence key signaling pathways that drive GBM progression and malignancy.

membrane-binding ability, thereby altering protein structure, localization, transport, and function. The combination of S-palmitoylation modification and its reverse reaction—depalmitoylation—leads to periodic changes in the structure and function of modified proteins, aligning with specific biological functions of individuals (Li et al., 2022).

As the most malignant primary tumor of the brain, Glioblastoma has long been a challenge for neurologists, oncologists, clinicians and so on Shergalis et al., 2018. Understanding its pathogenesis has been a focal and challenging aspect of scientific research. The significant role of zDHHC-mediated palmitoylation in GBM is increasingly recognized, particularly in key proteins associated with its occurrence and progression (Chen et al., 2020a). Increasing evidence suggests that palmitoylation or palmitoyltransferases could become novel targets for cancer therapy (Chen et al., 2018). This review comprehensively analyzes how zDHHC-mediated S-palmitoylation modification regulates the occurrence and development of GBM (Figure 1). Additionally, it explores the potential of developing protein S-palmitoylation-targeted drugs for more precise personalized therapy.

2 S-palmitoylation

S-palmitoylation is a reversible protein lipid modification that widely exists in eukaryotic cells and participates in regulating downstream gene transcription, expression, and signal transduction, thereby influencing cellular activities (Resh, 2013). Discovered in the 1980s, palmitoylation is catalyzed by the protein acyltransferase (PAT) family, which attaches palmitic acid (PA), a 16-carbon saturated fatty acid, to cysteine residues on target proteins (Schmidt et al., 1979; Chen et al., 1985; Mitchell et al., 2006). PA, which is one of the important substrates involved in palmitoylation modification, is the most common saturated fatty acid in the human body and constitutes 20%–30% of total fatty acids and can be obtained from dietary sources like meat and dairy (Carta et al., 2017; Carta et al., 2015) or synthesized endogenously through *de novo* lipogenesis (DNL) from other metabolites (Song et al., 2018; Kersten, 2001). Of note, PA plays essential physiological roles, serving as a component of cell membranes in the form of phospholipids and sphingolipids, providing energy through β-oxidation, or being esterified into triglycerides for lipid storage (Carta et al., 2017). Changes in PA concentration directly affect the efficiency and function of palmitoylation (Pei et al., 2016; Wang et al., 2023). Upon modification, the hydrophobicity and affinity of target proteins for the cell membrane surface increase.

Unlike irreversible lipid modifications such as myristoylation or prenylation, which involve amide or thioether bonds, respectively, S-palmitoylation forms thioester bonds (Robert and Vagner, 2018; Yuan et al., 2020a). These bonds are less stable due to the larger atomic radius and weaker bonding energy of sulfur compared to oxygen (Ashenhurst, 2015; van Bergen et al., 2016). Additionally, sulfur atoms are prone to oxidation and reduction reactions, leading to the instability of thioester bonds and enabling proteins to cycle between palmitoylated and depalmitoylated forms, thus spatio-temporal control of protein function can be conferred (Ko and Dixon, 2018; Wang et al., 2020). Depalmitoylation, the reverse reaction of palmitoylation, is catalyzed by palmitoyl-protein thioesterases (PPTs), including acyl-protein thioesterases (APT1/2), palmitoyl-protein thioesterases (PPT1/2), or proteins containing α/β hydrolase domains (ABHD17A/B/C) (Hornemann, 2015). Overall, depalmitoylation serves as an essential complement to palmitoylation, necessary for protein function and cellular processes. The dynamic balance between palmitoylation and depalmitoylation allows cells to regulate protein activity, stability, and localization to adapt to different cellular environments and physiological needs. Many key proteins in cellular signaling

pathways undergo palmitoylation, and depalmitoylation can alter their activity and stability, thereby affecting signal transduction. Through depalmitoylation, cells can timely adjust the activity of signaling pathways to adapt to different cellular stimuli.

3 ZDHHC

The enzymes catalyzing S-palmitoylation modification, known as PATs, are referred to as DHHC-PATs (Mitchell et al., 2006). In most cases, these enzymes contain a cysteine-rich domain (CRD) comprising 51 amino acids. Within the CRD, there's a highly conserved catalytic domain called the aspartate-histidine-histidine-cysteine (DHHC) motif, which is crucial for maintaining the palmitoylation activity of the PAT molecule (Tabaczar et al., 2017; Gottlieb et al., 2015). The DHHC domain serves as the catalytic center of the enzyme, and mutations in this domain can weaken the palmitoyl transfer capability (Ko and Dixon, 2018; Resh, 2006a). Additionally, the CRD contains two zinc-binding sites that coordinate with two Zn^{2+} ions which do not directly participate in protein catalysis play a vital role in maintaining the integrity and functionality of the domain (Zmuda and Chamberlain, 2020; Stix et al., 2020). Due to the presence of the zinc finger DHHC domain, this class of PATs is also referred to as zinc finger DHHC domain-containing palmitoyl transferases (zDHHC).

Up to present, There are 23 zDHHC genes identified in the human genome, named zDHHC1-24 (excluding zDHHC10) (Ko and Dixon, 2018). These zDHHC proteins constitute the zDHHC protein family, primarily responsible for the catalytic activity of palmitoyl transferases (Linder and Deschenes, 2007). These enzymes are mainly localized in the endoplasmic reticulum (ER), Golgi apparatus, with a smaller fraction found on the plasma membrane (PM), mitochondria and perinuclear regions (Chen et al., 2021; Greaves and Chamberlain, 2011; Jansen and Beaumelle, 2022). The localization of DHHC-PATs partly depends on structure and motif. For example, lysine-based sorting signals on the sequences of DHHC4 and DHHC6 enable them to form KXX and KKXX motifs, thus guiding them to the ER membrane (Gorleku et al., 2011).

Structurally, the molecular structure of zDHHC proteins which as a type of polytopic integral membrane protein consists of an N-terminal domain facing the cytoplasm, 4-7 transmembrane domains (TMDs), and a C-terminal domain also facing the cytoplasm (Ohno et al., 2006). N-terminal and C-terminal sequences exhibit significant diversity, mediating protein-protein interactions and facilitating acyl transfer processes (Jiang et al., 2018; Malgapo and Linder, 2021). The TMDs collectively form a cavity resembling a tent, facilitating the binding of fatty acyl chains (Rana et al., 2018). The DHHC domain, crucial for maintaining catalytic activity, is located between the second and third TMDs (Jiang et al., 2018; Rana et al., 2019). Besides the DHHC motif, other conserved motifs such as Asp-Pro-Gly (DPG), Thr-Thr-x-Glu (TTxE), and PaCCT have been reported (Mitchell et al., 2006). The second serine in the TTxE motif can directly interact with the aspartate of the DHHC motif to form hydrogen bonds, while Asn266 in the PaCCT motif utilizes its hydrogen bonding capability to assist inside chain amide formation (Rana et al., 2018). This suggests that these conserved residues may also participate in substrate protein recognition and catalysis processes by making key contacts with the DHHC domain (Rana et al., 2018).

Some zDHHC proteins require interaction with auxiliary factors to form complexes, such as the DHHC9/GCP16 complex (Swarthout et al., 2005) and the DHHC6/Selk complex (Fredericks et al., 2014). Additionally, certain zDHHC enzymes require palmitoylation by other family members to initiate S-palmitoylation cascades, thereby regulating substrate palmitoylation (Abrami et al., 2017; Plain et al., 2020). Specific zDHHC molecules may exhibit unique substrate binding preferences, as evidenced by zDHHC13 and zDHHC17's strong selectivity for synaptic-related proteins (Rodenburg et al., 2017; Zhang and Hang, 2017). Notably, zDHHC13 and zDHHC17 contain an ankyrin repeat domain (AR) within their N-terminal domains (Lemonidis et al., 2017), facilitating membrane localization and enhancing the palmitoylation activity of other zDHHC enzymes (Lemonidis et al., 2014; Lemonidis et al., 2015). These findings underscore the complex and efficient structure-function relationship within the zDHHC family, providing insights into the mechanism underlying protein palmitoylation.

4 Mechanisms of S-palmitoylation

Although 23 zDHHCs have been identified to catalyze protein S-palmitoylation, the specific process remains unclear (Yuan et al., 2024). Previous studies have found that in most cases, zDHHCs catalyze substrate S-palmitoylation through a two-step process, known as the ping-pong kinetic mechanism (Rana et al., 2019; Roth et al., 2002). First, zDHHC proteins undergo auto-palmitoylation, where the cysteine residue in the DHHC-CRD domain of zDHHC protein covalently binds with palmitoyl coenzyme A to form an acyl-enzyme intermediate (Malgapo and Linder, 2021). Subsequently, the crucial second step reaction occurs, where zDHHC proteins catalyze the transfer of the palmitoyl group from their own binding site to the cysteine thiol group of the protein substrate. Simultaneously, zDHHC proteins revert to their initial state, and the protein substrate forms an unstable thioester bond, completing the palmitoylation modification (Rana et al., 2018; Jennings and Linder, 2012; Chamberlain and Shipston, 2015; Salaun et al., 2020). The aforementioned reaction mechanisms apply to the catalytic processes of most zDHHC molecules (Stix et al., 2020). However, there is still insufficient evidence to determine whether zDHHC13, 17, and 19 can catalyze auto-palmitoylation or not. Further investigation may be needed to explore common mechanisms underlying zDHHC catalyzed palmitoylation modification and whether auto-palmitoylation does not occur in all zDHHCs (Lemonidis et al., 2014; Verardi et al., 2017).

Most proteins undergo palmitoylation on multiple amino acid residues, with reports suggesting up to 5-6 individual palmitoylated cysteine residues in some proteins (Zhang et al., 2018). While the above two-step kinetic mechanism applies to the majority of proteins, it has been observed that some proteins undergo auto-palmitoylation independently of the zDHHC protein family. Examples include yeast proteins (Swf1 and Pfa4) (Das et al., 2021), myelin protein P0 (Bharadwaj and Bizzozero, 1995) and others, which possess acyl-CoA within their structures. Due to the

unique characteristics of their structures, some of these proteins exhibit intrinsic "enzyme-like" activities and can directly bind with palmitoyl-CoA to undergo auto-palmitoylation (Smotrys and Linder, 2004; Chan et al., 2016).

In conclusion, our comprehension of the mechanisms by which ZDHHC and APT enlist their protein substrates remains incomplete (Rana et al., 2018; Verardi et al., 2017; Lee et al., 2022). Data from the SwissPalm database reveals that palmitoylation encompasses over 2,400 mammalian proteins, as evidenced by findings from more than 100 proteomics and biochemical studies (Blanc et al., 2015). These proteins encompass integral membrane proteins, peripheral membrane proteins, cytoplasmic signaling proteins, and transcription factors. Oncoproteins such as NRAS, KRAS4A, HRAS, epidermal growth factor receptor (EGFR), and p53 depend on S-palmitoylation cycling to modulate their localization, activity, or interaction partners (Tate et al., 2019).

5 Functions of S-palmitoylation

From a biochemical perspective, the binding of the lipid molecule palmitic acid can enhance the hydrophobicity of specific domains of target proteins (Jiang et al., 2018). Additionally, due to the instability of thioester bonds, palmitoylation and depalmitoylation can rapidly switch, acting as a protein switch or modulator similar to protein ubiquitination or phosphorylation (Blaskovic et al., 2013). Changes in hydrophobicity/hydrophilicity may affect various properties and functions of proteins. It can modulate the affinity of protein molecules for membrane structures, thereby determining protein localization (Levental and Lyman, 2023). Through different localizations, protein-protein interactions (PPI) may be altered, leading to activation or inhibition of downstream signaling pathways, affecting processes such as gene replication, transcription, expression, or protein secretion (Zhang Z. et al., 2021; Pei et al., 2022; Lee et al., 2021).

Palmitoylation modification plays a crucial role in the pathological and physiological functions of key proteins in signaling pathways such as RAS, MET, STING, EGFR and Hedgehog, etc. (Runkle et al., 2016; Lin et al., 2017). Taking the Ras protein family as an example, newly synthesized Ras proteins undergo palmitoylation modification during transport to the Golgi apparatus to enter the secretory pathway and transfer to the PM After activation at the PM, depalmitoylation occurs, weakening the protein's binding to the membrane, leading to its transport back to the Golgi apparatus (Shahinian and Silvius, 1995; Busquets-Hernández and Triola, 2021). The dynamic palmitoylation of Ras regulates its cycling between the PM and Golgi apparatus, preventing nonspecific residence on the PM and facilitating the transmission of activated Ras signals downstream (Rocks et al., 2005). Moreover, there is evidence suggesting that EGFR signaling can be both promoted and inhibited by its palmitoylation. This might be due to the presence of multiple palmitoylation sites, which are regulated by different ZDHHC proteins and have distinct functional effects (Bollu et al., 2015). Palmitoylation also regulates the activity of G protein-coupled receptors, including the melanocortin-1 receptor (MC1R), which drives melanin production and DNA repair following UV exposure (Chen S. et al., 2017). Therefore, impaired palmitoylation of MC1R increases the risk of melanoma. Additionally, palmitoylation is involved in regulating the tumor microenvironment, including crucial processes such as angiogenesis and immune evasion (Lee et al., 2021; Pan and Chen, 2022). S-palmitoylation imparts different characteristics to proteins by attaching palmitic acid molecules, thereby affecting protein hydrophobicity, structural stability, localization, migration between membrane regions, and interactions with effectors, enzyme activity, protein storage, and more.

S-palmitoylation is also associated with resistance to cancer treatments. For example, ZDHHC2-mediated palmitoylation of mitochondrial acylglycerol kinase (AGK) increases sunitinib resistance in renal cell carcinoma by activating the AKT–mTOR signaling pathway (Sun et al., 2023). Similarly, ZDHHC16-mediated palmitoylation of PCSK9 induces sorafenib resistance in cancer through activation of the PI3K–AKT pathway (Sun et al., 2022).

6 Glioblastoma

Glioblastoma (GBM) is a common malignant tumor originating from the central nervous system, which remains essentially incurable. Arising from neural glial cells, GBM can occur in various parts of the brain, accounting for approximately 51% of all malignant tumors of the brain. According to statistics, the incidence of GBM is 5 per 100,000 individuals, with a rising trend annually. While the affected population primarily comprises middle-aged and elderly individuals, GBM can occur across all age groups, including children (Xiao et al., 2023). Despite the standard treatment regimen for GBM, which involves maximal safe surgical resection followed by adjuvant therapies such as temozolomide (TMZ) chemotherapy, adjunctive radiotherapy, and tumor-treating fields (TTF) therapy, the median survival period post-diagnosis remains merely 8 months, with a 5-year survival rate of less than 7% (Zhong et al., 2021; Ostrom et al., 2022). Additionally, the majority of GBM patients experience tumor recurrence (Jiang et al., 2020; Daniel et al., 2022). The high recurrence rate may be attributed to the presence of dormant tumor-seeding cells located distant from the initial tumor or tumor cells infiltrating the normal brain parenchyma at the tumor's periphery (Darmanis et al., 2017; Johnson et al., 2014).

GBM, also known as glioblastoma multiforme, was classified by the World Health Organization (WHO) in 2016 into IDH-wildtype and IDH-mutant subtypes based on the mutation status of isocitrate dehydrogenase (IDH) (Louis et al., 2016). Due to the closer resemblance of IDH-mutant GBM to anaplastic astrocytoma, some scholars have proposed renaming IDH-mutant GBM as astrocytoma, IDH-mutant, grade 4 (White et al., 2020). The 2021 WHO classification defines GBM as lacking IDH mutation, along with alterations related to telomerase reverse transcriptase (TERT) promoter mutation, epidermal growth factor receptor (EGFR) amplification, chromosome 10 copy number loss, and chromosome 7 copy number gain (Louis et al., 2021).

As a typical solid tumor, GBM exhibits characteristics of sustained cell proliferation, resistance to cell death, continuous angiogenesis, increased cellular invasion and metastasis. These features are accompanied by genomic instability and mutations,

altered cellular metabolism, replicative immortality, sustained inflammation, evasion of growth suppression, and immune suppression (Hanahan, 2022; Verdugo et al., 2022). The mechanisms underlying its occurrence involve multiple aspects such as genetics, epigenetics, and transcriptomics, ultimately leading to significant alterations in crucial signaling pathways. The entire process can be summarized as follows: (Tabaczar et al., 2017): External signaling molecules activate signaling pathways via membrane proteins; (Zhang Y. et al., 2021); Protein kinases activate signals from the membrane to the nucleus; (Sobocińska et al., 2018); Transcription factors activate effector genes, resulting in cellular biological effects (Blanc et al., 2015; Lothrop et al., 2013; Nakada et al., 2011).

Current research has revealed several key factors contributing to the occurrence and development of GBM, including gene mutations (Brennan et al., 2013), tumor microenvironment (TME) (Quail and Joyce, 2017; Erices et al., 2023) and aberrant signaling pathways (Anido et al., 2010; Stommel et al., 2007). These processes are typically controlled by various oncogenes and/or tumor suppressor genes, many of which undergo post-translational modifications (PTMs) such as phosphorylation (Huang et al., 2021; Wang et al., 2021), methylation (Li et al., 2023; Leske et al., 2023), acetylation (Li et al., 2024; Liu X. et al., 2023), ubiquitination (Chang et al., 2023) and so on, which to some extent affect protein localization, stability, and function (Fhu and Ali, 2021). Protein lipidation is a significant and diverse class of post-translational modifications (PTMs) that involves the covalent attachment of specific lipid molecules to various amino acid residues on target proteins (Jiang et al., 2018; Liu et al., 2021). Lipidated proteins typically exhibit a stronger affinity for non-polar structures, such as lipid bilayers, influencing the localization, diffusion, and interactions of these modified proteins (Chen et al., 2018; Lanyon-Hogg et al., 2017; Burnaevskiy et al., 2015). It is widely recognized that this modification can regulate various biological processes in eukaryotic cells, including cell division, differentiation, and immune responses (Resh, 2006b). Most protein lipidation modifications are considered irreversible, including N-myristoylation (Lin et al., 2012), S-farnesylation (Kmiec et al., 2021), O-palmitoylation (Zou et al., 2011), and N-palmitoylation (Chen et al., 2004), etc. In contrast, protein S-palmitoylation is a reversible post-translational modification (PTM) (Jiang et al., 2018; Linder and Jennings, 2013). This characteristic allows the modified proteins to cycle between palmitoylated and depalmitoylated forms, making it essential for understanding how protein palmitoylation affects the function of individual proteins in both normal and cancer cells (Martin et al., 2011; Won and Martin, 2018).

7 zDHHC and GBM

Similar to lipid imbalance situations, palmitoylation not only participates in numerous physiological processes but also associates with various diseases, including neurological disorders, viral infections, cardiovascular diseases, immune system disorders, and the onset and progression of tumors (Mesquita et al., 2021; Ramos et al., 2023; Chong et al., 2023; Zhou et al., 2022). The importance of protein palmitoylation in tumorigenesis has been a subject of attention and research over the past decade. Palmitoylation influences multiple aspects of cancer, including cancer cell proliferation and survival, invasion and metastasis, and anti-tumor immunity (Ko and Dixon, 2018; Jin et al., 2021; Tang et al., 2022a). The crucial role of zDHHC-mediated palmitoylation in GBM is also increasingly recognized, particularly in key proteins associated with its occurrence and progression (Chen et al., 2020a). In our preliminary study, we compared the expression of various zDHHC molecules in normal brain tissue and brain tumors by querying the GENT database (Figure 2). Accumulating evidence suggests that palmitoylation or palmitoyltransferases may serve as novel targets for cancer therapy (Liu et al., 2020).

Understanding the cellular signaling and molecular mechanisms of palmitoylation modification, as well as its pathological effects, is crucial. Here, we will review the latest research to unveil the mechanisms by which zDHHC-mediated S-palmitoylation modification regulates protein function in the occurrence and progression of GBM. Finally, we aim to explore opportunities and new strategies for therapeutic intervention targeting protein palmitoylation (Table 1).

7.1 zDHHC3

GBM is considered a "cold tumor," where tumor cells, microglia, macrophages, T cells, and myeloid-derived suppressor cells (MDSCs) collectively form an immunosuppressive microenvironment (Da Ros et al., 2018; Lim et al., 2018). Programmed Death Ligand-1 (PD-L1) is expressed on the surface of GBM cells, which, upon binding to Programmed Death-1 (PD-1) on T cell surfaces, resists T cell cytotoxicity, ultimately leading to tumor immune evasion (Yao et al., 2021; Yang Y. et al., 2019; Freeman et al., 2000; Feng et al., 2023). Tang et al. demonstrated the colocalization and physical interaction between ZDHH3 and PD-L1 in GBM cell lines using immunofluorescence and co-immunoprecipitation in their glioma research (Tang W. et al., 2022). zDHHC3-mediated palmitoylation modification plays a crucial role in regulating tumor immune evasion and tumor cell survival. Through palmitoylation modification of PD-L1 protein (Cys272), zDHHC3 inhibits PD-L1 monoubiquitination, preventing its delivery to lysosomes for degradation, thereby increasing PD-L1 expression levels within cells. This results in increased surface expression of PD-L1 on tumor cells, which, by binding to PD-1, inhibits T cell activity, reducing tumor cell immune clearance (Yao et al., 2019). Blocking PD-L1 palmitoylation modification pharmacologically promotes PD-L1 lysosomal degradation, thereby activating anti-tumor immune responses and enhancing T cell cytotoxicity. Additionally, palmitoylation modification by zDHHC3 affects the intracellular distribution of PD-L1, depleting its intracellular pool, making anti-PD-L1 therapy more effective (Tang W. et al., 2022). Therefore, zDHHC3-mediated palmitoylation modification regulates the stability and function of PD-L1, influencing tumor immune evasion and survival. Current antibody therapies mainly target surface expression of PD-L1, while targeting PD-L1 palmitoylation simultaneously reduces its expression levels on the cell membrane and within cells. This strategy sensitizes tumor cells to T cell cytotoxicity more effectively, thereby more efficiently inhibiting tumor growth (Kinney et al., 2017).

10.3389/fcell.2024.1413708

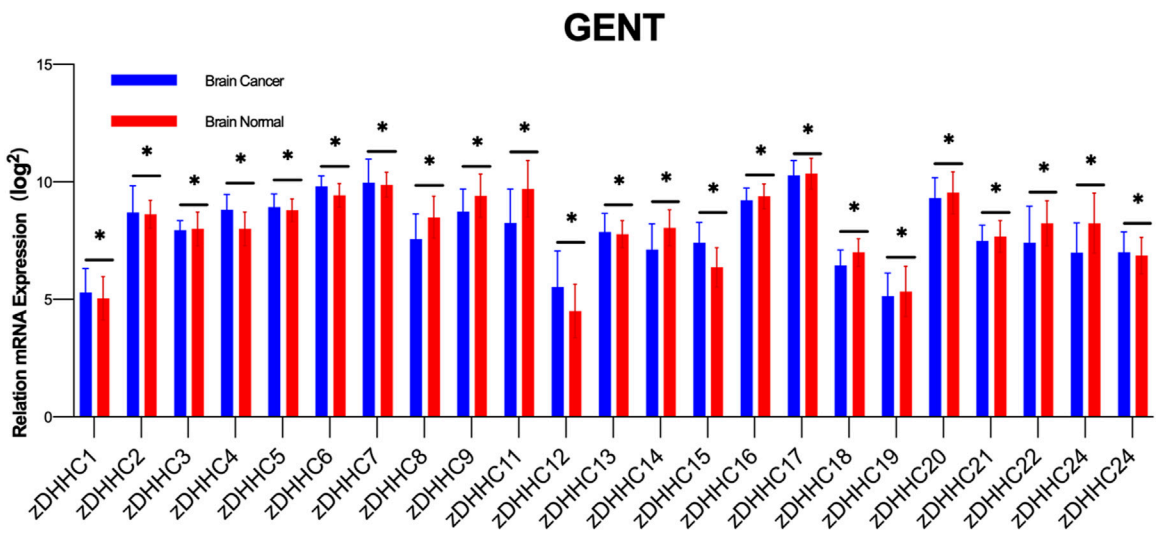


FIGURE 2
Differential Expression of zDHHC Genes in Normal Brain Tissue and Glioblastoma. The figure shows the differential expression of various zDHHC genes between normal brain tissues (n = 873) and glioblastoma tumor tissues (n = 3439) as analyzed from the GENT database. Multiple unpaired t-tests were conducted to compare the expression levels of each zDHHC gene in the two types of tissues. The bar graph shows the mean values with error bars representing the standard deviation (SD). The results indicate significant statistical differences in the expression of zDHHC genes between normal and tumor tissues, highlighting their potential role in the pathogenesis of glioblastoma. Each group showed significant *p*-values, denoting strong evidence against the null hypothesis of no difference in expression levels.

TABLE 1 Target proteins, pathways and biological roles ZDHHC protein-mediated protein palmiylation

ZDHHCs	Target proteins	Pathways	Actions	Biological effects
zDHHC3	PD-L1, Cys272	Immune evasion pathway	Increases the stability and intracellular expression of PD-L1 through palmitoylation, inhibiting T cell activity	Regulates immune evasion and survival of tumor cells
zDHHC4	GSK-3B,-	GSK-3B/EZH2/ STAT3 pathway	Modulates the phosphorylation status of GSK-3ß through palmitoylation, affecting self-renewal and drug resistance of GSCs	Participation in GSCs self-renewal and regulation of resistance to TMZ
zDHHC5	EZH2, Cys571 and Cys576	EZH2/S21 signaling pathway	Modulates the phosphorylation status of EZH2 and membrane localization of FAK1 through palmitoylation, affecting cell growth and migration	Regulates self-renewal of glioblastoma stem cells and epithelial- mesenchymal transition
zDHHC7	STAT3,-	JAK2/STAT3 signaling pathway	Affects membrane localization and activation of STAT3 through palmitoylation, regulating cell signaling	Involved in sustained activation of STAT3 signaling pathway in glioblastoma
zDHHC9	GLUT1, Cys207	Glucose metabolism pathway	Promotes membrane localization of GLUT1 through palmitoylation, increasing glucose uptake and cell proliferation	Regulates energy metabolism and proliferation of GBM cells
zDHHC15	gp130, -	IL-6/STAT3 signaling pathway	Modulates gp130 signaling by palmitoylation, inhibiting IL-6/ STAT3 signaling pathway	Involved in regulation of glioblastoma stem cell sphere formation, proliferation, and growth
zDHHC16	SETD2	DNA damage repair pathway	Modulates DNA damage repair signaling by affecting SETD2 palmitoylation	Involved in regulation of DNA damage repair signaling in GBM
zDHHC17	MAP2K4, -	JNK/p38 signaling pathway	Activates JNK/p38 pathway through interaction with MAP2K4, regulating development and progression of GBM	Involved in regulation of malignant characteristics and growth of GBM
zDHHC18	BMI1 and RNF144A,-	BMII signaling pathway	Affects state transition of GSCs by modulating BMI1 ubiquitination levels	Regulates state transition of GBM stem cells
zDHHC23	BMI1 and RNF144A,-	BMII signaling pathway	Affects stability of BMI1 in susceptible neural stem cells by increasing BMI1 ubiquitination levels	Regulates state transition of GBM stem cell
zDHHC19	Smad3,-	TGF-B signaling pathway	Promotes Smad3 activation through palmitoylation, regulating TGF-ß signaling pathway	Involved in regulation of GBM cell tendency and mesenchymal subtype

7.2 zDHHC4

Glycogen Synthase Kinase-3β (GSK-3β) belongs to the serine/threonine protein kinase family and is primarily localized in the cytoplasm, with distribution in the nucleus and mitochondria as well. Apart from its initial discovery in regulating the activity of glycogen synthase (GS), GSK-3β also influences the structure and function of various signaling proteins and transcription factors, contributing to tumor formation and progression. Depending on the different molecular modifications it undergoes, GSK-3β exhibits diverse effects on tumor cells (T et al., 2016; He et al., 2020). Zhao et al. (C et al., 2022) found that zDHHC4 catalyzes the palmitoylation modification of GSK-3β, followed by phosphorylation of EZH2 at S21. This process further regulates the phosphorylation and methylation of STAT3. Through the GSK-3β-EZH2-STAT3 axis, it is involved in the self-renewal of Glioblastoma Stem Cells (GSCs) and the development of resistance to TMZ (a chemotherapy drug) in GBM. Additionally, this modification affects the interaction between GSK3β and AKT and p70S6K, thereby regulating their phosphorylation status. This discovery reveals the critical role of GSK-3β palmitoylation modification in the regulation of tumor stem cells, providing a new theoretical basis for understanding and intervening in tumor development.

7.3 zDHHC5

The ZDHHC5 gene is located in an unstable chromosomal region and is upregulated in breast cancer and lung adenocarcinoma (Tian et al., 2015). Chen et al. (Chen X. et al., 2017) found that the p53 gene controls the transcriptional activation of the ZDHHC5 promoter in GBM, suggesting an association between ZDHHC5 expression, p53 regulation, and tumorigenesis. Furthermore, it was discovered for the first time that in GBM, both the mRNA and protein levels of ZDHHC5 increase with tumor grading, correlating with increased p53 mutation frequency. Further functional studies revealed that mutant p53 can physically interact with the transcription factor NF-Y, forming an enhanced functional complex independent of the type of p53 mutation, leading to aberrant upregulation of ZDHHC5. Additionally, overexpression of ZDHHC5, along with mutations in KRAS, TERT, and p53 oncogenes, is sufficient to trigger comprehensive and rapid malignant transformation of GBM. EZH2 is a histone methyltransferase targeting H3K27 and is thus considered a suppressor of tumor suppressor genes. When ZDHHC5 catalyzes the palmitoylation modification of EZH2 at Cys571 and Cys576, it affects the phosphorylation level of EZH2 at the S21 position, and the levels of these two modifications are inversely correlated. Palmitoylation is a necessary condition for EZH2 localization to the Golgi apparatus, while phosphorylation occurs at the Golgi apparatus. Decreasing the levels of ZDHHC5 and EZH2 palmitoyltransferase significantly inhibits the growth of glioma tumors (Tang et al., 2022c). ZDHHC5 can inhibit the expression of other pluripotency-related transcription factors by inhibiting EZH2 activation, thus preventing the self-renewal of GBM stem cells. In a recent study, Wang et al. (Wang et al., 2024) discovered that ZDHHC5 can also catalyze the S-palmitoylation of the tumor-related protein Focal Adhesion Kinase 1 (FAK1), thereby enhancing FAK's localization on the cell membrane and promoting epithelial-mesenchymal transition (EMT) of cells. EMT is a crucial process in tumor progression, enabling tumor cells to acquire invasive and migratory capabilities (Pastushenko and Blanpain, 2019; Thiery et al., 2009). Therefore, ZDHHC5-mediated palmitoylation modification of FAK may promote the development and metastasis of GBM by promoting the EMT process. This discovery provides important clues for understanding the molecular mechanisms of tumor development and offers new targets for therapeutic strategies targeting this modification process. Overall, the upregulation of ZDHHC5 in GBM is associated with p53 mutations and plays a role in influencing the self-renewal of GSCs and tumorigenesis. These findings suggest that ZDHHC5 may be a potential therapeutic target for treating p53-mutant GBM.

7.4 zDHHC7

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor located in the cytoplasm, whose activation by JAK, MAPK, or mTOR kinases can lead to phosphorylation of tyrosine or serine residues in the C-terminal domain of the STAT3 protein and its dimerization, thereby activating STAT3 dimers to enter the nucleus and initiate transcription of target genes (Becker and Wilting, 2019). Typically, STAT3 activation is transient and modulated. However, in tumor cells, 50%–90% of tumor cells exhibit sustained activation (LI et al., 2019) and in 66%–83% of GBM, the STAT3 pathway is constitutively activated (Carro et al., 2010; Kim et al., 2013). Moreover, the level of STAT3 phosphorylation is closely related to tumor grading, with significant differences observed between low-grade and high-grade tumors (Mizoguchi et al., 2006).

Advancements in research on palmitoylation mediated by zDHHC7 in GBM have demonstrated its significant role in the transcription factor STAT3. Studies have shown that cysteine 108 of STAT3 is palmitoylated by zDHHC7, which promotes the membrane localization of STAT3 and phosphorylation of JAK2, thereby affecting STAT3 activation and cellular signaling (Zhang et al., 2020). Additionally, it has been found that APT2 selectively depalmitoylates p-STAT3, promoting its nuclear translocation. This research reveals the crucial role of the palmitoylation-depalmitoylation cycle in regulating STAT3 activation, providing important clues for understanding the role of palmitoylation in the pathogenesis of GBM. These findings may provide a basis for the development of new therapeutic strategies. For example, therapeutic strategies targeting zDHHC7 and APT2 may help intervene in the STAT3 signaling pathway, thereby impacting the development and treatment of GBM.

7.5 zDHHC9

Glucose transporter 1 (GLUT1) belongs to the glucose transporter protein family and is responsible for the transmembrane uptake of glucose under normal conditions (Hg et al., 2002; Mueckler et al., 1985). It is widely expressed in various

tissues, with the highest expression levels observed in endothelial cells of barrier tissues such as red blood cells and the blood-brain barrier (Uldry and Thorens, 2004). In the central nervous system, GLUT1 plays a crucial role in endothelial cells and astrocytes, facilitating glucose uptake in astrocytes and glucose transport across the blood-brain barrier, making it considered the primary energy transporter in the brain.

A prominent characteristic of cancer is altered metabolism (Pavlova et al., 2022) and GLUT1 is aberrantly expressed in various cancers, including lung cancer, brain cancer, breast cancer, and bladder cancer (Ganapathy et al., 2009; Macheda et al., 2005). In the case of GBM, studies have shown high levels of GLUT1 expression in GBM cells to meet their high energy demands for glucose uptake (Mg et al., 2009; Komaki et al., 2019). DHHC9-mediated S-palmitoylation of GLUT1 is crucial for maintaining its localization on the cytoplasmic membrane. This palmitoylation promotes glucose uptake, glycolysis rate, and lactate production in GBM cells, thereby facilitating cell proliferation and tumor formation (Zhang Z. et al., 2021). This discovery is correlated with patient prognosis, highlighting the importance of zDHHC9 in regulating GLUT1 function in GBM.

7.6 zDHHC15

Glycoprotein 130 (gp130) is a glycoprotein located on the cell membrane and is one of the signaling receptor subunits of the interleukin-6 (IL-6) family (Cron et al., 2016; Wagener et al., 2014). It directly regulates signaling pathways such as STAT, MAPK, and PI3K/AKT and activates the SOCS negative feedback regulatory mechanism (Nogueira-Silva et al., 2013). As a crucial protein mediating cell survival, abnormal stabilization and overexpression of gp130 is closely associated with tumor progression. In normal cells, the level of gp130 protein is intricately regulated through various mechanisms, including ubiquitin-dependent degradation, endocytosis, and Caspase-induced protein cleavage. Specifically, the tetraspanin CD9 forms a complex with gp130, reducing its ubiquitination and maintaining high levels of gp130 in GSCs, ensuring sustained activation of STAT3 (Shi et al., 2017).

zDHHC15 plays a role by palmitoylating the IL-6 receptor subunit gp130, inhibiting the IL-6/STAT3 signaling pathway. Through a positive feedback mechanism, zDHHC15 effectively suppresses GSCs' sphere formation, cell proliferation, and growth (Fan et al., 2021a). Local anesthetics such as lidocaine, bupivacaine, mepivacaine, or ropivacaine can disrupt the transcription of ZDHHC15, reducing the palmitoylation level of gp130 and its localization on the cell membrane, thereby inhibiting the activation of the IL-6/STAT3 signal. This study emphasizes the crucial role of zDHHC15 in regulating the IL-6/STAT3 signaling pathway, which has a beneficial impact on the biological behavior of GSCs. Furthermore, the expression of ZDHHC15 in GBM is positively correlated with tumor grade, and high expression levels are associated with GSC self-renewal (Liu Z-Y. et al., 2023). Therefore, ZDHHC15 is not only an important regulatory factor for GSCs but also a potentially useful biomarker for GBM diagnosis and prognosis.

7.7 zDHHC16

Fan et al. (Fan et al., 2022) demonstrated that the ZDHHC16/SETD2/H3K36me3 signaling axis is inactivated in EGFR-altered GBM. Specifically, ZDHHC16 is significantly downregulated in GBM compared to normal brain tissue, which is closely associated with changes in EGFR. These events lead to the activation of p53, causing cells to arrest at the G1/S checkpoint. Additionally, in EGFR-amplified GBM, ionizing radiation-induced DNA damage affects DNA damage repair signaling, involving reduced palmitoylation of SETD2 and methylation of its target H3K36.

7.8 zDHHC17

ZDHHC17 is primarily localized in the Golgi apparatus, and its deficiency leads to arrest at the G2/M transition, along with an increase in the proportion of cells with dense Golgi. Studies have shown that ZDHHC17 is upregulated in GBM and interacts with MAP2K4 through its N-terminal signal transduction and protein-protein interaction ankyrin domain, forming a signaling module that activates the JNK/p38 pathway, thereby regulating the malignant development and progression of GBM (Chen et al., 2020b). This finding suggests the importance of the ZDHHC17-MAP2K4 signaling pathway in GBM and provides clues for further understanding the pathogenesis of GBM. Importantly, the JNK/p38 activation promoted by ZDHHC17 is independent of PAT, and glioma cells with suppressed ZDHHC17 expression are insensitive to 2-BP inhibition. This discovery highlights the importance of ZDHHC17 in regulating the JNK/p38 signaling pathway through a unique mechanism in GBM development.

7.9 zDHHC18 and zDHHC23

Bmi1 belongs to the Polycomb group (PcG) and is a key marker of stem cells (Xu et al., 2022). Initially discovered as an oncogene in mouse lymphomas, Bmi1 was later identified as an important regulator of hematopoietic and neural stem cell self-renewal (Wang et al., 2007). In this context, the role of zDHHC23 becomes evident as it recruits BMI1 and RNF144A (an E3 ligase involved in post-translational regulation of BMI1) to increase the ubiquitination level of BMI1 in susceptible neural stem cells, ensuring the stability of BMI1 protein in different subtypes of GSCs (Chen et al., 2019). On the other hand, ZDHHC18 affects the binding of BMI1 and RNF144A and reduces the polyubiquitination level of BMI1 in mesenchymal neural stem cells through competitive interaction with RNF144A. Overall, the relative changes in the abundance of zDHHC18 and zDHHC23 can regulate the expression pattern of BMI1, playing an important promoting role in the transition of glioblastoma stem cell states (Chen et al., 2019). These findings provide a new perspective for understanding the pathogenesis of GBM and lay the theoretical foundation for developing therapeutic strategies.

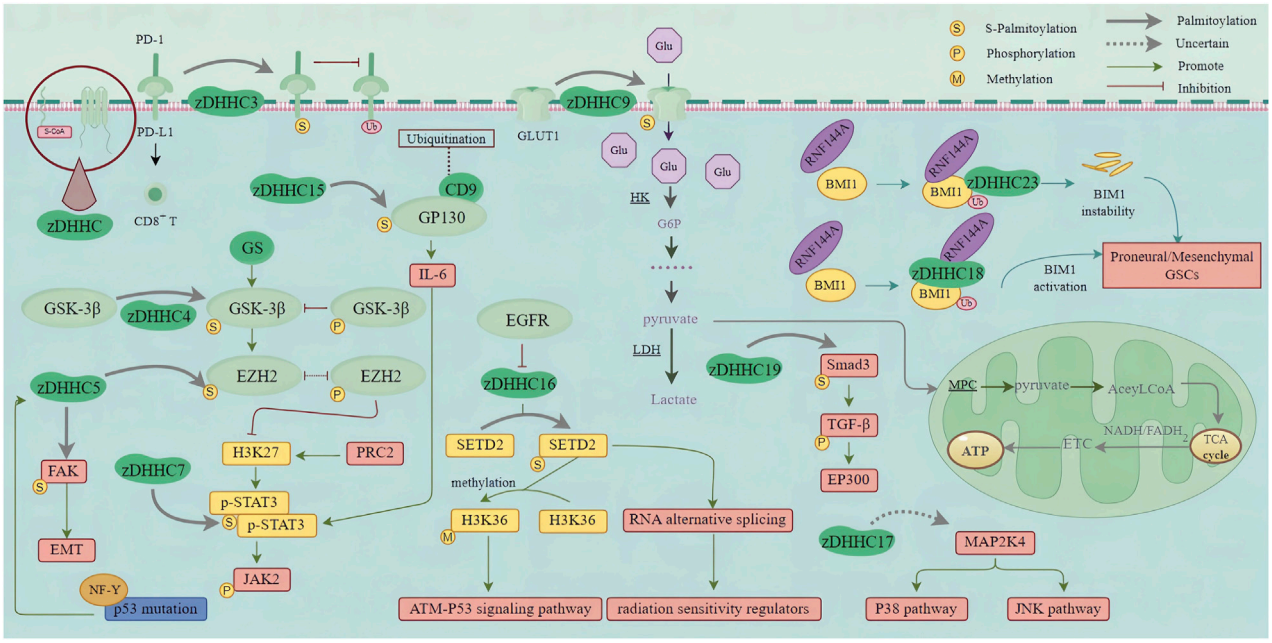


FIGURE 3
zDHHC-mediated S-palmitoylation in GBM Oncogenic Pathways. This figure illustrates the role of zDHHC enzymes in the S-palmitoylation of proteins involved in major oncogenic pathways in glioblastoma multiforme (GBM). zDHHC enzymes catalyze the addition of palmitoyl groups to specific proteins, such as EGFR, HRAS, and so on, which are critical in GBM pathogenesis. S-palmitoylation of these proteins affects their localization, stability, and interaction with other signaling molecules, thereby modulating key signaling pathways that promote GBM progression and malignancy. (PD-1, Programmed death receptor 1; PD-L1, Programmed death ligand 1; GSK-3β, Glycogen synthase kinase-3β; EZH2, Enhancer of Zeste homolog 2; GS, Glycogen synthase; FAK, Focal adhesion kinase; EMT, Epithelial-Mesenchymal Transition; NF-Y, Nuclear factor Y; H3K27, Histone 3 Lys 27; PRC2, Polycomb Repressive Complex 2; STAT3, Signal transducer and activator of transcription 3; JAK2, Janus kinase-2; CD9, Cluster of differentiation 9; GP130, Glycoprotein 130; IL-6, Interleukin 6; Glu, Glucose; GLUT1, Glucose transporter; HK, Hexokinase; G6P, Glucose 6-phosphate; LDH, lactate dehydrogenase; MPC, Mitochondrial pyruvate carrier; TCA cycle, Tricarboxylic acid cycle; ETC., Electron transfer chain; ATP, Adenosine triphosphate; Smad3, Small mother against decapentaplegic 3; TGF-β, Transforming growth factor-β; EP300, E1A-binding protein P300; MAP2K4, Mitogen-activated protein kinase kinase 4; JNK pathway, C-jun N-terminal kinase pathway; EGFR, Epidermal growth factor receptor; SETD2, Set2 in yeast; H3K36, Histone H3 on lysine 36; ATM, Ataxia Telangiectasia Mutated; RNA, Ribonucleic Acid; RNF144A, Ring Finger Protein 144; BMI1, B cell-specific Moloney murine leukemia virus integration site 1; GSCs, Glioma stem cells).

7.10 zDHHC19

Due to the significant intra- and inter-tumoral heterogeneity of GBM (including cellular and molecular complexity, therapies targeting a single molecular pathway are ineffective for GBM (Balça-Silva et al., 2019; Lah et al., 2020). GBM contains glioblastoma stem cells (GSCs), which are highly resistant to radiotherapy and chemotherapy, leading to high recurrence rates (Bao et al., 2006; Sharifzad et al., 2019). Targeting GSCs and identifying new markers are crucial issues in developing innovative strategies to eradicate GBM. IDH1 mutation induces the activation of HIF-1α and reduces the expression of TGF-β1 in proneural GSCs. However, a mechanism has been identified in mesenchymal-type GSCs whereby Smad3 activation is controlled through the regulation of Smad3 palmitoylation. This palmitoylation, facilitated by membrane localization and TGF-β1/2 phosphorylation, promotes Smad3 activation (Fan et al., 2021b). Inhibiting the activity of HIF-1α and Smad3 may effectively suppress the proneural and mesenchymal subtypes of GBM. EP300, a histone acetyltransferase (HAT), is involved in the formation of transcription complexes and plays crucial roles in cell cycle regulation and DNA damage repair (Snowden and Perkins, 1998). Interaction between activated TGF-β and EP300 further enhances the expression of corresponding markers in mesenchymal-type GBM (Fan et al., 2021b). This discovery reveals the intricate regulatory relationship between IDH1 mutation, the TGF-β signaling pathway, and mesenchymal features (Figure 3).

7.11 Other zDHHC molecules

zDHHC2 is an important gene associated with GBM. Studies have identified zDHHC2 as a significant prognostic gene for GBM, closely linked to its prognosis (Yang H. et al., 2019). High expression of zDHHC2 in GBM is strongly associated with the “surfactant metabolism” pathway and has been validated as a prognostic marker for GBM. Additionally, zDHHC2 has been found to be prognostically relevant in various cancers. For instance, in gastric cancer, its downregulation is associated with lymph node metastasis and poor prognosis (Yan et al., 2013). While the exact mechanisms of zDHHC2 in GBM remain incompletely understood, research suggests that zDHHC2 may play a pivotal role in the pathogenesis and prognosis of GBM.

Previous studies have linked zDHHC12 to neurological disorders such as Huntington’s disease, Alzheimer’s disease, and

schizophrenia (Liao et al., 2024; Young et al., 2012). Knocking out zDHHC12 in ovarian cancer significantly inhibits the precise membrane localization and protein stability of CLDN3, as well as tumor occurrence in ovarian cancer cells (Yuan et al., 2020b). Lu et al. (Lu et al., 2022) demonstrated the significant role of zDHHC12 in GBM. Firstly, their comprehensive analysis revealed upregulation of ZDHHC12 in various cancers, predicting poor prognosis in GBM and low-grade glioma (LGG). Further cell experiments indicated that zDHHC12 promotes the occurrence and progression of gliomas. Moreover, the study found that zDHHC12 exhibits a hypomethylated state in GBM, potentially being a mechanism underlying its overexpression. Hypomethylation of the zDHHC12 gene is particularly significant in high-grade glioma samples and is associated with IDH wild-type samples, suggesting that the methylation status of the zDHHC12 gene may occur before the increase in zDHHC12 expression, providing a potential biomarker for early screening of gliomas. While *in vitro* cell experiments demonstrate the role of zDHHC12 in the occurrence and progression of gliomas, further research is needed to collect more glioma samples and relevant clinical data to assess the performance of zDHHC12 in glioma diagnosis and prognosis.

8 Advances and strategies for targeting S-palmitoylation with drugs

Currently, research on the translational aspects of S-palmitoylation is progressing relatively slowly compared to other lipidation studies. However, selectively regulating one or more of the dozens of proteins involved in palmitoylation offers a broad and promising opportunity for the future. This regulation could potentially leverage the dynamic palmitoylation cycle to upregulate or downregulate the activity of palmitoylated tumor proteins or tumor suppressors that are otherwise difficult to target with drugs. The plasticity of palmitoylation indicates that a deeper understanding of its regulatory mechanisms could aid in the discovery of novel therapeutics.

Identifying inhibitors for several ZDHHCs or APTs is similar to developing "selective hybrid" kinase inhibitors, which already have recognized therapeutic value (Morphy, 2010). Although membrane-proximal cysteine is a common modification site, the lack of a consensus sequence for palmitoylation poses significant challenges for analysis. This variable and hydrophobic PTM makes it difficult to quantify the true extent and stoichiometry of palmitoylation. Therefore, new technological innovations may be required to enable knowledge-guided selection of specific ZDHHCs for drug discovery. For instance, recently reported chemical genetics methods have linked the metabolic labeling of palmitoylation with the activity of individual ZDHHCs, identifying specific relationships between ZDHHCs and their substrates (Ocasio et al., 2024; Tomić et al., 2024; Puthenveetil et al., 2023).

The ZDHHC proteins have a conserved hydrophobic lipid-binding site, indicating that these proteins can bind to small molecule targets (Rana et al., 2018). Although there are currently no fully validated, highly selective ZDHHC inhibitors, some APT inhibitors have demonstrated varying degrees of efficacy and selectivity (Won et al., 2016; Remsberg et al., 2021; Lan et al., 2021). Unfortunately, recent studies have tended to misuse nonspecific active compounds, such as 2-BP or cerulenin, as so-called ZDHHC inhibitors. These compounds primarily target lipid biosynthetic enzymes and have highly nonspecific and pleiotropic effects on ZDHHC activity (Lanyon-Hogg et al., 2017; Ergun et al., 2019; Davda et al., 2013).

The recent high-throughput screening for ZDHHC2 inhibitors represents the first step towards developing more selective ZDHHC inhibitors. However, the disclosed compounds were initially reported as inhibitors for another class of enzymes, so their selectivity needs further optimization (Salaun et al., 2022). For example, ZDHHC9 maintains the palmitoylation and plasma membrane expression of GLUT1, which is crucial for glucose transport in glioblastoma cells. Additionally, ZDHHC9 enhances anticancer immune suppression by catalyzing the palmitoylation of PD1 and PDL1 (67).

Directly targeting palmitoylation sites is an attractive alternative strategy due to its potential for high specificity. However, in practice, this approach may be limited to a few sites with well-defined binding pockets that are relatively buried, similar to TEAD inhibitors (Hagenbeek et al., 2023).

9 Conclusion and future perspectives

Glioblastoma (GBM) is a highly recurrent primary malignant tumor of the brain, which has long been a focus of clinical and research attention. In the studies of GBM, S-palmitoylation modification, as an important protein lipid modification, plays a crucial role in the occurrence, development, and treatment of tumors. This review summarizes the biological processes of S-palmitoylation modification and its key roles in GBM. Specifically, we focus on the zDHHC family as key enzymes of S-palmitoylation modification, their research progress, and potential roles in GBM.

Firstly, S-palmitoylation modification affects various cellular functions and signaling pathways by increasing the hydrophobicity and altering the localization of proteins. This modification plays a vital role in cell cycle regulation, cell proliferation, metastasis, and signal transduction, and its abnormal expression in tumors may lead to tumor initiation and progression.

Secondly, the zDHHC family, as crucial enzymes of S-palmitoylation modification, has attracted widespread attention in GBM research. Members of the zDHHC family may play different roles in the occurrence and development of GBM. We summarize the functions of molecules such as zDHHC3, zDHHC4, zDHHC5, zDHHC7, zDHHC9, zDHHC15, zDHHC16, zDHHC17, zDHHC18, and zDHHC23 in GBM and the signaling pathways they regulate. These molecules participate in the occurrence and progression of GBM by affecting tumor cell immune evasion, stem cell self-renewal, and activation of signaling pathways. We particularly highlight the regulation of key proteins' palmitoylation modification by these molecules and their effects on tumor biological behaviors. For example, palmitoylation modification of PD-L1 mediated by zDHHC3 affects tumor cell immune evasion; palmitoylation modification of GSK-3β mediated by zDHHC4 is involved in glioblastoma stem cell self-renewal and

resistance to chemotherapy drugs; palmitoylation modification of EZH2 mediated by zDHHC5 affects the self-renewal and metastasis ability of tumor stem cells.

With further research on the mechanisms of action of the zDHHC family in GBM, we can gain a deeper understanding of the roles of these molecules in tumor initiation and progression, providing new insights and targets for developing therapeutic strategies for GBM. For example, inhibiting tumor cell growth and metastasis by interfering with palmitoylation modification mediated by zDHHC molecules may become one of the future treatment strategies. Furthermore, exploring the relationship between the expression levels of these molecules in tumor tissues and clinical features can explore their potential value as diagnostic and prognostic markers.

However, S-palmitoylation faces some challenges and difficulties in GBM research. Firstly, the complexity of this field requires interdisciplinary cooperation, integrating expertise from biology, chemistry, bioinformatics, and other fields. Secondly, as a membrane modification process, although some progress has been made in research, a comprehensive understanding of the precise mechanism of S-palmitoylation in cells involves many complex biochemical processes and interactions, including specific steps of protein modification, enzymes involved, signaling pathways, etc. Current scientific research still needs to delve deeper to reveal the detailed mechanism of S-palmitoylation. Additionally, the heterogeneity and highly malignant characteristics of GBM itself add to the complexity of research, requiring more refined experimental design and data analysis.

Given the importance of S-palmitoylation mediated by the zDHHC family in GBM, future research can focus on several aspects: firstly, in-depth exploration of the expression patterns of each zDHHC molecule in GBM and their association with tumor development; secondly, studying the mechanisms by which each zDHHC molecule affects biological behaviors such as proliferation, invasion, and metastasis of GBM cells; finally, exploring the potential role of regulating or inhibiting the zDHHC family in GBM treatment, aiming to provide a theoretical basis and clinical application prospects for developing new therapeutic strategies.

Overall, the study of S-palmitoylation in GBM has broad prospects and important clinical application value. By overcoming the corresponding challenges, we hope to provide more effective and personalized treatment strategies for GBM patients, promoting the development of the tumor research field.

Author contributions

BT: Data curation, Writing–original draft, Writing–review and editing. WK: Data curation, Writing–original draft, Writing–review and editing. QD: Funding acquisition, Methodology, Visualization, Writing–review and editing. ZQ: Supervision, Validation, Writing–review and editing. LD: Supervision, Validation, Writing–review and editing. XZ: Supervision, Validation, Writing–review and editing. GY: Supervision, Validation, Writing–review and editing. YP: Formal Analysis, Methodology, Visualization, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by grants from Project of Healty and Famliy Planing Commission of Gansu (grant nos. S-62000000024-2024-047), Cuiying Scientific and Technological Innovation Program of Lanzhou University Second Hospital (grant nos. CY2022-YB-A05) and The Gansu Provincial Natural Science Foundation Project (No. 24JRRA332).

Acknowledgments

We are immensely grateful to all those who contributed to the success of this research. We thank the members of the Department of Neurosurgery at the Second Hospital of Lanzhou University for their invaluable assistance and support. Special thanks go to our colleagues at the Key Laboratory of Neurology of Gansu Province for providing critical insights and resources that greatly enhanced the quality of our work.

Conflict of interest

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

Publisher's note

References

Abrami, L., Dallavilla, T., Sandoz, P. A., Demir, M., Kunz, B., Savoglidis, G., et al. (2017). Identification and dynamics of the human ZDHHC16-ZDHHC6 palmitoylation cascade. *eLife* 6, e27826. doi:10.7554/eLife.27826

Anido, J., Sáez-Borderías, A., Gonzàlez-Juncà, A., Rodón, L., Folch, G., Carmona, M. A., et al. (2010). TGF-B receptor inhibitors target the CD44(high)/id1(high) glioma-initiating cell population in human glioblastoma. *Cancer Cell* 18, 655–668. doi:10.1016/j.ccr.2010.10.023

Ashenhurst, J. (2015). Thiols and thioethers. *Master Org. Chem.* Available at: https://www.masterorganicchemistry.com/2015/07/05/thiols-and-thioethers/ (Accessed July 5, 2024).

Balça-Silva, J., Matias, D., Carmo, A. do, Sarmento-Ribeiro, A. B., Lopes, M. C., and Moura-Neto, V. (2019). Cellular and molecular mechanisms of glioblastoma malignancy: implications in resistance and therapeutic strategies. *Semin. Cancer Biol.* 58, 130–141. doi:10.1016/j.semcancer.2018.09.007

Bao, S., Wu, Q., McLendon, R. E., Hao, Y., Shi, Q., Hjelmeland, A. B., et al. (2006). Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444, 756–760. doi:10.1038/nature05236

Becker, J., and Wilting, J. (2019). WNT signaling in neuroblastoma. *Cancers* 11, 1013. doi:10.3390/cancers11071013

Bharadwaj, M., and Bizzozero, O. A. (1995). Myelin P0 glycoprotein and a synthetic peptide containing the palmitoylation site are both autoacylated. *J. Neurochem.* 65, 1805–1815. doi:10.1046/j.1471-4159.1995.65041805.x

Blanc, M., David, F., Abrami, L., Migliozzi, D., Armand, F., Bürgi, J., et al. (2015). SwissPalm: protein palmitoylation database. *F1000Research* 4, 261. doi:10.12688/f1000research.6464.1

Blaskovic, S., Blanc, M., and van der Goot, F. G. (2013). What does S-palmitoylation do to membrane proteins? *FEBS J.* 280, 2766–2774. doi:10.1111/febs.12263

Bollu, L. R., Katreddy, R. R., Blessing, A. M., Pham, N., Zheng, B., Wu, X., et al. (2015). Intracellular activation of EGFR by fatty acid synthase dependent palmitoylation. *Oncotarget* 6, 34992–35003. doi:10.18632/oncotarget.5252

Brennan, C. W., Verhaak, R. G. W., McKenna, A., Campos, B., Noushmehr, H., Salama, S. R., et al. (2013). The somatic genomic landscape of glioblastoma. *Cell* 155, 462–477. doi:10.1016/j.cell.2013.09.034

Burnaevskiy, N., Peng, T., Reddick, L. E., Hang, H. C., and Alto, N. M. (2015). Myristoylome profiling reveals a concerted mechanism of ARF GTPase deacylation by the bacterial protease IpaJ. *Mol. Cell* 58, 110–122. doi:10.1016/j.molcel.2015.01.040

Busquets-Hernández, C., and Triola, G. (2021). Palmitoylation as a key regulator of ras localization and function. *Front. Mol. Biosci.* 8, 659861. doi:10.3389/fmolb.2021.659861

Carro, M. S., Lim, W. K., Alvarez, M. J., Bollo, R. J., Zhao, X., Snyder, E. Y., et al. (2010). The transcriptional network for mesenchymal transformation of brain tumours. *Nature* 463, 318–325. doi:10.1038/nature08712

Carta, G., Murru, E., Banni, S., and Manca, C. (2017). Palmitic acid: physiological role, metabolism and nutritional implications. *Front. Physiol.* 8, 902. doi:10.3389/fphys.2017.00902

Carta, G., Murru, E., Lisai, S., Sirigu, A., Piras, A., Collu, M., et al. (2015). Dietary triacylglycerols with palmitic acid in the sn-2 position modulate levels of N-acylethanolamides in rat tissues. *PloS One* 10, e0120424. doi:10.1371/journal.pone.0120424

Chamberlain, L. H., and Shipston, M. J. (2015). The physiology of protein S-acylation. *Physiol. Rev.* 95, 341–376. doi:10.1152/physrev.00032.2014

Chan, P., Han, X., Zheng, B., DeRan, M., Yu, J., Jarugumilli, G. K., et al. (2016). Autopalmitoylation of TEAD proteins regulates transcriptional output of the Hippo pathway. *Nat. Chem. Biol.* 12, 282–289. doi:10.1038/nchembio.2036

Chang, G., Xie, G. S., Ma, L., Li, P., Li, L., and Richard, H. T. (2023). USP36 promotes tumorigenesis and drug sensitivity of glioblastoma by deubiquitinating and stabilizing ALKBH5. *Neuro-Oncol* 25, 841–853. doi:10.1093/neuonc/noac238

Charollais, J., and Van Der Goot, F. G. (2009). Palmitoylation of membrane proteins (Review). *Mol. Membr. Biol.* 26, 55–66. doi:10.1080/09687680802620369

Chen, B., Sun, Y., Niu, J., Jarugumilli, G. K., and Wu, X. (2018). Protein lipidation in cell signaling and diseases: function, regulation, and therapeutic opportunities. *Cell Chem. Biol.* 25, 817–831. doi:10.1016/j.chembiol.2018.05.003

Chen, J. J., Fan, Y., and Boehning, D. (2021). Regulation of dynamic protein S-acylation. *Front. Mol. Biosci.* 8, 656440. doi:10.3389/fmolb.2021.656440

Chen, M.-H., Li, Y.-J., Kawakami, T., Xu, S.-M., and Chuang, P.-T. (2004). Palmitoylation is required for the production of a soluble multimeric Hedgehog protein complex and long-range signaling in vertebrates. *Genes Dev.* 18, 641–659. doi:10.1101/gad.1185804

Chen, S., Zhu, B., Yin, C., Liu, W., Han, C., Chen, B., et al. (2017a). Palmitoylation-dependent activation of MC1R prevents melanomagenesis. *Nature* 549, 399–403. doi:10.1038/nature23887

Chen, X., Hao, A., Li, X., Ye, K., Zhao, C., Yang, H., et al. (2020b). Activation of JNK and p38 MAPK mediated by ZDHHC17 drives glioblastoma multiforme development and malignant progression. *Theranostics* 10, 998–1015. doi:10.7150/thno.40076

Chen, X., Hu, L., Yang, H., Ma, H., Ye, K., Zhao, C., et al. (2019). DHHC protein family targets different subsets of glioma stem cells in specific niches. *J. Exp. Clin. Cancer Res. CR* 38, 25. doi:10.1186/s13046-019-1033-2

Chen, X., Li, H., Fan, X., Zhao, C., Ye, K., Zhao, Z., et al. (2020a). Protein palmitoylation regulates cell survival by modulating XBP1 activity in glioblastoma multiforme. *Mol. Ther. Oncolytics* 17, 518–530. doi:10.1016/j.omto.2020.05.007

Chen, X., Ma, H., Wang, Z., Zhang, S., Yang, H., and Fang, Z. (2017b). EZH2 palmitoylation mediated by ZDHHC5 in p53-mutant glioma drives malignant development and progression. *Cancer Res.* 77, 4998–5010. doi:10.1158/0008-5472.CAN-17-1139

Chen, Z. Q., Ulsh, L. S., DuBois, G., and Shih, T. Y. (1985). Posttranslational processing of p21 ras proteins involves palmitylation of the C-terminal tetrapeptide containing cysteine-186. *J. Virol.* 56, 607–612. doi:10.1128/JVI.56.2.607-612.1985

Chong, X., Zhu, L., Yu, D., Chen, S., Wang, G., Yu, Q., et al. (2023). ZDHHC9 promotes colon tumor growth by inhibiting effector T cells. *Oncol. Lett.* 25, 5. doi:10.3892/ol.2022.13591

Cron, L., Allen, T., and Febbraio, M. A. (2016). The role of gp130 receptor cytokines in the regulation of metabolic homeostasis. *J. Exp. Biol.* 219, 259–265. doi:10.1242/jeb.129213

C, Z., H, Y., X, F., W, N., J, F., S, S., et al. (2022). GSK3β palmitoylation mediated by ZDHHC4 promotes tumorigenicity of glioblastoma stem cells in temozolomide-resistant glioblastoma through the EZH2-STAT3 axis. *Oncogenesis*, 11. doi:10.1038/s41389-022-00402-w

Daniel, P., Meehan, B., Sabri, S., Jamali, F., Sarkaria, J. N., Choi, D., et al. (2022). Detection of temozolomide-induced hypermutation and response to PD-1 checkpoint inhibitor in recurrent glioblastoma. *Neurooncol Adv.* 4, vdac076. doi:10.1093/noajnl/vdac076

Darmanis, S., Sloan, S. A., Croote, D., Mignardi, M., Chernikova, S., Samghababi, P., et al. (2017). Single-cell RNA-seq analysis of infiltrating neoplastic cells at the migrating front of human glioblastoma. *Cell Rep.* 21, 1399–1410. doi:10.1016/j.celrep.2017.10.030

Da Ros, M., De Gregorio, V., Iorio, A. L., Giunti, L., Guidi, M., De Martino, M., et al. (2018). Glioblastoma chemoresistance: the double play by microenvironment and blood-brain barrier. *Int. J. Mol. Sci.* 19, 2879. doi:10.3390/ijms19102879

Das, T., Yount, J. S., and Hang, H. C. (2021). Protein S-palmitoylation in immunity. *Open Biol.* 11, 200411. doi:10.1098/rsob.200411

Davda, D., El Azzouny, M. A., Tom, CTMB, Hernandez, J. L., Majmudar, J. D., Kennedy, R. T., et al. (2013). Profiling targets of the irreversible palmitoylation inhibitor 2-bromopalmitate. *ACS Chem. Biol.* 8, 1912–1917. doi:10.1021/cb400380s

Ergun, S. L., Fernandez, D., Weiss, T. M., and Li, L. (2019). STING polymer structure reveals mechanisms for activation, hyperactivation, and inhibition. *Cell* 178, 290–301. doi:10.1016/j.cell.2019.05.036

Erices, J. I., Bizama, C., Niechi, I., Uribe, D., Rosales, A., Fabres, K., et al. (2023). Glioblastoma microenvironment and invasiveness: new insights and therapeutic targets. *Int. J. Mol. Sci.* 24, 7047. doi:10.3390/ijms24087047

Fan, X., Fan, J., Yang, H., Zhao, C., Niu, W., Fang, Z., et al. (2021b). Heterogeneity of subsets in glioblastoma mediated by Smad3 palmitoylation. *Oncogenesis* 10, 72. doi:10.1038/s41389-021-00361-8

Fan, X., Sun, S., Yang, H., Ma, H., Zhao, C., Niu, W., et al. (2022). SETD2 palmitoylation mediated by ZDHHC16 in epidermal growth factor receptor-mutated glioblastoma promotes ionizing radiation-induced DNA damage. *Int. J. Radiat. Oncol. Biol. Phys.* 113, 648–660. doi:10.1016/j.ijrobp.2022.02.018

Fan, X., Yang, H., Zhao, C., Hu, L., Wang, D., Wang, R., et al. (2021a). Local anesthetics impair the growth and self-renewal of glioblastoma stem cells by inhibiting ZDHHC15-mediated GP130 palmitoylation. *Stem Cell Res. Ther.* 12, 107. doi:10.1186/s13287-021-02175-2

Feng, C., Zhang, L., Chang, X., Qin, D., and Zhang, T. (2023). Regulation of post-translational modification of PD-L1 and advances in tumor immunotherapy. *Front. Immunol.* 14, 1230135. doi:10.3389/fimmu.2023.1230135

Fhu, C. W., and Ali, A. (2021). Protein lipidation by palmitoylation and myristoylation in cancer. *Front. Cell Dev. Biol.* 9, 673647. doi:10.3389/fcell.2021.673647

Fredericks, G. J., Hoffmann, F. W., Rose, A. H., Osterheld, H. J., Hess, F. M., Mercier, F., et al. (2014). Stable expression and function of the inositol 1,4,5-triphosphate receptor requires palmitoylation by a DHHC6/selenoprotein K complex. *Proc. Natl. Acad. Sci. U. S. A.* 111, 16478–16483. doi:10.1073/pnas.1417176111

Freeman, G. J., Long, A. J., Iwai, Y., Bourque, K., Chernova, T., Nishimura, H., et al. (2000). Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.* 192, 1027–1034. doi:10.1084/jem.192.7.1027

Ganapathy, V., Thangaraju, M., and Prasad, P. D. (2009). Nutrient transporters in cancer: relevance to Warburg hypothesis and beyond. *Pharmacol. Ther.* 121, 29–40. doi:10.1016/j.pharmthera.2008.09.005

Gorleku, O. A., Barns, A.-M., Prescott, G. R., Greaves, J., and Chamberlain, L. H. (2011). Endoplasmic reticulum localization of DHHC palmitoyltransferases mediated by lysine-based sorting signals. *J. Biol. Chem.* 286, 39573–39584. doi:10.1074/jbc.M111.272369

Gottlieb, C. D., Zhang, S., and Linder, M. E. (2015). The cysteine-rich domain of the DHHC3 palmitoyltransferase is palmitoylated and contains tightly bound zinc. *J. Biol. Chem.* 290, 29259–29269. doi:10.1074/jbc.M115.691147

Greaves, J., and Chamberlain, L. H. (2011). DHHC palmitoyl transferases: substrate interactions and (patho)physiology. *Trends Biochem. Sci.* 36, 245–253. doi:10.1016/j.tibs.2011.01.003

Hagenbeek, T. J., Zbieg, J. R., Hafner, M., Mroue, R., Lacap, J. A., Sodir, N. M., et al. (2023). An allosteric pan-TEAD inhibitor blocks oncogenic YAP/TAZ signaling and overcomes KRAS G12C inhibitor resistance. *Nat. Cancer* 4, 812–828. doi:10.1038/s43018-023-00577-0

Hanahan, D. (2022). Hallmarks of cancer: new dimensions. *Cancer Discov.* 12, 31–46. doi:10.1158/2159-8290.CD-21-1059

He, R., Du, S., Lei, T., Xie, X., and Wang, Y. (2020). Glycogen synthase kinase 3β in tumorigenesis and oncotherapy (Review). *Oncol. Rep.* 44, 2373–2385. doi:10.3892/or.2020.7817

Hg, J., Gi, B., Jd, B., Mj, B., Mj, C., Yt, C., et al. (2002). Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. *Am. J. Physiol. Endocrinol. Metab.*, 282. doi:10.1152/ajpendo.00407.2001

Hornemann, T. (2015). Palmitoylation and depalmitoylation defects. *J. Inherit. Metab. Dis.* 38, 179–186. doi:10.1007/s10545-014-9753-0

Huang, T., Yang, Y., Song, X., Wan, X., Wu, B., Sastry, N., et al. (2021). PRMT6 methylation of RCC1 regulates mitosis, tumorigenicity, and radiation response of glioblastoma stem cells. *Mol. Cell* 81, 1276–1291.e9. doi:10.1016/j.molcel.2021.01.015

Jansen, M., and Beaumelle, B. (2022). How palmitoylation affects trafficking and signaling of membrane receptors. *Biol. Cell* 114, 61–72. doi:10.1111/boc.202100052

Jennings, B. C., and Linder, M. E. (2012). DHHC protein S-acyltransferases use similar ping-pong kinetic mechanisms but display different acyl-CoA specificities. *J. Biol. Chem.* 287, 7236–7245. doi:10.1074/jbc.M111.337246

Jiang, H., Yu, K., Li, M., Cui, Y., Ren, X., Yang, C., et al. (2020). Classification of progression patterns in glioblastoma: analysis of predictive factors and clinical implications. *Front. Oncol.* 10, 590648. doi:10.3389/fonc.2020.590648

Jiang, H., Zhang, X., Chen, X., Aramsangtienchai, P., Tong, Z., and Lin, H. (2018). Protein lipidation: occurrence, mechanisms, biological functions, and enabling technologies. *Chem. Rev.* 118, 919–988. doi:10.1021/acs.chemrev.6b00750

Jin, J., Zhi, X., Wang, X., and Meng, D. (2021). Protein palmitoylation and its pathophysiological relevance. *J. Cell Physiol.* 236, 3220–3233. doi:10.1002/jcp.30122

Johnson, B. E., Mazor, T., Hong, C., Barnes, M., Aihara, K., McLean, C. Y., et al. (2014). Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. *Science* 343, 189–193. doi:10.1126/science.1239947

Kersten, S. (2001). Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Rep.* 2, 282–286. doi:10.1093/embo-reports/kve071

Kim, E., Kim, M., Woo, D.-H., Shin, Y., Shin, J., Chang, N., et al. (2013). Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. *Cancer Cell* 23, 839–852. doi:10.1016/j.ccr.2013.04.008

Kinney, N., Varghese, R. T., Anandakrishnan, R., and Garner, H. R. S. (2017). ZDHHC3 as a risk and mortality marker for breast cancer in african American women. *Cancer Inf.* 16, 1176935117746644. doi:10.1177/1176935117746644

Kmiec, D., Lista, M. J., Ficarelli, M., Swanson, C. M., and Neil, S. J. D. (2021). S-farnesylation is essential for antiviral activity of the long ZAP isoform against RNA viruses with diverse replication strategies. *PLoS Pathog.* 17, e1009726. doi:10.1371/journal.ppat.1009726

Ko, P.-J., and Dixon, S. J. (2018). Protein palmitoylation and cancer. *EMBO Rep.* 19, e46666. doi:10.15252/embr.201846666

Komaki, S., Sugita, Y., Furuta, T., Yamada, K., Moritsubo, M., Abe, H., et al. (2019). Expression of GLUT1 in pseudopalisaded and perivascular tumor cells is an independent prognostic factor for patients with glioblastomas. *J. Neuropathol. Exp. Neurol.* 78, 389–397. doi:10.1093/jnen/nly124

Lah, T. T., Novak, M., and Breznik, B. (2020). Brain malignancies: glioblastoma and brain metastases. *Semin. Cancer Biol.* 60, 262–273. doi:10.1016/j.semcancer.2019.10.010

Lan, T., Delalande, C., and Dickinson, B. C. (2021). Inhibitors of DHHC family proteins. *Curr. Opin. Chem. Biol.* 65, 118–125. doi:10.1016/j.cbpa.2021.07.002

Lanyon-Hogg, T., Faronato, M., Serwa, R. A., and Tate, E. W. (2017). Dynamic protein acylation: new substrates, mechanisms, and drug targets. *Trends Biochem. Sci.* 42, 566–581. doi:10.1016/j.tibs.2017.04.004

Lee, C.-J., Stix, R., Rana, M. S., Shikwana, F., Murphy, R. E., Ghirlando, R., et al. (2022). Bivalent recognition of fatty acyl-CoA by a human integral membrane palmitoyltransferase. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2022050119. doi:10.1073/pnas.2022050119

Lee, J.-W., Hur, J., Kwon, Y.-W., Chae, C.-W., Choi, J.-I., Hwang, I., et al. (2021). KAI1(CD82) is a key molecule to control angiogenesis and switch angiogenic milieu to quiescent state. *J. Hematol. OncolJ Hematol. Oncol. (2021)* 14, 148. doi:10.1186/s13045-021-01147-6

Lemonidis, K., Gorleku, O. A., Sanchez-Perez, M. C., Grefen, C., and Chamberlain, L. H. (2014). The Golgi S-acylation machinery comprises zDHHC enzymes with major differences in substrate affinity and S-acylation activity. *Mol. Biol. Cell* 25, 3870–3883. doi:10.1091/mbc.E14-06-1169

Lemonidis, K., Salaun, C., Kouskou, M., Diez-Ardanuy, C., Chamberlain, L. H., and Greaves, J. (2017). Substrate selectivity in the zDHHC family of S-acyltransferases. *Biochem. Soc. Trans.* 45, 751–758. doi:10.1042/BST20160309

Lemonidis, K., Sanchez-Perez, M. C., and Chamberlain, L. H. (2015). Identification of a novel sequence motif recognized by the ankyrin repeat domain of zDHHC17/13 S-acyltransferases. *J. Biol. Chem.* 290, 21939–21950. doi:10.1074/jbc.M115.657668

Leske, H., Camenisch Gross, U., Hofer, S., Neidert, M. C., Leske, S., Weller, M., et al. (2023). MGMT methylation pattern of long-term and short-term survivors of glioblastoma reveals CpGs of the enhancer region to be of high prognostic value. *Acta Neuropathol. Commun.* 11, 139. doi:10.1186/s40478-023-01622-w

Levental, I., and Lyman, E. (2023). Regulation of membrane protein structure and function by their lipid nano-environment. *Nat. Rev. Mol. Cell Biol.* 24, 107–122. doi:10.1038/s41580-022-00524-4

Li, H., Song, C., Zhang, Y., Liu, G., Mi, H., Li, Y., et al. (2024). Transgelin promotes glioblastoma stem cell hypoxic responses and maintenance through p53 acetylation. *Adv. Sci. Weinh Baden-Wurtt Ger.* 11, e2305620. doi:10.1002/advs.202305620

Li, X., Shen, L., Xu, Z., Liu, W., Li, A., and Xu, J. (2022). Protein palmitoylation modification during viral infection and detection methods of palmitoylated proteins. *Front. Cell Infect. Microbiol.* 12, 821596. doi:10.3389/fcimb.2022.821596

Li, Y., Gao, Z., Wang, Y., Pang, B., Zhang, B., Hu, R., et al. (2023). Lysine methylation promotes NFAT5 activation and determines temozolomide efficacy in glioblastoma. *Nat. Commun.* 14, 4062. doi:10.1038/s41467-023-39845-z

Liao, D., Huang, Y., Liu, D., Zhang, H., Shi, X., Li, X., et al. (2024). The role of s-palmitoylation in neurological diseases: implication for zDHHC family. *Front. Pharmacol.* 14, 1342830. doi:10.3389/fphar.2023.1342830

Li, J., Tong, L., Yi, L., Liu, P., Yang, X., Wang, X., et al. (2019). Experimental study on ACT001 inhibiting the spheroidization ability and stemness maintenance of U87-MG glioma stem cells through the STAT3 signaling pathway. *Chin. J. Neurosurg.* 35, 1160–1166. doi:10.3760/cma.j.issn.1001-2346.2019.11.019

Lim, M., Xia, Y., Bettegowda, C., and Weller, M. (2018). Current state of immunotherapy for glioblastoma. *Nat. Rev. Clin. Oncol.* 15, 422–442. doi:10.1038/s41571-018-0003-5

Lin, D. T. S., Davis, N. G., and Conibear, E. (2017). Targeting the Ras palmitoylation/depalmitoylation cycle in cancer. *Biochem. Soc. Trans.* 45, 913–921. doi:10.1042/BST20160303

Lin, H., Su, X., and He, B. (2012). Protein lysine acylation and cysteine succination by intermediates of energy metabolism. *ACS Chem. Biol.* 7, 947–960. doi:10.1021/cb3001793

Linder, M. E., and Deschenes, R. J. (2007). Palmitoylation: policing protein stability and traffic. *Nat. Rev. Mol. Cell Biol.* 8, 74–84. doi:10.1038/nrm2084

Linder, M. E., and Jennings, B. C. (2013). Mechanism and function of DHHC S-acyltransferases. *Biochem. Soc. Trans.* 41, 29–34. doi:10.1042/BST20120328

Liu, R., Lee, J.-H., Li, J., Yu, R., Tan, L., Xia, Y., et al. (2021). Choline kinase alpha 2 acts as a protein kinase to promote lipolysis of lipid droplets. *Mol. Cell* 81, 2722–2735.e9. doi:10.1016/j.molcel.2021.05.005

Liu, X., Guo, C., Leng, T., Fan, Z., Mai, J., Chen, J., et al. (2023a). Differential regulation of H3K9/H3K14 acetylation by small molecules drives neuron-fate-induction of glioma cell. *Cell Death Dis.* 14, 142. doi:10.1038/s41419-023-05611-8

Liu, Z., Liu, C., Xiao, M., Han, Y., Zhang, S., and Xu, B. (2020). Bioinformatics analysis of the prognostic and biological significance of ZDHHC-protein acyltransferases in kidney renal clear cell carcinoma. *Front. Oncol.* 10, 565414. doi:10.3389/fonc.2020.565414

Liu, Z.-Y., Lan, T., Tang, F., He, Y.-Z., Liu, J.-S., Yang, J.-Z., et al. (2023b). ZDHHC15 promotes glioma malignancy and acts as a novel prognostic biomarker for patients with glioma. *BMC Cancer* 23, 420. doi:10.1186/s12885-023-10883-6

Lothrop, A. P., Torres, M. P., and Fuchs, S. M. (2013). Deciphering post-translational modification codes. *FEBS Lett.* 587, 1247–1257. doi:10.1016/j.febslet.2013.01.047

Louis, D. N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., et al. (2016). The 2016 World health organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol. Berl.* 131, 803–820. doi:10.1007/s00401-016-1545-1

Louis, D. N., Perry, A., Wesseling, P., Brat, D. J., Cree, I. A., Figarella-Branger, D., et al. (2021). The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro-Oncol* 23, 1231–1251. doi:10.1093/neuonc/noab106

Lu, F., Shen, S.-H., Wu, S., Zheng, P., Lin, K., Liao, J., et al. (2022). Hypomethylation-induced prognostic marker zinc finger DHHC-type palmitoyltransferase 12 contributes to glioblastoma progression. *Ann. Transl. Med.* 10, 334. doi:10.21037/atm-22-520

Macheda, M. L., Rogers, S., and Best, J. D. (2005). Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J. Cell Physiol.* 202, 654–662. doi:10.1002/jcp.20166

Malgapo, M. I. P., and Linder, M. E. (2021). Substrate recruitment by zDHHC protein acyltransferases. *Open Biol.* 11, 210026. doi:10.1098/rsob.210026

Martin, B. R., Wang, C., Adibekian, A., Tully, S. E., and Cravatt, B. F. (2011). Global profiling of dynamic protein palmitoylation. *Nat. Methods* 9, 84–89. doi:10.1038/nmeth.1769

Mesquita, F. S., Abrami, L., Sergeeva, O., Turelli, P., Qing, E., Kunz, B., et al. (2021). S-acylation controls SARS-CoV-2 membrane lipid organization and enhances infectivity. *Dev. Cell* 56, 2790–2807.e8. doi:10.1016/j.devcel.2021.09.016

Mg, V. H., Lc, C., and Cb, T. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*, 324. doi:10.1126/science.1160809

Mitchell, D. A., Vasudevan, A., Linder, M. E., and Deschenes, R. J. (2006). Protein palmitoylation by a family of DHHC protein S-acyltransferases. *J. Lipid Res.* 47, 1118–1127. doi:10.1194/jlr.R600007-JLR200

Mizoguchi, M., Betensky, R. A., Batchelor, T. T., Bernay, D. C., Louis, D. N., and Nutt, C. L. (2006). Activation of STAT3, MAPK, and AKT in malignant astrocytic gliomas: correlation with EGFR status, tumor grade, and survival. *J. Neuropathol. Exp. Neurol.* 65, 1181–1188. doi:10.1097/01.jnen.0000248549.14962.b2

Morphy, R. (2010). Selectively nonselective kinase inhibition: striking the right balance. *J. Med. Chem.* 53, 1413–1437. doi:10.1021/jm901132v

Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H. R., et al. (1985). Sequence and structure of a human glucose transporter. *Science* 229, 941–945. doi:10.1126/science.3839598

Nakada, M., Kita, D., Watanabe, T., Hayashi, Y., Teng, L., Pyko, I. V., et al. (2011). Aberrant signaling pathways in glioma. *Cancers* 3 (3), 3242–3278. doi:10.3390/cancers3033242

Nogueira-Silva, C., Piairo, P., Carvalho-Dias, E., Veiga, C., Moura, R. S., and Correia-Pinto, J. (2013). The role of glycoprotein 130 family of cytokines in fetal rat lung development. *PloS One* 8, e67607. doi:10.1371/journal.pone.0067607

Ocasio, C. A., Baggelaar, M. P., Sipthorp, J., Losada de la Lastra, A., Tavares, M., Volarić, J., et al. (2024). A palmitoyl transferase chemical-genetic system to map ZDHHC-specific S-acylation. *Nat. Biotechnol.* 42, 1548–1558. doi:10.1038/s41587-023-02030-0

Ohno, Y., Kihara, A., Sano, T., and Igarashi, Y. (2006). Intracellular localization and tissue-specific distribution of human and yeast DHHC cysteine-rich domain-containing proteins. *Biochim. Biophys. Acta* 1761, 474–483. doi:10.1016/j.bbalip.2006.03.010

Ostrom, Q. T., Price, M., Neff, C., Cioffi, G., Waite, K. A., Kruchko, C., et al. (2022). CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2015-2019. *Neuro-Oncol* 24, v1–v95. doi:10.1093/neuonc/noac202

Pan, S., and Chen, R. (2022). Pathological implication of protein post-translational modifications in cancer. *Mol. Asp. Med.* 86, 101097. doi:10.1016/j.mam.2022.101097

Pastushenko, I., and Blanpain, C. (2019). EMT transition states during tumor progression and metastasis. *Trends Cell Biol.* 29, 212–226. doi:10.1016/j.tcb.2018.12.001

Pavlova, N. N., Zhu, J., and Thompson, C. B. (2022). The hallmarks of cancer metabolism: still emerging. *Cell Metab.* 34, 355–377. doi:10.1016/j.cmet.2022.01.007

Pei, X., Li, K.-Y., Shen, Y., Li, J.-T., Lei, M.-Z., Fang, C.-Y., et al. (2022). Palmitoylation of MDH2 by ZDHHC18 activates mitochondrial respiration and accelerates ovarian cancer growth. *Sci. China Life Sci.* 65, 2017–2030. doi:10.1007/s11427-021-2048-2

Pei, Z., Xiao, Y., Meng, J., Hudmon, A., and Cummins, T. R. (2016). Cardiac sodium channel palmitoylation regulates channel availability and myocyte excitability with implications for arrhythmia generation. *Nat. Commun.* 7, 12035. doi:10.1038/ncomms12035

Plain, F., Howie, J., Kennedy, J., Brown, E., Shattock, M. J., Fraser, N. J., et al. (2020). Control of protein palmitoylation by regulating substrate recruitment to a zDHHC-protein acyltransferase. *Commun. Biol.* 3, 411. doi:10.1038/s42003-020-01145-3

Puthenveetil, R., Auger, S. A., Gómez-Navarro, N., Rana, M. S., Das, R., Healy, L. B., et al. (2023). Orthogonal enzyme-substrate design strategy for discovery of human protein palmitoyltransferase substrates. *J. Am. Chem. Soc.* 145, 22287–22292. doi:10.1021/jacs.3c04359

Qu, M., Zhou, X., Wang, X., and Li, H. (2021). Lipid-induced S-palmitoylation as a vital regulator of cell signaling and disease development. *Int. J. Biol. Sci.* 17, 4223–4237. doi:10.7150/ijbs.64046

Quail, D. F., and Joyce, J. A. (2017). The microenvironmental landscape of brain tumors. *Cancer Cell* 31, 326–341. doi:10.1016/j.ccell.2017.02.009

Ramos, A. K. S., Caldas-Rosa, E. C. C., Ferreira, B. M., Versiani, B. R., Moretti, P. N., de Oliveira, S. F., et al. (2023). ZDHHC9 X-linked intellectual disability: clinical and molecular characterization. *Am. J. Med. Genet. A* 191, 599–604. doi:10.1002/ajmg.a.63052

Rana, M. S., Kumar, P., Lee, C.-J., Verardi, R., Rajashankar, K. R., and Banerjee, A. (2018). Fatty acyl recognition and transfer by an integral membrane S-acyltransferase. *Science* 359, eaao6326. doi:10.1126/science.aao6326

Rana, M. S., Lee, C.-J., and Banerjee, A. (2019). The molecular mechanism of DHHC protein acyltransferases. *Biochem. Soc. Trans.* 47, 157–167. doi:10.1042/BST20180429

Remsberg, J. R., Suciu, R. M., Zambetti, N. A., Hanigan, T. W., Firestone, A. J., Inguva, A., et al. (2021). ABHD17 regulation of plasma membrane palmitoylation and N-Ras-dependent cancer growth. *Nat. Chem. Biol.* 17, 856–864. doi:10.1038/s41589-021-00785-8

Resh, M. D. (2006a). Palmitoylation of ligands, receptors, and intracellular signaling molecules. *Sci. STKE Signal Transduct. Knowl. Environ.* 2006, re14. doi:10.1126/stke.3592006re14

Resh, M. D. (2006b). Trafficking and signaling by fatty-acylated and prenylated proteins. *Nat. Chem. Biol.* 2, 584–590. doi:10.1038/nchembio834

Resh, M. D. (2013). Covalent lipid modifications of proteins. *Curr. Biol. CB* 23, R431–R435. doi:10.1016/j.cub.2013.04.024

Robert, C., and Vagner, S. (2018). Boosting immunity by targeting post-translational prenylation of small GTPases. *Cell* 175, 901–902. doi:10.1016/j.cell.2018.10.032

Rocks, O., Peyker, A., Kahms, M., Verveer, P. J., Koerner, C., Lumbierres, M., et al. (2005). An acylation cycle regulates localization and activity of palmitoylated Ras isoforms. *Science* 307, 1746–1752. doi:10.1126/science.1105654

Rodenburg, R. N. P., Snijder, J., van de Waterbeemd, M., Schouten, A., Granneman, J., Heck, A. J. R., et al. (2017). Stochastic palmitoylation of accessible cysteines in membrane proteins revealed by native mass spectrometry. *Nat. Commun.* 8, 1280. doi:10.1038/s41467-017-01461-z

Roth, A. F., Feng, Y., Chen, L., and Davis, N. G. (2002). The yeast DHHC cysteine-rich domain protein Akr1p is a palmitoyl transferase. *J. Cell Biol.* 159, 23–28. doi:10.1083/jcb.200206120

Runkle, K. B., Kharbanda, A., Stypulkowski, E., Cao, X.-J., Wang, W., Garcia, B. A., et al. (2016). Inhibition of DHHC20-mediated EGFR palmitoylation creates a dependence on EGFR signaling. *Mol. Cell* 62, 385–396. doi:10.1016/j.molcel.2016.04.003

Salaun, C., Locatelli, C., Zmuda, F., Cabrera González, J., and Chamberlain, L. H. (2020). Accessory proteins of the zDHHC family of S-acylation enzymes. *J. Cell Sci.* 133, jcs251819. doi:10.1242/jcs.251819

Salaun, C., Takizawa, H., Galindo, A., Munro, K. R., McLellan, J., Sugimoto, I., et al. (2022). Development of a novel high-throughput screen for the identification of new inhibitors of protein S-acylation. *J. Biol. Chem.* 298, 102469. doi:10.1016/j.jbc.2022.102469

Schmidt, M. F., Bracha, M., and Schlesinger, M. J. (1979). Evidence for covalent attachment of fatty acids to Sindbis virus glycoproteins. *Proc. Natl. Acad. Sci. U. S. A.* 76, 1687–1691. doi:10.1073/pnas.76.4.1687

Shahinian, S., and Silvius, J. R. (1995). Doubly-lipid-modified protein sequence motifs exhibit long-lived anchorage to lipid bilayer membranes. *Biochemistry* 34, 3813–3822. doi:10.1021/bi00011a039

Sharifzad, F., Ghavami, S., Verdi, J., Mardpour, S., Mollapour Sisakht, M., Azizi, Z., et al. (2019). Glioblastoma cancer stem cell biology: potential theranostic targets. *Drug Resist Updat Rev. Comment Antimicrob. Anticancer Chemother.* 42, 35–45. doi:10.1016/j.drup.2018.03.003

Shergalis, A., Bankhead, A., Luesakul, U., Muangsin, N., and Neamati, N. (2018). Current challenges and opportunities in treating glioblastoma. *Pharmacol. Rev.* 70, 412–445. doi:10.1124/pr.117.014944

Shi, Y., Zhou, W., Cheng, L., Chen, C., Huang, Z., Fang, X., et al. (2017). Tetraspanin CD9 stabilizes gp130 by preventing its ubiquitin-dependent lysosomal degradation to promote STAT3 activation in glioma stem cells. *Cell Death Differ.* 24, 167–180. doi:10.1038/cdd.2016.110

Smotrys, J. E., and Linder, M. E. (2004). Palmitoylation of intracellular signaling proteins: regulation and function. *Annu. Rev. Biochem.* 73, 559–587. doi:10.1146/annurev.biochem.73.011303.073954

Snowden, A. W., and Perkins, N. D. (1998). Cell cycle regulation of the transcriptional coactivators p300 and CREB binding protein. *Biochem. Pharmacol.* 55, 1947–1954. doi:10.1016/S0006-2952(98)00020-3

Sobocińska, J., Roszczenko-Jasińska, P., Ciesielska, A., and Kwiatkowska, K. (2018). Protein palmitoylation and its role in bacterial and viral infections. *Front. Immunol.* 8, 2003. doi:10.3389/fimmu.2017.02003

Song, Z., Xiaoli, A. M., and Yang, F. (2018). Regulation and metabolic significance of *de novo* lipogenesis in adipose tissues. *Nutrients* 10, 1383. doi:10.3390/nu10101383

Stix, R., Lee, C.-J., Faraldo-Gómez, J. D., and Banerjee, A. (2020). Structure and mechanism of DHHC protein acyltransferases. *J. Mol. Biol.* 432, 4983–4998. doi:10.1016/j.jmb.2020.05.023

Stommel, J. M., Kimmelman, A. C., Ying, H., Nabioullin, R., Ponugoti, A. H., Wiedemeyer, R., et al. (2007). Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science* 318, 287–290. doi:10.1126/science.1142946

Sun, Y., Zhang, H., Meng, J., Guo, F., Ren, D., Wu, H., et al. (2022). S-palmitoylation of PCSK9 induces sorafenib resistance in liver cancer by activating the PI3K/AKT pathway. *Cell Rep.* 40, 111194. doi:10.1016/j.celrep.2022.111194

Sun, Y., Zhu, L., Liu, P., Zhang, H., Guo, F., and Jin, X. (2023). ZDHHC2-Mediated AGK palmitoylation activates AKT-mTOR signaling to reduce sunitinib sensitivity in renal cell carcinoma. *Cancer Res.* 83, 2034–2051. doi:10.1158/0008-5472.CAN-22-3105

Swarthout, J. T., Lobo, S., Farh, L., Croke, M. R., Greentree, W. K., Deschenes, R. J., et al. (2005). DHHC9 and GCP16 constitute a human protein fatty acyltransferase with specificity for H- and N-Ras. *J. Biol. Chem.* 280, 31141–31148. doi:10.1074/jbc.M504113200

Tabaczar, S., Czogalla, A., Podkalicka, J., Biernatowska, A., and Sikorski, A. F. (2017). Protein palmitoylation: palmitoyltransferases and their specificity. *Exp. Biol. Med. Maywood N. J.* 242, 1150–1157. doi:10.1177/1535370217707732

Tang, F., Liu, Z., Chen, X., Yang, J., Wang, Z., and Li, Z. (2022a). Current knowledge of protein palmitoylation in gliomas. *Mol. Biol. Rep.* 49, 10949–10959. doi:10.1007/s11033-022-07809-z

Tang, F., Yang, C., Li, F.-P., Yu, D.-H., Pan, Z.-Y., Wang, Z.-F., et al. (2022c). Palmitoyl transferases act as potential regulators of tumor-infiltrating immune cells and

glioma progression. *Mol. Ther. Nucleic Acids* 28, 716–731. doi:10.1016/j.omtn.2022.04.030

Tang, W., Xu, N., Zhou, J., He, Z., Lenahan, C., Wang, C., et al. (2022b). ALKBH5 promotes PD-L1-mediated immune escape through m6A modification of ZDHHC3 in glioma. *Cell Death Discov.* 8, 497. doi:10.1038/s41420-022-01286-w

Tate, J. G., Bamford, S., Jubb, H. C., Sondka, Z., Beare, D. M., Bindal, N., et al. (2019). COSMIC: the catalogue of somatic mutations in cancer. *Nucleic Acids Res.* 47, D941-D947–D947. doi:10.1093/nar/gky1015

T, D., Iv, P., T, F., K, M., M, U., T, S., et al. (2016). Glycogen synthase kinase-3β is a pivotal mediator of cancer invasion and resistance to therapy. *Cancer Sci.*, 107. doi:10.1111/cas.13028

Thiery, J. P., Acloque, H., Huang, R. Y. J., and Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell* 139, 871–890. doi:10.1016/j.cell.2009.11.007

Tian, H., Lu, J.-Y., Shao, C., Huffman, K. E., Carstens, R. M., Larsen, J. E., et al. (2015). Systematic siRNA screen unmasks NSCLC growth dependence by palmitoyltransferase DHHC5. *Mol. Cancer Res. MCR* 13, 784–794. doi:10.1158/1541-7786.MCR-14-0608

Tomić, G., Sheridan, C., Refermat, A. Y., Baggelaar, M. P., Sipthorp, J., Sudarshan, B., et al. (2024). Palmitoyl transferase ZDHHC20 promotes pancreatic cancer metastasis. *Cell Rep.* 43, 114224. doi:10.1016/j.celrep.2024.114224

Uldry, M., and Thorens, B. (2004). The SLC2 family of facilitated hexose and polyol transporters. *Pflüg Arch.* 447, 480–489. doi:10.1007/s00424-003-1085-0

van Bergen, L. A. H., Alonso, M., Palló, A., Nilsson, L., De Proft, F., and Messens, J. (2016). Revisiting sulfur H-bonds in proteins: the example of peroxiredoxin AhpE. *Sci. Rep.* 6, 30369. doi:10.1038/srep30369

Verardi, R., Kim, J.-S., Ghirlando, R., and Banerjee, A. (2017). Structural basis for substrate recognition by the ankyrin repeat domain of human DHHC17 palmitoyltransferase. *Struct. Lond Engl.* 25, 1337–1347. doi:10.1016/j.str.2017.06.018

Verdugo, E., Puerto, I., and Medina, M. Á. (2022). An update on the molecular biology of glioblastoma, with clinical implications and progress in its treatment. *Cancer Commun. Lond Engl.* 42, 1083–1111. doi:10.1002/cac2.12361

Wagener, E.-M., Aurich, M., Aparicio-Siegmund, S., Floss, D. M., Garbers, C., Breusing, K., et al. (2014). The amino acid exchange R28E in ciliary neurotrophic factor (CNTF) abrogates interleukin-6 receptor-dependent but retains CNTF receptor-dependent signaling via glycoprotein 130 (gp130)/Leukemia inhibitory factor receptor (LIFR). *J. Biol. Chem.* 289, 18442–18450. doi:10.1074/jbc.M114.568857

Wang, G. G., Allis, C. D., and Chi, P. (2007). Chromatin remodeling and cancer, part I: covalent histone modifications. *Trends Mol. Med.* 13, 363–372. doi:10.1016/j.molmed.2007.07.003

Wang, L.-B., Karpova, A., Gritsenko, M. A., Kyle, J. E., Cao, S., Li, Y., et al. (2021). Proteogenomic and metabolomic characterization of human glioblastoma. *Cancer Cell* 39, 509–528.e20. doi:10.1016/j.ccell.2021.01.006

Wang, X., Zhang, C., and Bao, N. (2023). Molecular mechanism of palmitic acid and its derivatives in tumor progression. *Front. Oncol.* 13, 1224125. doi:10.3389/fonc.2023.1224125

Wang, Y., Lu, H., Fang, C., and Xu, J. (2020). Palmitoylation as a signal for delivery. *Adv. Exp. Med. Biol.* 1248, 399–424. doi:10.1007/978-981-15-3266-5_16

Wang, Y., Shen, N., Yang, Y., Xia, Y., Zhang, W., Lu, Y., et al. (2024). ZDHHC5-mediated S-palmitoylation of FAK promotes its membrane localization and epithelial-mesenchymal transition in glioma. *Cell Commun. Signal CCS* 22, 46. doi:10.1186/s12964-023-01366-z

White, K., Connor, K., Clerkin, J., Murphy, B. M., Salvucci, M., O'Farrell, A. C., et al. (2020). New hints towards a precision medicine strategy for IDH wild-type glioblastoma. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* 31, 1679–1692. doi:10.1016/j.annonc.2020.08.2336

Won, S. J., Davda, D., Labby, K. J., Hwang, S. Y., Pricer, R., Majmudar, J. D., et al. (2016). Molecular mechanism for isoform-selective inhibition of acyl protein thioesterases 1 and 2 (APT1 and APT2). *ACS Chem. Biol.* 11, 3374–3382. doi:10.1021/acschembio.6b00720

Won, S. J., and Martin, B. R. (2018). Temporal profiling establishes a dynamic S-palmitoylation cycle. *ACS Chem. Biol.* 13, 1560–1568. doi:10.1021/acschembio.8b00157

Xiao, D., Yan, C., Li, D., Xi, T., Liu, X., Zhu, D., et al. (2023). National Brain Tumour Registry of China (NBTRC) statistical report of primary brain tumours diagnosed in China in years 2019-2020. *Lancet Reg. Health West Pac* 34, 100715. doi:10.1016/j.lanwpc.2023.100715

Xu, J., Li, L., Shi, P., Cui, H., and Yang, L. (2022). The crucial roles of bmi-1 in cancer: implications in pathogenesis, metastasis, drug resistance, and targeted therapies. *Int. J. Mol. Sci.* 23, 8231. doi:10.3390/ijms23158231

Yan, S.-M., Tang, J.-J., Huang, C.-Y., Xi, S.-Y., Huang, M.-Y., Liang, J.-Z., et al. (2013). Reduced expression of ZDHHC2 is associated with lymph node metastasis and poor prognosis in gastric adenocarcinoma. *PloS One* 8, e56366. doi:10.1371/journal.pone.0056366

Yang, H., Jin, L., and Sun, X. (2019b). A thirteen-gene set efficiently predicts the prognosis of glioblastoma. *Mol. Med. Rep.* 19, 1613–1621. doi:10.3892/mmr.2019.9801

Yang, Y., Hsu, J.-M., Sun, L., Chan, L.-C., Li, C.-W., Hsu, J. L., et al. (2019a). Palmitoylation stabilizes PD-L1 to promote breast tumor growth. *Cell Res.* 29, 83–86. doi:10.1038/s41422-018-0124-5

Yao, H., Lan, J., Li, C., Shi, H., Brosseau, J.-P., Wang, H., et al. (2019). Inhibiting PD-L1 palmitoylation enhances T-cell immune responses against tumours. *Nat. Biomed. Eng.* 3, 306–317. doi:10.1038/s41551-019-0375-6

Yao, H., Li, C., He, F., Song, T., Brosseau, J.-P., Wang, H., et al. (2021). A peptidic inhibitor for PD-1 palmitoylation targets its expression and functions. *RSC Chem. Biol.* 2, 192–205. doi:10.1039/d0cb00157k

Young, F. B., Butland, S. L., Sanders, S. S., Sutton, L. M., and Hayden, M. R. (2012). Putting proteins in their place: palmitoylation in Huntington disease and other neuropsychiatric diseases. *Prog. Neurobiol.* 97, 220–238. doi:10.1016/j.pneurobio.2011.11.002

Yuan, M., Chen, X., Sun, Y., Jiang, L., Xia, Z., Ye, K., et al. (2020b). ZDHHC12-mediated claudin-3 S-palmitoylation determines ovarian cancer progression. *Acta Pharm. Sin. B* 10, 1426–1439. doi:10.1016/j.apsb.2020.03.008

Yuan, M., Song, Z.-H., Ying, M.-D., Zhu, H., He, Q.-J., Yang, B., et al. (2020a). N-myristoylation: from cell biology to translational medicine. *Acta Pharmacol. Sin.* 41, 1005–1015. doi:10.1038/s41401-020-0388-4

Yuan, Y., Li, P., Li, J., Zhao, Q., Chang, Y., and He, X. (2024). Protein lipidation in health and disease: molecular basis, physiological function and pathological implication. *Signal Transduct. Target Ther.* 9, 60. doi:10.1038/s41392-024-01759-7

Zhang, J., Huang, Y., Chen, J., Zhu, H., and Whiteheart, S. W. (2018). Dynamic cycling of t-SNARE acylation regulates platelet exocytosis. *J. Biol. Chem.* 293, 3593–3606. doi:10.1074/jbc.RA117.000140

Zhang, M., Zhou, L., Xu, Y., Yang, M., Xu, Y., Komaniecki, G. P., et al. (2020). A STAT3 palmitoylation cycle promotes TH17 differentiation and colitis. *Nature* 586, 434–439. doi:10.1038/s41586-020-2799-2

Zhang, M. M., and Hang, H. C. (2017). Protein S-palmitoylation in cellular differentiation. *Biochem. Soc. Trans.* 45, 275–285. doi:10.1042/BST20160236

Zhang, Y., Qin, Z., Sun, W., Chu, F., and Zhou, F. (2021a). Function of protein S-palmitoylation in immunity and immune-related diseases. *Front. Immunol.* 12, 661202. doi:10.3389/fimmu.2021.661202

Zhang, Z., Li, X., Yang, F., Chen, C., Liu, P., Ren, Y., et al. (2021b). DHHC9-mediated GLUT1 S-palmitoylation promotes glioblastoma glycolysis and tumorigenesis. *Nat. Commun.* 12, 5872. doi:10.1038/s41467-021-26180-4

Zhong, B., Yu, J., Hou, Y., Ai, N., Ge, W., Lu, J. J., et al. (2021). A novel strategy for glioblastoma treatment by induction of noptosis, an NQO1-dependent necrosis. *Free Radic. Biol. Med.* 166, 104–115. doi:10.1016/j.freeradbiomed.2021.02.014

Zhou, B., Hao, Q., Liang, Y., and Kong, E. (2022). Protein palmitoylation in cancer: molecular functions and therapeutic potential. *Mol. Oncol.* 17, 3–26. doi:10.1002/1878-0261.13308

Zmuda, F., and Chamberlain, L. H. (2020). Regulatory effects of post-translational modifications on zDHHC S-acyltransferases. *J. Biol. Chem.* 295, 14640–14652. doi:10.1074/jbc.REV120.014717

Zou, C., Ellis, B. M., Smith, R. M., Chen, B. B., Zhao, Y., and Mallampalli, R. K. (2011). Acyl-CoA:Lysophosphatidylcholine acyltransferase I (Lpcat1) catalyzes histone protein O-palmitoylation to regulate mRNA synthesis. *J. Biol. Chem.* 286, 28019–28025. doi:10.1074/jbc.M111.253385

Frontiers in
Cell and Developmental Biology

Explores the fundamental biological processes of life, covering intracellular and extracellular dynamics.

The world's most cited developmental biology journal, advancing our understanding of the fundamental processes of life. It explores a wide spectrum of cell and developmental biology, covering intracellular and extracellular dynamics.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact