frontiers | Research Topics

New anti-cancer strategies targeting epigenetic modifications and associated metabolism reprogramming

Edited by
Lixiang Xue, Zhengquan Yu, Ke-Wu Zeng, Fei Chen and Na Li

Published in
Frontiers in Pharmacology
Frontiers in Oncology

ISSN 1664-8714
ISBN 978-2-8325-2895-2
DOI 10.3389/978-2-8325-2895-2

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

New anti-cancer strategies targeting epigenetic modifications and associated metabolism reprogramming

Topic editors

Lixiang Xue — Peking University Third Hospital, China
Zhengquan Yu — China Agricultural University, China
Ke-Wu Zeng — Peking University, China
Fei Chen — Stony Brook University, United States
Na Li — University of California, San Diego, United States

Citation

Xue, L., Yu, Z., Zeng, K.-W., Chen, F., Li, N., eds. (2023). *New anti-cancer strategies targeting epigenetic modifications and associated metabolism reprogramming*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2895-2

Table of contents

frontiers in Pharmacology

REVIEW
published: 30 March 2022
doi: 10.3389/fphar.2022.855488

Noncoding RNAs in the Glycolysis of Ovarian Cancer

*Chunmei Zhang and Ning Liu**

Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University, Shenyang, China

Energy metabolism reprogramming is the characteristic feature of tumors. The tumorigenesis, metastasis, and drug resistance of ovarian cancer (OC) is dependent on energy metabolism. Even under adequate oxygen conditions, OC cells tend to convert glucose to lactate, and glycolysis can rapidly produce ATP to meet their metabolic energy needs. Non-coding RNAs (ncRNAs) interact directly with DNA, RNA, and proteins to function as an essential regulatory in gene expression and tumor pathology. Studies have shown that ncRNAs regulate the process of glycolysis by interacting with the predominant glycolysis enzyme and cellular signaling pathway, participating in tumorigenesis and progression. This review summarizes the mechanism of ncRNAs regulation in glycolysis in OC and investigates potential therapeutic targets.

Keywords: circular RNAs, long non-coding RNAs, microRNAs, glycolysis, ovarian cancer

OPEN ACCESS

Edited by:
Na Li,
University of California, San Diego, United States

Reviewed by:
Patricia Zancan,
Federal University of Rio de Janeiro, Brazil
Shiv Verma,
Case Western Reserve University, United States

****Correspondence:***
Ning Liu
ningliu@cmu.edu.cn

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *15 January 2022*
Accepted: *15 March 2022*
Published: *30 March 2022*

Citation:
Zhang C and Liu N (2022) Noncoding RNAs in the Glycolysis of Ovarian Cancer. Front. Pharmacol. 13:855488. doi: 10.3389/fphar.2022.855488

1 INTRODUCTION

Ovarian cancer (OC) is currently the most deadly gynecologic malignancy with insidious and rapidly progressive onset. Most patients have advanced pelvic and abdominal metastases by the time of diagnosis, and the 5-years survival rate is only 20–30% worldwide (Vafadar et al., 2020; DiSilvestro et al., 2021; Vergote et al., 2021). OC account for 5% of all cancer deaths in women (Yang et al., 2021; Youssef et al., 2021) due to the low survival rates resulting from late diagnosis. The standard treatment for OC is tumor resection combined with platinum-based chemotherapy. However, the majority with advanced disease will replase or even develop drug resistance, leading to curative failure and ultimately mortality (Giudice et al., 2021; Xie H et al., 2021; Xu et al., 2021). Therefore, it is essential to investigate new treatment options to improve the outcome of OC.

Tumorigenesis is considered an energy metabolic disease. Compared with metabolism of healthy and neoplastic cells, researchers found the oxidative phosphorylation pathway is dominant to provide ATP in normal cells, while the glycolytic pathway is the primary energy supply in tumor cells (Nakagawa et al., 2020; Tyagi et al., 2021). Even in the presence of sufficient oxygen, the glycolytic pathway, an alteration known as the Warburg effect, or aerobic glycolysis, accounts for over 95% of energy supply (Sun et al., 2018; Harris and Fenton 2019; Lu 2019). The altered glycolytic pathway is a characteristic difference between neoplastic and healthy cells (Icard et al., 2018). Tumor cells can produce more nucleotides, fatty acids, proteins, and ATP through enhanced aerobic glycolysis as the

Abbreviations: BZW1, basic leucine zipper and W2 domains 1; circRNAs, circular RNAs; CAF, cancer-associated fibroblasts; DNMT3A, DNA methyltransferase 3 alpha; F26BP, Fructose 2, 6-bisphosphate; GHET1, gastric carcinoma proliferation enhancing transcript 1; HK2, hexokinase 2; HIF-1, hypoxia-inducible factor-1; lncRNAs, long non-coding RNAs; LDHA, lactate dehydrogenase A; miRNAs, microRNAs; ncRNAs, Non-coding RNAs; NEAT1, nuclear paraspeckle assembly transcript 1; OC, Ovarian cancer; PKM, pyruvate kinase M1/2; PFKFB2, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2; RBP, RNA-binding protein; SNHG3, small nucleolar RNA host gene 3; VHL, von Hippel-Lindau; YAP1, Yes1 associated transcriptional regulator.

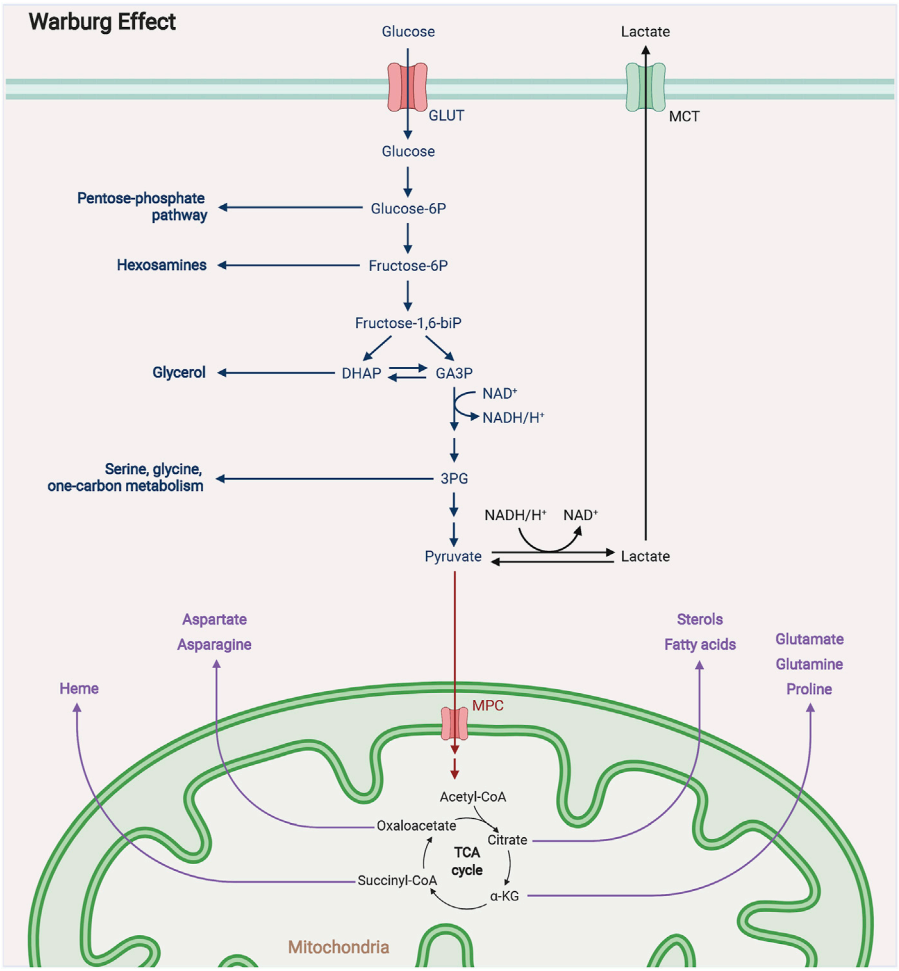


FIGURE 1 | The mechanism diagram of Warburg effect. The Warburg effect states that in the presence of sufficient oxygen supply, tumor cells still prefer glycolysis for energy to the more efficient oxidative phosphorylation, a phenomenon known as the Warburg effect.

material basis for rapid proliferation and invasiveness (Poff et al., 2019). Meanwhile, the Warburg effect reduces reactive oxygen species production, improves cellular antioxidant capacity, and reduces apoptosis (Yue et al., 2016; Shulman and Rothman 2017; Yue et al., 2019). In addition, aerobic glycolysis can produce large amounts of lactic acid, which creates an acidic microenvironment to facilitate invasion and metastasis of the tumor cells (Schwartz et al., 2017; Tekade and Sun 2017; Chen et al., 2018).

Noncoding RNAs (ncRNAs) primarily include microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs) (Jusic et al., 2020; Deogharia and Gurha 2021; Rahimian et al., 2021). The ncRNAs bind to multiple molecular targets to form regulatory networks in various biological activities, including initiating specific cellular biological responses, regulating gene expression, intracellular signaling, and epigenetic modifications (Ding et al., 2021; Ducoli and Detmar 2021). NcRNAs are involved in a variety of life activities such as regulation of gene expression, intracellular signaling and epigenetic modifications. Apart from participation in tumorigenesis, ncRNAs also account paramount role in the glycolytic process of tumors (Li Q et al., 2021; Lu et al., 2021; Park et al., 2021; Razavi et al., 2021; Wang et al., 2021). This review summarizes the possible molecular mechanisms of ncRNAs in the process of glycolysis and potentially effective targeted therapies for OC.

2 GLUCOSE METABOLISM IN NEOPLASTIC CELLS

Reprogramming of energy metabolism is the hallmark of cancer. Healthy cells generally undergo glycolysis to produce lactate only under anaerobic conditions with limited energy production, while the glycolysis of tumor cells in aerobic conditions (Chandel 2021; Reinfeld et al., 2021). Although glycolysis produces low levels of ATP compared to oxidative phosphorylation, cancer cells can rapidly uptake the available ATP and intermediates from glycolysis for the transduction of the biosynthetic pathway (Bacigalupa and Rathmell 2020; Cao et al., 2020). The reprogrammed metabolism contributes to tumor cell

metastasis, preventing apoptosis and promoting other malignant features.

2.1 Warburg Effect

Warburg effect is mainly a compensatory activity of tumor to adapt to the external environment (Lu et al., 2015; Cassim et al., 2020) (**Figure 1**) Efficient aerobic glycolysis facilitates tumor cell proliferation allowing tumor cells to produce abundant ATP from extracellular nutrients. Although the total energy produced per glucose during the Warburg effect is less than that by oxidative phosphorylation, ATP production by aerobic glycolysis can exceed that of oxidative phosphorylation with glucose available (Linehan and Rouault 2013; Hitosugi and Chen 2014). On the other hand, the Warburg effect provides tumor cells with intermediates for biosynthetic pathways, including ribose for nucleotide synthesis, glycerol, citrate, and nonessential amino acids for lipid synthesis (Ward and Thompson 2012; Upadhyay et al., 2013). Glucose can also produce nicotinamide adenine dinucleotide phosphate *via* the pentose phosphate pathway. Therefore, the Warburg effect is vital for facilitating tumor cell bioenergetics and biosynthesis.

2.2 Factors Affecting Aerobic Glycolysis

2.2.1 GLUTs

Compared with healthy cells, tumor cells exhibit an efficient aerobic glycolysis rate, which requires increased glucose flux to improve the efficiency of glucose uptake (Yang et al., 2020). Therefore, the expression and activity of Glucose Transporters (GLUTs) and glycolytic rate-limiting enzymes, such as HK, PFK and PK were significantly upregulated in tumor cells to facilitate the inevitably increased glucose consumption (Foltynie 2019; Bommer et al., 2020; Faustman 2020). Oncogenes regulate GLUT1 to intervene the glucose intake and tumor cell metabolism. The c-myc induces GLUT1 overexpression leading to increased glucose uptake (Leen et al., 2013; Huang L et al., 2021; Su et al., 2021). P53 can inhibit GLUT1 expression in cells, resulting in decreased glucose uptake and thus inhibiting tumor development (Feng et al., 2018). GLUT3 is expressed in most cancer cells but rarely in normal cells, facilitating glucose consumption (Cazzato et al., 2021; Libby et al., 2021). Targeting GLUT can inhibit the degree of aerobic glycolysis, affecting tumorigenesis (Fu et al., 2021; Kim E et al., 2021).

2.2.2 HK Isoforms

Glycolysis is a complex process that starts with glucose catalyzation by various non-rate limiting and rate-limiting enzymes to form lactate (Ganapathy-Kanniappan 2018; Fan et al., 2019). The classical glycolysis involves three rate-limiting enzymes, HK, PFK, and PK, mediating different processes and playing essential roles in glucose metabolism (Shakespear et al., 2018; Yellen 2018), HK has four isoforms, HKI, HKII, HKIII, and HKIV, catalyzing glucose to glucose-6-phosphate (G6P) (Zuo et al., 2021). HKI and HKII present high affinity for mitochondria, and HK1 expression is present in most mammalian tissues (Zhong and Zhou 2017; Garcia et al., 2019). HKII is abundantly present in fat, heart, and skeletal muscle (Mathupala et al., 2009; Tan and Miyamoto 2015). with a higher glycolytic rate than HKI(Tan and Miyamoto 2015). HKIV, also known as glucokinase, is present in hepatocytes with the lowest affinity for glucose and no inhibition by G6P (Xu and Herschman 2019; Kasprzak 2021). HKII is essential for tumor metabolism. Increased expression of HKII promotes proliferation and is associated with poor prognosis in tumor patients (Roberts and Miyamoto 2015; Tan and Miyamoto 2015).

2.2.3 PFK and PK

Fructose 2, 6-bisphosphate (F26BP) can diminish the inhibition of ATP and increase glucose uptake by interacting with PFK1(Kalezic et al., 2021; Zuo et al., 2021). The substrate can abnormally inhibit PFK, and ATP has a dual effect on PFK (PK is an evolutionarily conserved metabolic enzyme that catalyzes pyruvate production from phosphoenolpyruvate) (Shen et al., 2020; Zhao et al., 2020). Almost all mammalian genomes, including humans, encode two PK genes, PKLR and PKM, which express four PK isoforms (L, R, M1, and M2) (Jyoti et al., 2020; Yang et al., 2021). PKL and PKR are encoded by the PKLR gene and are expressed in hepatocytes and erythrocytes, respectively (Park et al., 2020; Storkus et al., 2021). The PKM gene encodes PKM1 and PKM2 through selective splicing (Chen k et al., 2021; Itoyama et al., 2021). PKM1 is expressed in normal differentiated tissues (Zhong et al., 2021), while PKM2 is expressed in highly proliferative cells such as embryonic cells, stem cells and tumor cells (Wang et al., 2021). Physiologically, PKM1 exists as a tetramer, while PKM2 can exist as a tetramer or a dimer (Hu et al., 2020; Rai et al., 2020). Fructose 1,6-2 phosphate is a transactivator of PKM2 but has little effect on PKM1 (Xu et al., 2019; Angiari et al., 2020).

3 TUMOR AEROBIC GLYCOLYTIC SIGNALING PATHWAY

C-myc can regulate the transcriptional process of various glycolytic genes (Gu et al., 2017). C-myc can bind to the regulatory region of hexokinase 2 (HK2) and thus play an essential role in tumor aerobic glycolysis (Huang WL et al., 2021; Su et al., 2021). PK catalyzes the final step of glycolysis, PKM2, which is only found in self-renewable groups such as stem cells and tumors (Li et al., 2017; van Niekerk and Engelbrecht 2018). C-myc can directly activate the PKM2 promoter region and upregulate PKM2 expression, thus promoting tumor aerobic glycolysis (Li et al., 2017; Yin et al., 2019). In addition, c-myc can induce PKM2 splicing by indirectly regulating hnRNP protein, thus promoting aerobic glycolysis (Gu et al., 2017). Glucose-6-phosphate dehydrogenase is a key enzyme in the glucose metabolism pathway. C-myc binds to the promoter region of glucose-6-phosphate dehydrogenase to promote its expression and thus the pentose phosphate pathway (Tang et al., 2021).

Ras-mediated metabolic reprogramming provides vital functions in tumorigenesis (Lin et al., 2021). The Ras

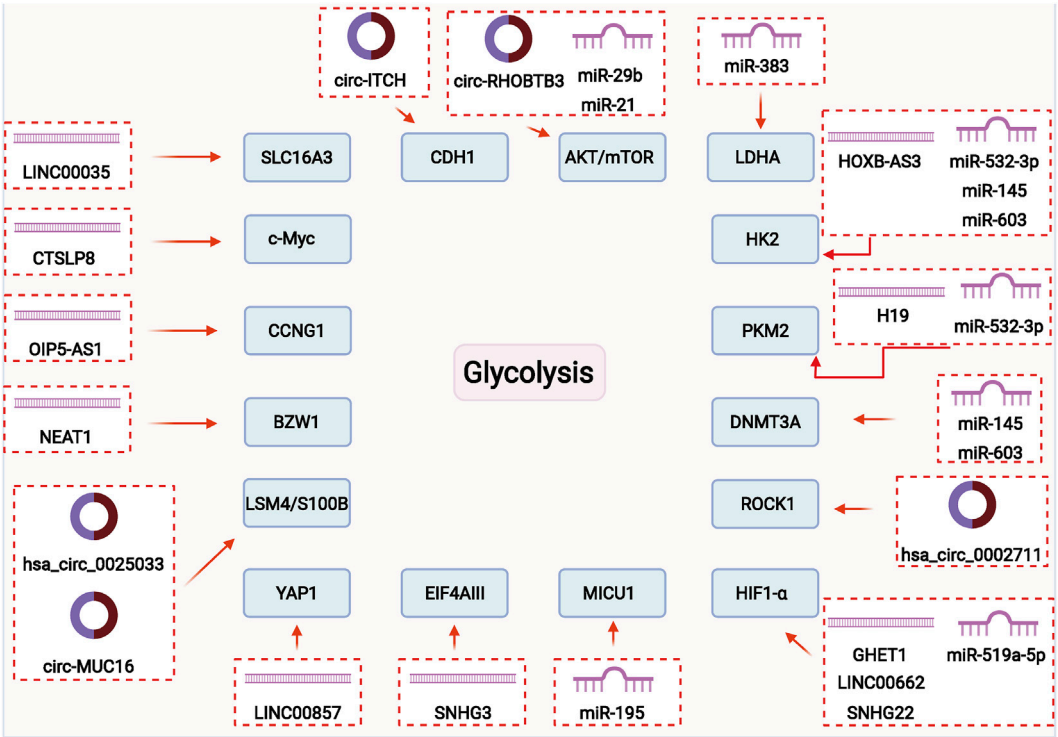


FIGURE 2 | ncRNAs may play a vital role in regulating glycolysis of ovarian cancer through different signal pathways and mechanisms.

signaling pathway can promote aerobic glycolysis and provide lactate and α-ketoglutarate through various enzymes (Campbell and Philips 2021; Chen B et al., 2021). Ras can promote glucose uptake by upregulating the expression of GLUT1 on the cell membrane surface, which in turn increases aerobic glycolysis efficiency (Healy et al., 2021). In addition, PI3K-Akt-mTOR signaling is also a significant regulator of glucose uptake, promoting GLUT1 expression and protein translocation from the inner membrane to the cell surface (Krencz et al., 2021; Sanaei et al., 2021). P53 is the most critical oncogene, affecting the cell cycle by encoding transcription factors (Liu et al., 2019; Alvarado-Ortiz et al., 2020). P53 can inhibit aerobic glycolysis by regulating TP53-mediated glycolysis and apoptosis-inducing factor expression (Strycharz et al., 2017; Itahana and Itahana 2018; Smiles and Camera 2018), regulating mitochondrial respiratory function, pentose phosphate pathway, and glycolysis-related enzymes (Kruiswijk et al., 2015; Werner et al., 2016). PTEN proteins exert their tumor-suppressive effects through three predominant signaling pathways, PI3K/AKT, local adherens spot kinase and mitogen-activated protein kinase (Mendes et al., 2016). PTEN inhibits tumorigenesis by activating PI3K/AKT pathway (Ortega-Molina and Serrano 2013). Phosphoglycerate kinase 1 (PGK1) can function as a glycolytic enzyme or phosphorylated as a protein kinase (He et al., 2019; Zhang et al., 2019). PTEN directly interacts with PGK1 to control aerobic glycolysis in tumors, and PTEN encodes a protein with phosphatase activity that inhibits phosphorylated PGK1, which ultimately inhibits aerobic glycolysis and tumor cell proliferation (Nie et al., 2020; Chu et al., 2021).

4 THE REGULATORY MECHANISM OF NCRNAS IN THE GLYCOLYSIS OF OVARIAN CANCER

The ncRNAs can regulate the expression of criticalgenes or enzymes of glycolytic pathway through different cellular signaling pathways, which promote the malignant development by regulating glucose metabolism in OC. Here, we summarize the mechanisms of miRNAs, lncRNAs and circRNAs in the regulation of glycolysis in OC (**Figure 2**).

4.1 MicorRNAs in the Glycolysis of Ovarian Cancer

The miRNAs are a group of 18–24 nucleotide noncoding RNAs that bind to the 3-terminal noncoding region of the target mRNA, altering gene expression (Sakshi et al., 2021; Yang et al., 2021) (**Figure 3**). The aberrant expression of miRNA in tumor cells revealed that miRNAs play an essential role in tumor development by regulating the expression and function of their associated target genes and participating in a variety of physiological and pathological processes (Barrera-Rojas et al., 2021; Pidikova and Herichova 2021; Roy et al., 2021). Abundant

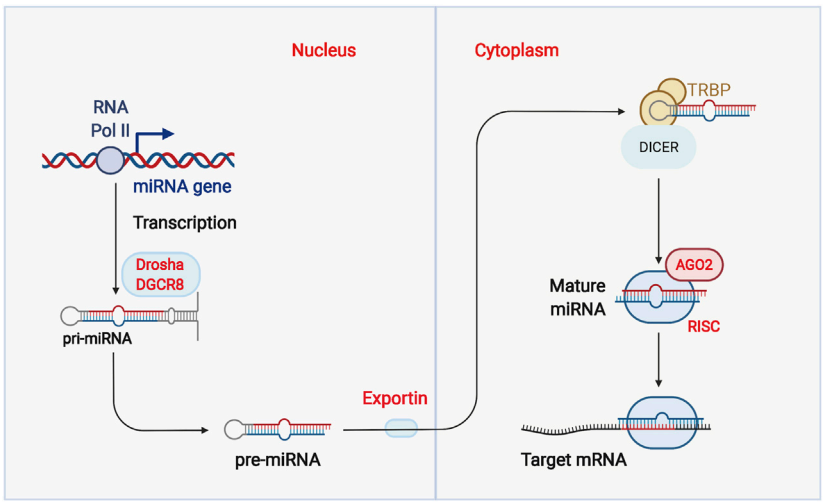


FIGURE 3 | Biogenesis of micro RNAs (miRNAs). RNA polymerase II regulates the transcription of miRNAs. As pri-miRNAs are transcribed, pri-miRNAs are processed by several sequential cleavages to produce mature miRNAs. Finally, mature miRNAs are integrated into Argonaute to form the miRNA-induced silencing complex (RISC).

TABLE 1 | miRNAs involved in glycolysis in ovarian cancer.

MiRNAs	Role	Expression	Target	Mechanism	Type of model	References
miR-29b	Tumor suppressor	Down	AKT2/AKT3	Inhibit HK2/PKM2 expression and Warburg effect	SKOV3, A2780	Teng et al. (2015)
miR-383	Tumor suppressor	Down	LDHA	Inhibit LDHA expression	Human samples	Han et al. (2017)
miR-21	Oncogene	Up	/	Promote AKT phosphorylation and glycolysis enzymes expression	SKOV3, TOV21G	Guo et al. (2017)
miR-532–3p	Oncogene	Up	HK2 and PKM2	Inhibit HK2 and PKM2 expression	SKOV3	Zhou et al. (2018)
miR-145	Tumor suppressor	Down	HK2 and DNMT3A	DNMT3A-miR-145-HK2 regulatory axis	Human samples	Zhang et al. (2018)
miR-603	Tumor suppressor	Down	HK2 and DNMT3A	DNMT3A-miR-603-HK2 regulatory axis	/	Lu et al. (2019)
miR-1180	Oncogene	Up	/	Activate the Wnt signaling pathway	SKOV3, COC1	Gu et al. (2019)
miR-519a-5p	Tumor suppressor	Down	HIF1-α	Inhibit HK2/PKM2 expression and Warburg effect	SKOV3	Lu et al. (2020)
miR-195	Tumor suppressor	Down	MICU1	/	OVCAR4, A2780-CP20	Rao et al. (2020)

miRNAs have been proved to regulate tumor metabolism and function as an essential role in the process of glycolysis in OC (**Table 1**).

Studies have shown that miRNAs control the expression of several key enzymes of glycolysis to regulate the glycolytic process. As the critical rate-limiting enzymes of glycolysis, HK2 catalyzes the first irreversible step of glycolysis, which increases at significantly elevated levels in a variety of tumor cells. HK2 can significantly inhibit the function of mitochondria from regulating tumor growth, survival, and metastasis (Huang L et al., 2021; Yu et al., 2021). PKM2 becomes an essential component of tumorigenesis by providing a metabolic advantage that tumor cells can utilize the upstream lipids of glycolytic intermediates as precursors for lipid, amino acid, and nucleic acid synthesis (Xia et al., 2021; Yuan et al., 2021). Zhou et al., found that 20(S)-Rg3 significantly attenuated DNA methyltransferase 3 alpha (DNMT3A)-mediated methylation and promoted the inhibition of HK2 and PKM2 by miR-532–3p, thereby antagonizing the Warburg effect in OC cells (Zhou et al., 2018). Zhang et al., found that miR-145 could target DNMT3A to reduce methylation of the pre-miR-145 promoter region. The feedback loop between these two miRNA was a characteristic feature of the Warburg effect, promising a potential therapeutic target for OC(Mirzaei et al., 2016; Zhang et al., 2018). Lu et al., reported a similar regulatory machanism between miR-603 and DNMT3A, and the DNMT3A-miR-603-HK2 regulatory axis may be the critical

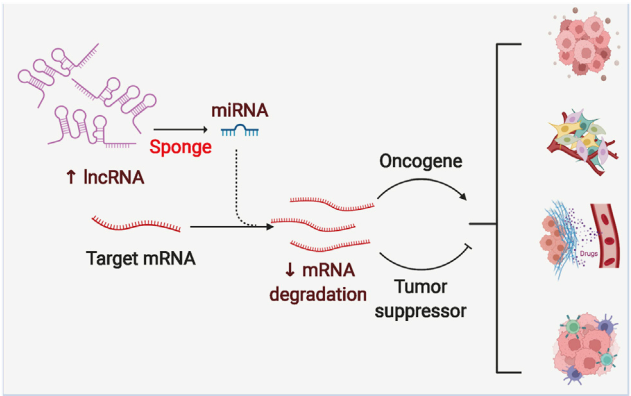


FIGURE 4 | The competing endogenous RNA mechanism of Long noncoding RNAs (lncRNAs). LncRNAs can inhibit the degradation of downstream mRNAs by binding different miRNAs, which in turn regulates the expression of pro- or oncogenes, ultimately leading to malignant progression of tumors.

molecular mechanism in the glycolytic pathway of OC(Lu et al., 2019; Pourhanifeh et al., 2020).

Lactate dehydrogenase A (LDHA) is an important metabolic enzyme belonging to the 2-hydroxy acid oxidoreductase family that plays a crucialrole in intracellular anaerobic sugar metabolism (Guan H et al., 2021; Huo et al., 2021). Hypoxic conditions induced the overexpression of LDHA, which shifts the metabolic pathway of ATP synthesis from oxidative phosphorylation to aerobic glycolysis. Therefore, the inhibition of LDHA is considered a promising strategy for tumor therapy (Jiang et al., 2021; Martinez-Ordonez et al., 2021). Han et al., demonstrated that miR-383 regulates LDHA expression in OC cells, impeding glycolysis, cell proliferation and invasion (Han et al., 2017). Tumor glycolytic activity is enhanced to adapt to ischemic and hypoxic environment by inducing an energy metabolic switch as the metabolic basis of its hypoxia tolerance (Wang et al., 2021). This process activateshypoxia-inducible factor-1 (HIF-1), a widely present dominant oxygen regulator in mammals, triggers various biological events, including glycolytic activation and tumorigenesis (Favier et al., 2015; Moldogazieva et al., 2020). Lu et al., reported that 20(S)-Rg3 upregulates miR-519a-5p expression by reducing DNMT3A-mediated DNA methylation of miR-519a-5p, thereby inhibiting HIF-1α and promoting the Warburg effect, leading to malignant progression of OC(Lu et al., 2020).

Aberrant activation and inactivation of oncogenes regulate abnormal energy metabolism to adapt to tumor growth demands (Yeung et al., 2008; Meijer et al., 2012). Teng et al., demonstrated that inhibition of miR-29b promotes the expression of AKT2/3, pakt2/3, HK2, and PKM2 and regulates pyruvate and NAD+/NADH levels (Teng et al., 2015). The miR-29b regulates the Warburg effect in OC by modulating AKT2/AKT3, which is a potential therapeutic target for OC. Moreover, miR-21 could promote AKT phosphorylation and glycolysis enzymes expression in OC(Guo et al., 2017). The miR-1180 could activate the Wnt signaling pathway and regulate the glycolysis progression of OC(Gu et al., 2019). Rao et al., demonstrated that miR-195 significantly inhibited tumor growth, increased tumor proliferation time, and improved overall survival by targeting MICU1 to inhibite glycolysis and chemoresistance (Rao et al., 2020).

4.2 LncRNAs in the Glycolysis of Ovarian Cancer

LncRNAs are a category of noncoding RNAs with over 200 nucleotides in length, tissue specificity and low species conservation (Jalaiei et al., 2021; Zhao et al., 2021). LncRNAs bind to proteins through their unique secondary structure to form RNA-protein complexes (Dashti et al., 2021; Janaththani et al., 2021; Mardani et al., 2021) and interact with multiple RNAs to form complex gene expression regulatory networks (Sun and Feinberg 2021; Wu et al., 2021). LncRNAs also target miRNAs through their 3′UTR region to regulate the effective concentration and activity, which affects the repressive effect on the target mRNAs(Sun and Feinberg 2021; Wu et al., 2021). (**Figure 4**). Above all, lncRNAs are the critical regulators in the process of glycolysis in OC (**Table 2**).

Small nucleolar RNA host gene 3 (SNHG3) promotes glycolysis and oxidative phosphorylation to induce OC drug resistance by binding to miR-186–5p and upregulating EIF4AIII expression (Li et al., 2018). H19 promotes glycolysis and malignant progression of OC by binding miR-324–5p to promote PKM2 expression (Zheng et al., 2018). LINC00857 acts as a pro-oncogene by binding miR-486–5p to promote Yes1 associated transcriptional regulator (YAP1) expression, promoting OC cell proliferation, migration, invasion, and glycolytic progression (Lin et al., 2020). Nuclear paraspeckle assembly transcript 1 (NEAT1) can play an essential role in OC malignant growth, metastasis and glycolysis by binding to miR-4500 and thus promoting basic leucine zipper and W2 domains 1 (BZW1) expression (Xu et al., 2020). HOXB-AS3 regulates both LDHA and ECAR expression by binding to miR-378a-3p in the glycolytic process of OC(Xu et al., 2021). OIP5-AS1 binds miR-128–3p to promote the expression of CCNG1, which leads to the malignant progression of OCthrough the glycolytic process (Liu et al., 2021). Moreover, LINC00504 is involved in the glycolytic process of OC by binding miR-1244. However, the specific downstream genes need more elaboration (Liu et al., 2020).

HIF is a nuclear transcription factor that facilitates cells to adapt to the hypoxic environment (Knutson et al., 2021; Cowman and Koh 2022). Liu et al., found that upregulation of gastric carcinoma proliferation enhancing transcript 1 (GHET1) positively correlated with tumor size, metastasis, proliferation, and colony formation in OC patients (Liu and Li 2019). Further studies confirmed that GHET1 interacted with von Hippel-Lindau (VHL) to prevent VHL-mediated hypoxia-inducible factor-1α (HIF-1α) degradation and increased HIF1α protein levels in OC cells. The up-regulated HIF-1α promoted glucose uptake and lactate production in OC cells. Tao et al., reported that LINC00662 was highly expressed in OC cells and was strongly associated with overall survival of

TABLE 2 | lncRNAs involved in glycolysis in ovarian cancer.

LncRNAs	Role	Expression	Target	Mechanism	Type of model	References
LINC00092	Oncogene	Up	/	Bind to PFKFB2	Human samples, SKOV3	Zhao et al. (2017)
SNHG3	Oncogene	Up	miR-186–5p	Promote EIF4AIII expression	Human samples, SKOV3, TOV-21G, OVCAR3	Li et al. (2018)
H19	Oncogene	Up	miR-324–5p	Promote PKM2 expression	SKOV3	Zheng et al. (2018)
GHET1	Oncogene	Up	/	Interact with VHL and up-regulate HIF1-α	HOSEpiC, SKOV3, TOV-21G, 3AO, A2780	Liu and Li (2019)
LINC00504	Oncogene	Up	miR-1244	/	HOSEpiC, SKOV3, CAOV3, OVCAR3, HO-8910	Liu et al. (2020)
LINC00662	Oncogene	Up	miR-375	Promote HIF1-α expression	IOSE-29, SKOV3	Tao et al. (2020)
LINC00857	Oncogene	Up	miR-486–5p	Promote YAP1 expression	SKOV3, CAOV3, A2780, IOSE-29	Lin et al. (2020)
NEAT1	Oncogene	Up	miR-4500	Promote BZW1 expression	CAOV3, ES-2, iose80	Xu et al. (2020)
HOXB-AS3	Oncogene	Up	miR-378a-3p	Promote LDHA and ECAR expression	SKOV3, A2780	Xu et al. (2021)
OIP5-AS1	Oncogene	Up	miR-128–3p	Promote CCNG1 expression	IOSE-80, OVCAR-3, SKOV-3	Liu et al. (2021)
CTSLP8	Oncogene	Up	/	Promote c-Myc expression by binding to PKM2	SKOV-3, SKOV3-DDPee	Li X et al. (2021)
SNHG22	Oncogene	Up	/	SP1 and HIF1-α can promote SNHG22 expression	ES-2, HO8910, OVCAR-3, A2780	Guan N et al. (2021)
LINC00035	Oncogene	Up	/	Promote SLC16A3 expression by binding to CEBPB	IOES80, CAOV-3, A2780, SKOV3, CoC1	Yang et al. (2021)

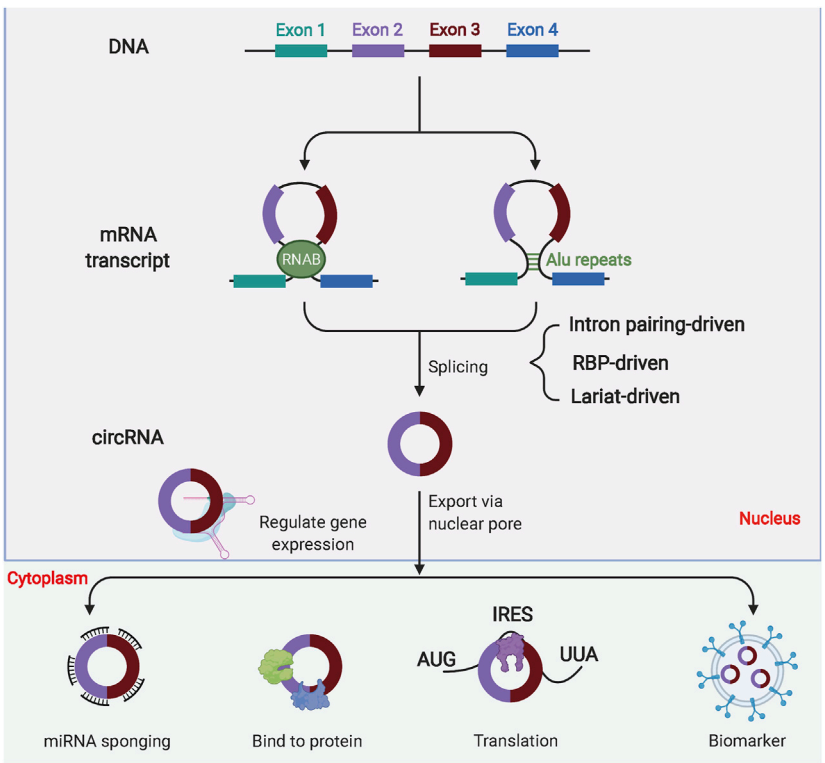


FIGURE 5 | Biogenesis of circular RNAs (circRNAs). Most circRNAs are derived from pre-mRNA. Due to their composition, circRNAs are classified into several types, including exonic circRNAs, exon-intron circRNAs and intronic circRNAs. CircRNAs can perform biological functions by binding miRNAs, binding proteins or translating into polypeptides. In addition, circRNAs are also enriched in exosomes and are good markers for disease diagnosis.

TABLE 3 | circRNAs involved in glycolysis in ovarian cancer.

CircRNAs	Role	Expression	Target	Mechanism	Type of model	References
Circ-ITCH	Tumor suppressor	Down	miR-106a	Promote CDH1 expression	A2780, OVCAR3, ISOE80	Lin et al. (2020)
RHOBTB3	Tumor suppressor	Down	/	Inactivate PI3K/AKT signaling pathway, Inhibit GLUT1, HK2 and LDHA expression	IOSE-80, OVCAR-3, SKOV-3	Yalan et al. (2020)
Hsa_circ_0025033	Oncogene	Up	miR-184	Promote LSM4 expression	A2780, OVCAR3, ISOE80	Hou and Zhang (2021)
Hsa_circ_0002711	Oncogene	Up	miR-1244	Promote ROCK1 expression	OVCAR-3	Xie W et al. (2021)
Circ-MUC16	Oncogene	Up	miR-1182	Promote S100B expression	A2780, SKOV-3, ISOE80	Yang GJ et al. (2021)

OC patients (Tao et al., 2020). Mechanistic studies confirmed that LINC00662 act as a competitive RNA to regulate HIF-1α expression by directly binding to miR-375, which in turn regulates the proliferation and glycolysis of OC cells. Guan et al., found that SP1 and HIF1-α can promote SNHG22 expression and promote the glycolytic process and malignant progression of OC(Guan H et al., 2021).

In addition, there are lncRNAs that can directly regulate the expression of genes involved in the glycolytic process of OC. LINC00092 binds 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (PFKFB2) and thus promotes malignant metastasis of OC by altering glycolysis and maintaining the local support function of cancer-associated fibroblasts (CAF) (Zhao et al., 2017; Hashemipour et al., 2021). Li et al., revealed that CTSLP8 expression increases in chemoresistant tumor tissues, which promotes c-Myc expression and thus upregulates glycolysis by facilitating the binding of PKM2 to the c-Myc promoter region (Li Q et al., 2021). Yang et al., demonstrated that LINC00035 promotes malignant progression of OC by regulating glycolysis and apoptosis through CEBPB-mediated SLC16A3 transcription (Yang et al., 2021).

4.3 circRNAs in the Glycolysis of Ovarian Cancer

Most circRNAs are expressed from known protein-coding genes and consist of exons forming a covalently closed loop structure by aberrant reverse splicing (**Figure 5**). CircRNA formation mechanisms included intron pairing-driven circularization, RNA-binding protein (RBP)-driven circularization, and lasso-driven circularization. The circRNAs play critical biological functions in eukaryotic organisms, which compete for miRNAs. By base-complementary pairing with the target mRNA 3-UTR, miRNAs can block the translation and stability of target RNA-binding Proteins (RBPs) can interact with circRNAs and regulate circRNA splicing, replication, folding, stabilization and localization (Huang and Zhu 2021; Zeng et al., 2021). In summary, the circRNAs act as miRNA sponges and interact with RBPs to perform transcriptional functions in organisms. The open reading frames in circRNAs enrich exosomes and can be translated into polypeptides for early diagnosis and prognosis (Kim H et al., 2021; Sinha et al., 2021; Wang et al., 2021). The circRNAs are critical in regulating the process of glycolysis in OC (**Table 3**).

Circ-ITCH was downregulated in OC and positively correlated with 5-years overall survival in OC patients (Lin et al., 2020) while the overexpression significantly inhibited proliferation, invasion, glycolysis and promoted apoptosis in OC cells. Sun et al., demonstrated the downregulation of circ-RHOBTB3 in OC tissues and cells, and overexpression significantly inhibited cell proliferation, metastasis, and glycolysis (Yalan et al., 2020). Circ-RHOBTB3 inhibited OC progression by inactivating the PI3K/AKT signaling pathway. The expression of hsa_circ_0025033 was found to be upregulated in OC, and downregulation of hsa_circ_0025033 significantly inhibited OC cell colony formation, migration/invasion and glycolytic metabolism (Hou and Zhang 2021). Hsa_circ_0025033 promotes LSM4 expression by binding miR-184. Xie et al., demonstrated that the hsa_circ_0002711/miR-1244/ROCK1 regulatory axis promotes malignant progression of OC *in vivo* by regulating Warburg effect and tumor growth (Xie W et al., 2021). Hsa_circ_MUC16 promotes OC cell proliferation, glycolytic metabolism, migration and invasion by targeting the miR-1182/S100B regulatory axis (Yang et al., 2021).

5 FUTURE PERSPECTIVESAND CONCLUSION

The development and progression of OC is a complex physiological process. The invasion and metastasis of OC is a complicated process, which poses difficulties for early detection, intervention, and treatment (Tymon-Rosario et al., 2021; Wang et al., 2021). The Warburg effect is one of the recognized metabolic features of tumor cells (Abi Zamer et al., 2021; Nakagawa et al., 2021). Active glycolysis remains a common feature of cancer metabolism, and metabolic reprogramming increases the expression of critical enzymes and, ultimately, lactate secretion. Lactate in the tumor microenvironment can promote malignant progression and tumor immune escape (Hashemian et al., 2020; Mirzaei and Hamblin 2020; Holloway and Marignani 2021; Nakagawa et al., 2021). Various oncogenes and signaling pathways regulate the glycolytic enzymes to affect the rate of

glycolysis (Almeida et al., 2021; Chandel 2021). Although the glycolytic process has drawn attention in the control of oncogenic features, the mechanisms of critical enzymes and complex interactions with signaling are not well studied in OC, considering the high heterogeneity of tumors.

Findings have confirmed the regulatory role of ncRNAs on the Warburg effect of tumor cells and highlight their significance in tumor biology research. The expression of specific ncRNAs in tumors predicts tumors' biological properties and their possible outcomes and prognosis. On the other hand, ncRNAs may also become target sites for tumor treatment. However, there are still relatively few discoveries lacking systematic content and reliable clinical evidence. In summary, ncRNAs play an essential role in OC aerobic glycolysis, regulating the activity and content of specific enzymes and acting as transcriptional activators to regulate the expression of metabolism-related genes. In addition, these ncRNAs interact with other critical factors related to glucose metabolism and initiate various oncogenic processes. In the future, it is vital to confirm and elucidate the role of ncRNAs in OC aerobic glycolysis and their potential as molecular biomarkers. Investigating the correlation of ncRNA and aerobic glycolysis is promising for the interaction network of ncRNAs and the feedback regulation in tumorigenesis. Elucidating the mechanism of ncRNAs in the aerobic glycolysis of OC will provide new insights into OC research and provide new strategies for clinical treatment.

AUTHOR CONTRIBUTIONS

Original draft preparation, allocation, supplementation and editing: CZ. Revision: NL and CZ, All authors have read and agreed to the published version of the manuscript.

FUNDING

This work was supported by the Shengjing Hospital of China Medical University (Shenyang) and China Medical University (Shenyang).

REFERENCES

Abi Zamer, B., Abumustafa, W., Hamad, M., Maghazachi, A. A., and Muhammad, J. S. (2021). Genetic Mutations and Non-coding RNA-Based Epigenetic Alterations Mediating the Warburg Effect in Colorectal Carcinogenesis. *Biology (Basel)* 10, 849. doi:10.3390/biology10090847

Almeida, L., Dhillon-LaBrooy, A., Carriche, G., Berod, L., and Sparwasser, T. (2021). CD4+ T-Cell Differentiation and Function: Unifying Glycolysis, Fatty Acid Oxidation, Polyamines NAD Mitochondria. *J. Allergy Clin. Immunol.* 148, 16–32. doi:10.1016/j.jaci.2021.03.033

Alvarado-Ortiz, E., de la Cruz-López, K. G., Becerril-Rico, J., Sarabia-Sánchez, M. A., Ortiz-Sánchez, E., and García-Carrancá, A. (2020). Mutant P53 Gain-Of-Function: Role in Cancer Development, Progression, and Therapeutic Approaches. *Front Cel Dev Biol* 8, 607670. doi:10.3389/fcell.2020.607670

Angiari, S., Runtsch, M. C., Sutton, C. E., Palsson-McDermott, E. M., Kelly, B., Rana, N., et al. (2020). Pharmacological Activation of Pyruvate Kinase M2 Inhibits CD4+ T Cell Pathogenicity and Suppresses Autoimmunity. *Cell Metab* 31, 391–e8. doi:10.1016/j.cmet.2019.10.015

Bacigalupa, Z. A., and Rathmell, W. K. (2020). Beyond Glycolysis: Hypoxia Signaling as a Master Regulator of Alternative Metabolic Pathways and the Implications in clear Cell Renal Cell Carcinoma. *Cancer Lett.* 489, 19–28. doi:10.1016/j.canlet.2020.05.034

Barrera-Rojas, C. H., Otoni, W. C., and Nogueira, F. T. S. (2021). Shaping the Root System: the Interplay between miRNA Regulatory Hubs and Phytohormones. *J. Exp. Bot.* 72, 6822–6835. doi:10.1093/jxb/erab299

Bommer, G. T., Van Schaftingen, E., and Veiga-da-Cunha, M. (2020). Metabolite Repair Enzymes Control Metabolic Damage in Glycolysis. *Trends Biochem. Sci.* 45, 228–243. doi:10.1016/j.tibs.2019.07.004

Campbell, S. L., and Philips, M. R. (2021). Post-translational Modification of RAS Proteins. *Curr. Opin. Struct. Biol.* 71, 180–192. doi:10.1016/j.sbi.2021.06.015

Cao, L., Wu, J., Qu, X., Sheng, J., Cui, M., Liu, S., et al. (2020). Glycometabolic Rearrangements-Aaerobic Glycolysis in Pancreatic Cancer: Causes, Characteristics and Clinical Applications. *J. Exp. Clin. Cancer Res.* 39, 267. doi:10.1186/s13046-020-01765-x

Cassim, S., Vučetić, M., Ždralević, M., and Pouyssegur, J. (2020). Warburg and beyond: The Power of Mitochondrial Metabolism to Collaborate or Replace Fermentative Glycolysis in Cancer. *Cancers (Basel)* 12, 19. doi:10.3390/cancers12051119

Cazzato, G., Colagrande, A., Cimmino, A., Abbatepaolo, C., Bellitti, E., Romita, P., et al. (2021). GLUT1, GLUT3 Expression and 18FDG-PET/CT in Human Malignant Melanoma: What Relationship Exists? New Insights and Perspectives. *Cells* 10, 11.

Chandel, N. S. (2021). Glycolysis. *Cold Spring Harb Perspect. Biol.* 13, 5. doi:10.1101/cshperspect.a040535

Chen, B., Deng, Y. N., Wang, X., Xia, Z., He, Y., Zhang, P., et al. (2021). miR-26a Enhances Colorectal Cancer Cell Growth by Targeting RREB1 Deacetylation to Activate AKT-Mediated Glycolysis. *Cancer Lett.* 521, 1–13. doi:10.1016/j.canlet.2021.08.017

Chen, K., Zhang, Y., Qian, L., and Wang, P. (2021). Emerging Strategies to Target RAS Signaling in Human Cancer Therapy. *J. Hematol. Oncol.* 14, 116. doi:10.1186/s13045-021-01127-w

Chen, Z., Liu, M., Li, L., and Chen, L. (2018). Involvement of the Warburg Effect in Non-tumor Diseases Processes. *J. Cel Physiol* 233, 2839–2849. doi:10.1002/jcp.25998

Chu, Z., Huo, N., Zhu, X., Liu, H., Cong, R., Ma, L., et al. (2021). FOXO3A-induced LINC00926 Suppresses Breast Tumor Growth and Metastasis through Inhibition of PGK1-Mediated Warburg Effect. *Mol. Ther.* 29, 2737–2753. doi:10.1016/j.ymthe.2021.04.036

Cowman, S. J., and Koh, M. Y. (2022). Revisiting the HIF Switch in the Tumor and its Immune Microenvironment. *Trends Cancer* 8, 28–42. doi:10.1016/j.trecan.2021.10.004

Dashti, F., Mirazimi, S. M. A., Rabiei, N., Fathazam, R., Rabiei, N., Piroozmand, H., et al. (2021). The Role of Non-coding RNAs in Chemotherapy for Gastrointestinal Cancers. *Mol. Ther. Nucleic Acids* 26, 892–926. doi:10.1016/j.omtn.2021.10.004

Deogharia, M., and Gurha, P. (2021). *The "guiding" Principles of Noncoding RNA Function*. Wiley Interdiscip Rev RNA, e1704.

Ding, L., Wang, R., Shen, D., Cheng, S., Wang, H., Lu, Z., et al. (2021). Role of Noncoding RNA in Drug Resistance of Prostate Cancer. *Cell Death Dis* 12, 590. doi:10.1038/s41419-021-03854-x

DiSilvestro, P., Colombo, N., Harter, P., González-Martín, A., Ray-Coquard, I., and Coleman, R. L. (2021). Maintenance Treatment of Newly Diagnosed Advanced Ovarian Cancer: Time for a Paradigm Shift? *Cancers (Basel)* 13, 756. doi:10.3390/cancers13225756

Ducoli, L., and Detmar, M. (2021). Beyond PROX1: Transcriptional, Epigenetic, and Noncoding RNA Regulation of Lymphatic Identity and Function. *Dev. Cel* 56, 406–426. doi:10.1016/j.devcel.2021.01.018

Fan, T., Sun, G., Sun, X., Zhao, L., Zhong, R., and Peng, Y. (2019). Tumor Energy Metabolism and Potential of 3-Bromopyruvate as an Inhibitor of Aerobic Glycolysis: Implications in Tumor Treatment. *Cancers (Basel)* 11, 317. doi:10.3390/cancers11030317

Faustman, D. L. (2020). Benefits of BCG-Induced Metabolic Switch from Oxidative Phosphorylation to Aerobic Glycolysis in Autoimmune and Nervous System Diseases. *J. Intern. Med.* 288 6, 641–650. doi:10.1111/joim.13050

Favier, F. B., Britto, F. A., Freyssenet, D. G., Bigard, X. A., and Benoit, H. (2015). HIF-1-driven Skeletal Muscle Adaptations to Chronic Hypoxia: Molecular Insights into Muscle Physiology. *Cell Mol Life Sci* 72, 4681–4696. doi:10.1007/s00018-015-2025-9

Feng, Y., Wu, M., Li, S., He, X., Tang, J., Peng, W., et al. (2018). The Epigenetically Downregulated Factor CYGB Suppresses Breast Cancer through Inhibition of Glucose Metabolism. *J. Exp. Clin. Cancer Res.* 37, 313. doi:10.1186/s13046-018-0979-9

Foltynie, T. (2019). Glycolysis as a Therapeutic Target for Parkinson's Disease. *Lancet Neurol.* 18, 1072–1074. doi:10.1016/S1474-4422(19)30404-1

Fu, C., Fu, Z., Jiang, C., Xia, C., Zhang, Y., Gu, X., et al. (2021). CD205 + Polymorphonuclear Myeloid-derived Suppressor Cells Suppress Antitumor Immunity by Overexpressing GLUT3. *Cancer Sci.* 112, 1011–1025. doi:10.1111/cas.14783

Ganapathy-Kanniappan, S. (2018). Molecular Intricacies of Aerobic Glycolysis in Cancer: Current Insights into the Classic Metabolic Phenotype. *Crit. Rev. Biochem. Mol. Biol.* 53, 667–682. doi:10.1080/10409238.2018.1556578

Garcia, S. N., Guedes, R. C., and Marques, M. M. (2019). Unlocking the Potential of HK2 in Cancer Metabolism and Therapeutics. *Curr. Med. Chem.* 26, 7285–7322. doi:10.2174/0929867326666181213092652

Giudice, E., Salutari, V., Ricci, C., Nero, C., Carbone, M. V., Ghizzoni, V., et al. (2021). Gut Microbiota and its Influence on Ovarian Cancer Carcinogenesis, Anticancer Therapy and Surgical Treatment: A Literature Review. *Crit. Rev. Oncol. Hematol.* 168, 103542. doi:10.1016/j.critrevonc.2021.103542

Gu, Z., Xia, J., Xu, H., Frech, I., Tricot, G., and Zhan, F. (2017). NEK2 Promotes Aerobic Glycolysis in Multiple Myeloma through Regulating Splicing of Pyruvate Kinase. *J. Hematol. Oncol.* 10, 17. doi:10.1186/s13045-017-0392-4

Gu, Z. W., He, Y. F., Wang, W. J., Tian, Q., and Di, W. (2019). MiR-1180 from Bone Marrow-Derived Mesenchymal Stem Cells Induces Glycolysis and Chemoresistance in Ovarian Cancer Cells by Upregulating the Wnt Signaling Pathway. *J. Zhejiang Univ. Sci. B* 20, 219–237. doi:10.1631/jzus.B1800190

Guan, H., Luo, W., Liu, Y., and Li, M. (2021). Novel Circular RNA circSLIT2 Facilitates the Aerobic Glycolysis of Pancreatic Ductal Adenocarcinoma via miR-510-5p/c-Myc/LDHA axis. *Cel Death Dis* 12, 645. doi:10.1038/s41419-021-03918-y

Guan, N., Zheng, H., Wu, X., Xie, L., and Tong, X. (2021). SP1-Regulated Non-coding RNA SNHG22 Promotes Ovarian Cancer Growth and Glycolysis. *Cancer Manag. Res.* 13, 7299–7309. doi:10.2147/CMAR.S318378

Guo, N. L., Zhang, J. X., Wu, J. P., and Xu, Y. H. (2017). Isoflurane Promotes Glucose Metabolism through Up-Regulation of miR-21 and Suppresses Mitochondrial Oxidative Phosphorylation in Ovarian Cancer Cells. *Biosci. Rep.* 37, 6. doi:10.1042/BSR20170818

Han, R. L., Wang, F. P., Zhang, P. A., Zhou, X. Y., and Li, Y. (2017). miR-383 Inhibits Ovarian Cancer Cell Proliferation, Invasion and Aerobic Glycolysis by Targeting LDHA. *Neoplasma* 64, 244–252. doi:10.4149/neo_2017_211

Harris, R. A., and Fenton, A. W. (20191871). A Critical Review of the Role of M2PYK in the Warburg Effect. *Biochim. Biophys. Acta Rev. Cancer* 1871, 225–239. doi:10.1016/j.bbcan.2019.01.004

Hashemian, S. M., Pourhanifeh, M. H., Fadaei, S., Velayati, A. A., Mirzaei, H., and Hamblin, M. R. (2020). Non-coding RNAs and Exosomes: Their Role in the Pathogenesis of Sepsis. *Mol. Ther. Nucleic Acids* 21, 51–74. doi:10.1016/j.omtn.2020.05.012

Hashemipour, M., Boroumand, H., Mollazadeh, S., Tajiknia, V., Nourollahzadeh, Z., Rohani Borj, M., et al. (2021). Exosomal microRNAs and Exosomal Long Non-coding RNAs in Gynecologic Cancers. *Gynecol. Oncol.* 161, 314–327. doi:10.1016/j.ygyno.2021.02.004

He, Y., Luo, Y., Zhang, D., Wang, X., Zhang, P., Li, H., et al. (2019). PGK1-mediated Cancer Progression and Drug Resistance. *Am. J. Cancer Res.* 9, 2280–2302.

Healy, F. M., Prior, I. A., and MacEwan, D. J. (2021). The Importance of Ras in Drug Resistance in Cancer. *Br. J. Pharmacol.* 15, 420. doi:10.1111/bph.15420

Hitosugi, T., and Chen, J. (2014). Post-translational Modifications and the Warburg Effect. *Oncogene* 33, 4279–4285. doi:10.1038/onc.2013.406

Holloway, R. W., and Marignani, P. A. (2021). Targeting mTOR and Glycolysis in HER2-Positive Breast Cancer. *Cancers (Basel)* 13, 122. doi:10.3390/cancers13122922

Hou, W., and Zhang, Y. (2021). Circ_0025033 Promotes the Progression of Ovarian Cancer by Activating the Expression of LSM4 via Targeting miR-184. *Pathol. Res. Pract.* 217, 153275. doi:10.1016/j.prp.2020.153275

Hu, X. K., Rao, S. S., Tan, Y. J., Yin, H., Luo, M. J., Wang, Z. X., et al. (2020). Fructose-coated Angstrom Silver Inhibits Osteosarcoma Growth and Metastasis via Promoting ROS-dependent Apoptosis through the Alteration of Glucose Metabolism by Inhibiting PDK. *Theranostics* 10, 7710–7729. doi:10.7150/thno.45858

Huang, L., He, C., Zheng, S., Wu, C., Ren, M., and Shan, Y. (2021). *AKT1/HK2 Axis-mediated Glucose Metabolism: A Novel Therapeutic Target of Sulforaphane in Bladder Cancer*. Weinheim, Germany: Mol Nutr Food Res, e2100738.

Huang, W. L., Abudureheman, T., Xia, J., Chu, L., Zhou, H., Zheng, W. W., et al. (2021). CDK9 Inhibitor Induces the Apoptosis of B-Cell Acute Lymphocytic Leukemia by Inhibiting C-Myc-Mediated Glycolytic Metabolism. *Front. Cel Dev Biol* 9, 641271. doi:10.3389/fcell.2021.641271

Huang, Y., and Zhu, Q. (2021). Mechanisms Regulating Abnormal Circular RNA Biogenesis in Cancer. *Cancers (Basel)* 13, 164185. doi:10.3390/cancers13164185

Huo, N., Cong, R., Sun, Z. J., Li, W. C., Zhu, X., Xue, C. Y., et al. (2021). STAT3/LINC00671 axis Regulates Papillary Thyroid Tumor Growth and Metastasis via LDHA-Mediated Glycolysis. *Cel Death Dis* 12, 799. doi:10.1038/s41419-021-04081-0

Icard, P., Shulman, S., Farhat, D., Steyaert, J. M., Alifano, M., and Lincet, H. (2018). How the Warburg Effect Supports Aggressiveness and Drug Resistance of Cancer Cells? *Drug Resist. Updat* 38, 1–11. doi:10.1016/j.drup.2018.03.001

Itahana, Y., and Itahana, K. (2018). Emerging Roles of P53 Family Members in Glucose Metabolism. *Int. J. Mol. Sci.* 19, 3. doi:10.3390/ijms19030776

Itoyama, R., Yasuda-Yoshihara, N., Kitamura, F., Yasuda, T., Bu, L., Yonemura, A., et al. (2021). Metabolic Shift to Serine Biosynthesis through 3-PG Accumulation and PHGDH Induction Promotes Tumor Growth in Pancreatic Cancer. *Cancer Lett.* 523, 29–42. doi:10.1016/j.canlet.2021.09.007

Jalaiei, A., Asadi, M. R., Sabaie, H., Dehghani, H., Gharesouran, J., Hussen, B. M., et al. (2021). Long Non-coding RNAs, Novel Offenders or Guardians in Multiple Sclerosis: A Scoping Review. *Front. Immunol.* 12, 774002. doi:10.3389/fimmu.2021.774002

Janaththani, P., Srinivasan, S. L., and Batra, J. (2021). Long Non-coding RNAs at the Chromosomal Risk Loci Identified by Prostate and Breast Cancer GWAS. *Genes (Basel)* 12, 122028. doi:10.3390/genes12122028

Jiang, Y., Li, F., Gao, B., Ma, M., Chen, M., Wu, Y., et al. (2021). KDM6B-mediated Histone Demethylation of LDHA Promotes Lung Metastasis of Osteosarcoma. *Theranostics* 11, 3868–3881. doi:10.7150/thno.53347

Jusic, A., Devaux, Y., and ActionU-CardioRNA Cost, E. (2020). Mitochondrial Noncoding RNA-Regulatory Network in Cardiovascular Disease. *Basic Res. Cardiol.* 115, 23. doi:10.1007/s00395-020-0783-5

Jyoti, P., Shree, M., Joshi, C., Prakash, T., Ray, S. K., Satapathy, S. S., et al. (2020). The Entner-Doudoroff and Nonoxidative Pentose Phosphate Pathways Bypass Glycolysis and the Oxidative Pentose Phosphate Pathway in Ralstonia Solanacearum. *mSystems* 5, 20. doi:10.1128/mSystems.00091-20

Kalezic, A., Udicki, M., Srdic Galic, B., Aleksic, M., Korac, A., Jankovic, A., et al. (2021). Tissue-Specific Warburg Effect in Breast Cancer and Cancer-Associated Adipose Tissue-Relationship between AMPK and Glycolysis. *Cancers (Basel)* 13, 112731. doi:10.3390/cancers13112731

Kasprzak, A. (2021). Insulin-Like Growth Factor 1 (IGF-1) Signaling in Glucose Metabolism in Colorectal Cancer. *Int. J. Mol. Sci.* 22, 126434. doi:10.3390/ijms22126434

Kim, E., Kim, Y. K., and Lee, S. V. (2021). Emerging Functions of Circular RNA in Aging. *Trends Genet.* 4, 14. doi:10.1016/j.tig.2021.04.014

Kim, H., Son, S., Ko, Y., and Shin, I. (2021). CTGF Regulates Cell Proliferation, Migration, and Glucose Metabolism through Activation of FAK Signaling in Triple-Negative Breast Cancer. *Oncogene* 40, 2667–2681. doi:10.1038/s41388-021-01731-7

Knutson, A. K., Williams, A. L., Boisvert, W. A., and Shohet, R. V. (2021). HIF in the Heart: Development, Metabolism, Ischemia, and Atherosclerosis. *J. Clin. Invest.* 131, 17. doi:10.1172/JCI137557

Krencz, I., Sztankovics, D., Danko, T., Sebestyen, A., and Khoor, A. (2021). Progression and Metastasis of Small Cell Lung Carcinoma: the Role of the

PI3K/Akt/mTOR Pathway and Metabolic Alterations. *Cancer Metastasis Rev.* 21, 12. doi:10.1007/s10555-021-10012-4

Kruiswijk, F., Labuschagne, C. F., and Vousden, K. H. (2015). p53 in Survival, Death and Metabolic Health: a Lifeguard with a Licence to Kill. *Nat. Rev. Mol. Cel Biol* 16, 393–405. doi:10.1038/nrm4007

Leen, W. G., Wevers, R. A., Kamsteeg, E. J., Scheffer, H., Verbeek, M. M., and Willemsen, M. A. (2013). Cerebrospinal Fluid Analysis in the Workup of GLUT1 Deficiency Syndrome: a Systematic Review. *JAMA Neurol.* 70, 1440–1444. doi:10.1001/jamaneurol.2013.3090

Li, K., Cheng, J., Lu, H., Yang, W., Zhou, J., and Cen, K. (2017). Transcriptome-based Analysis on Carbon Metabolism of Haematococcus pluvialis Mutant under 15% CO2. *Bioresour. Technol.* 233, 313–321. doi:10.1016/j.biortech.2017.02.121

Li, N., Zhan, X., and Zhan, X. (2018). The lncRNA SNHG3 Regulates Energy Metabolism of Ovarian Cancer by an Analysis of Mitochondrial Proteomes. *Gynecol. Oncol.* 150, 343–354. doi:10.1016/j.ygyno.2018.06.013

Li, Q., Sun, H., Luo, D., Gan, L., Mo, S., Dai, W., et al. (2021). Lnc-RP11-536 K7.3/SOX2/HIF-1α Signaling axis Regulates Oxaliplatin Resistance in Patient-Derived Colorectal Cancer Organoids. *J. Exp. Clin. Cancer Res.* 40, 348. doi:10.1186/s13046-021-02143-x

Li, X., Zhang, Y., Wang, X., Lin, F., Cheng, X., Wang, Z., et al. (2021). Long Non-coding RNA CTSLP8 Mediates Ovarian Cancer Progression and Chemotherapy Resistance by Modulating Cellular Glycolysis and Regulating C-Myc Expression through PKM2. *Cell Biol Toxicol* 21, 9. doi:10.1007/s10565-021-09650-9

Libby, C. J., Gc, S., Benavides, G. A., Fisher, J. L., Williford, S. E., Zhang, S., et al. (2021). A Role for GLUT3 in Glioblastoma Cell Invasion that Is Not Recapitulated by GLUT1. *Cell Adhes. Migration* 15, 101–115. doi:10.1080/19336918.2021.1903684

Lin, C., Xu, X., Yang, Q., Liang, L., and Qiao, S. (2020). Circular RNA ITCH Suppresses Proliferation, Invasion, and Glycolysis of Ovarian Cancer Cells by Up-Regulating CDH1 via Sponging miR-106a. *Cancer Cel Int* 20, 336. doi:10.1186/s12935-020-01420-7

Lin, L., Miao, L., Lin, H., Cheng, J., Li, M., Zhuo, Z., et al. (2021). Targeting RAS in Neuroblastoma: Is it Possible? *Pharmacol. Ther.* 236, 108054. doi:10.1016/j.pharmthera.2021.108054

Lin, X., Feng, D., Li, P., and Lv, Y. (2020). LncRNA LINC00857 Regulates the Progression and Glycolysis in Ovarian Cancer by Modulating the Hippo Signaling Pathway. *Cancer Med.* 9, 8122–8132. doi:10.1002/cam4.3322

Linehan, W. M., and Rouault, T. A. (2013). Molecular Pathways: Fumarate Hydratase-Deficient Kidney Cancer-Ttargeting the Warburg Effect in Cancer. *Clin. Cancer Res.* 19, 3345–3352. doi:10.1158/1078-0432.CCR-13-0304

Liu, D., and Li, H. (2019). Long Non-coding RNA GEHT1 Promoted the Proliferation of Ovarian Cancer Cells via Modulating the Protein Stability of HIF1α. *Biosci. Rep.* 39, 5. doi:10.1042/BSR20181650

Liu, J., Zhang, C., Hu, W., and Feng, Z. (2019). Tumor Suppressor P53 and Metabolism. *J. Mol. Cel Biol* 11, 284–292. doi:10.1093/jmcb/mjy070

Liu, Y., He, X., Chen, Y., and Cao, D. (2020). Long Non-coding RNA LINC00504 Regulates the Warburg Effect in Ovarian Cancer through Inhibition of miR-1244. *Mol. Cel Biochem* 464 (1-2), 39–50. doi:10.1007/s11010-019-03647-z

Liu, Y., Fu, X., Wang, X., Liu, Y., and Song, X. (2021). Long Noncoding RNA OIP5AS1 Facilitates the Progression of Ovarian Cancer via the miR1283p/CCNG1 axis. *Mol. Med. Rep.* 23, 5. doi:10.3892/mmr.2021.12027

Lu, J., Chen, H., He, F., You, Y., Feng, Z., Chen, W., et al. (2020). Ginsenoside 20(S)-Rg3 Upregulates HIF-1α-Targeting miR-519a-5p to Inhibit the Warburg Effect in Ovarian Cancer Cells. *Clin. Exp. Pharmacol. Physiol.* 47, 1455–1463. doi:10.1111/1440-1681.13321

Lu, J., Tan, M., and Cai, Q. (2015). The Warburg Effect in Tumor Progression: Mitochondrial Oxidative Metabolism as an Anti-metastasis Mechanism. *Cancer Lett.* 356, 156–164. doi:10.1016/j.canlet.2014.04.001

Lu, J. (2019). The Warburg Metabolism Fuels Tumor Metastasis. *Cancer Metastasis Rev.* 38 (1-2), 157–164. doi:10.1007/s10555-019-09794-5

Lu, J., Wang, L., Chen, W., Wang, Y., Zhen, S., Chen, H., et al. (2019). miR-603 Targeted Hexokinase-2 to Inhibit the Malignancy of Ovarian Cancer Cells. *Arch. Biochem. Biophys.* 661, 1–9. doi:10.1016/j.abb.2018.10.014

Lu, S., Han, L., Hu, X., Sun, T., Xu, D., Li, Y., et al. (2021). N6-methyladenosine Reader IMP2 Stabilizes the ZFAS1/OLA1 axis and Activates the Warburg Effect: Implication in Colorectal Cancer. *J. Hematol. Oncol.* 14, 188. doi:10.1186/s13045-021-01204-0

Mardani, M., Rashedi, S., Keykhaei, M., Farrokhpour, H., Azadnajafabad, S., Tavolinejad, H., et al. (2022). Long Non-coding RNAs (lncRNAs) as Prognostic and Diagnostic Biomarkers in Multiple Myeloma: A Systematic Review and Meta-Analysis. *Pathol. Res. Pract.* 229, 153726. doi:10.1016/j.prp.2021.153726

Martínez-Ordoñez, A., Seoane, S., Avila, L., Eiro, N., Macía, M., Arias, E., et al. (2021). POU1F1 Transcription Factor Induces Metabolic Reprogramming and Breast Cancer Progression via LDHA Regulation. *Oncogene* 40, 2725–2740. doi:10.1038/s41388-021-01740-6

Mathupala, S. P., Ko, Y. H., and Pedersen, P. L. (2009). Hexokinase-2 Bound to Mitochondria: Cancer's Stygian Link to the "Warburg Effect" and a Pivotal Target for Effective Therapy. *Semin. Cancer Biol.* 19, 17–24. doi:10.1016/j.semcancer.2008.11.006

Meijer, T. W., Kaanders, J. H., Span, P. N., and Bussink, J. (2012). Targeting Hypoxia, HIF-1, and Tumor Glucose Metabolism to Improve Radiotherapy Efficacy. *Clin. Cancer Res.* 18, 5585–5594. doi:10.1158/1078-0432.CCR-12-0858

Mendes, R. D., Canté-Barrett, K., Pieters, R., and Meijerink, J. P. (2016). The Relevance of PTEN-AKT in Relation to NOTCH1-Directed Treatment Strategies in T-Cell Acute Lymphoblastic Leukemia. *Haematologica* 101, 1010–1017. doi:10.3324/haematol.2016.146381

Mirzaei, H., and Hamblin, M. R. (2020). Regulation of Glycolysis by Non-coding RNAs in Cancer: Switching on the Warburg Effect. *Mol. Ther. Oncolytics* 19, 218–239. doi:10.1016/j.omto.2020.10.003

Mirzaei, H., Yazdi, F., Salehi, R., and Mirzaei, H. R. (2016). SiRNA and Epigenetic Aberrations in Ovarian Cancer. *J. Cancer Res. Ther.* 12, 498–508. doi:10.4103/0973-1482.153661

Moldogazieva, N. T., Mokhosoev, I. M., and Terentiev, A. A. (2020). Metabolic Heterogeneity of Cancer Cells: An Interplay between HIF-1, GLUTs, and AMPK. *Cancers (Basel)* 12, 40862. doi:10.3390/cancers12040862

Nakagawa, T., Lanaspa, M. A., Millan, I. S., Fini, M., Rivard, C. J., Sanchez-Lozada, L. G., et al. (2020). Fructose Contributes to the Warburg Effect for Cancer Growth. *Cancer Metab.* 8, 16. doi:10.1186/s40170-020-00222-9

Nakagawa, T., Sanchez-Lozada, L. G., Andres-Hernando, A., Kojima, H., Kasahara, M., Rodriguez-Iturbe, B., et al. (2021). Endogenous Fructose Metabolism Could Explain the Warburg Effect and the Protection of SGLT2 Inhibitors in Chronic Kidney Disease. *Front. Immunol.* 12, 694457. doi:10.3389/fimmu.2021.694457

Nie, H., Ju, H., Fan, J., Shi, X., Cheng, Y., Cang, X., et al. (2020). O-GlcNAcylation of PGK1 Coordinates Glycolysis and TCA Cycle to Promote Tumor Growth. *Nat. Commun.* 11, 36. doi:10.1038/s41467-019-13601-8

Ortega-Molina, A., and Serrano, M. (2013). PTEN in Cancer, Metabolism, and Aging. *Trends Endocrinol. Metab.* 24, 184–189. doi:10.1016/j.tem.2012.11.002

Park, J. S., Burckhardt, C. J., Lazcano, R., Solis, L. M., Isogai, T., Li, L., et al. (2020). Mechanical Regulation of Glycolysis via Cytoskeleton Architecture. *Nature* 578, 621–626. doi:10.1038/s41586-020-1998-1

Park, M. K., Zhang, L., Min, K.-W., Cho, J.-H., Yeh, C.-C., Moon, H., et al. (2021). NEAT1 Is Essential for Metabolic Changes that Promote Breast Cancer Growth and Metastasis. *Cel Metab.* 33, 2380–2397. doi:10.1016/j.cmet.2021.11.011

Pidíková, P., and Herichová, I. (2021). miRNA Clusters with Up-Regulated Expression in Colorectal Cancer. *Cancers (Basel)* 13, 122979. doi:10.3390/cancers13122979

Poff, A., Koutnik, A. P., Egan, K. M., Sahebjam, S., D'Agostino, D., and Kumar, N. B. (2019). Targeting the Warburg Effect for Cancer Treatment: Ketogenic Diets for Management of Glioma. *Semin. Cancer Biol.* 56, 135–148. doi:10.1016/j.semcancer.2017.12.011

Pourhanifeh, M. H., Darvish, M., Tabatabaeian, J., Fard, M. R., Mottaghi, R., Azadchehr, M. J., et al. (2020). Therapeutic Role of Curcumin and its Novel Formulations in Gynecological Cancers. *J. Ovarian Res.* 13, 130. doi:10.1186/s13048-020-00731-7

Rahimian, N., Razavi, Z. S., Aslanbeigi, F., Mirkhabbaz, A. M., Piroozmand, H., Shahrzad, M. K., et al. (2021). Non-coding RNAs Related to Angiogenesis in Gynecological Cancer. *Gynecol. Oncol.* 161, 896–912. doi:10.1016/j.ygyno.2021.03.020

Rai, G., Urban, D. J., Mott, B. T., Hu, X., Yang, S. M., Benavides, G. A., et al. (2020). Pyrazole-Based Lactate Dehydrogenase Inhibitors with Optimized Cell Activity and Pharmacokinetic Properties. *J. Med. Chem.* 63, 10984–11011. doi:10.1021/acs.jmedchem.0c00916

Rao, G., Dwivedi, S. K. D., Zhang, Y., Dey, A., Shameer, K., Karthik, R., et al. (2020). MicroRNA-195 Controls MICU1 Expression and Tumor Growth in Ovarian Cancer. *EMBO Rep.* 21, e48483. doi:10.15252/embr.201948483

Razavi, Z. S., Tajiknia, V., Majidi, S., Ghandali, M., Mirzaei, H. R., Rahimian, N., et al. (2021). Gynecologic Cancers and Non-coding RNAs: Epigenetic Regulators with Emerging Roles. *Crit. Rev. Oncol. Hematol.* 157, 103192. doi:10.1016/j.critrevonc.2020.103192

Reinfeld, B. I., Rathmell, W. K., Kim, T. K., and Rathmell, J. C. (2021). The Therapeutic Implications of Immunosuppressive Tumor Aerobic Glycolysis. *Cell Mol Immunol* 21, 727. doi:10.1038/s41423-021-00727-3

Roberts, D. J., and Miyamoto, S. (2015). Hexokinase II Integrates Energy Metabolism and Cellular protection: Akting on Mitochondria and TORCing to Autophagy. *Cell Death Differ* 22, 364–257. doi:10.1038/cdd.2014.208

Roy, B., Ghose, S., and Biswas, S. (2021). Therapeutic Strategies for miRNA Delivery to Reduce Hepatocellular Carcinoma. *Semin. Cel Dev Biol* 4, 6. doi:10.1016/j.semcdb.2021.04.006

Sakshi, S., Jayasuriya, R., Ganesan, K., Xu, B., and Ramkumar, K. M. (2021). Role of circRNA-miRNA-mRNA Interaction Network in Diabetes and its Associated Complications. *Mol. Ther. Nucleic Acids* 26, 1291–1302. doi:10.1016/j.omtn.2021.11.007

Sanaei, M. J., Baghery Saghchy Khorasani, A., Pourbagheri-Sigaroodi, A., Shahrokh, S., Zali, M. R., and Bashash, D. (2021). The PI3K/Akt/mTOR axis in Colorectal Cancer: Oncogenic Alterations, Non-coding RNAs, Therapeutic Opportunities, and the Emerging Role of Nanoparticles. *J. Cel Physiol*. [Online ahead of print] doi:10.1002/jcp.30655

Schwartz, L., Seyfried, T., Alfarouk, K. O., Da Veiga Moreira, J., and Fais, S. (2017). Out of Warburg Effect: An Effective Cancer Treatment Targeting the Tumor Specific Metabolism and Dysregulated pH. *Semin. Cancer Biol.* 43, 134–138. doi:10.1016/j.semcancer.2017.01.005

Shakespear, M. R., Iyer, A., Cheng, C. Y., Das Gupta, K., Singhal, A., Fairlie, D. P., et al. (2018). Lysine Deacetylases and Regulated Glycolysis in Macrophages. *Trends Immunol.* 39, 473–488. doi:10.1016/j.it.2018.02.009

Shen, J., Jin, Z., Lv, H., Jin, K., Jonas, K., Zhu, C., et al. (2020). PFKP Is Highly Expressed in Lung Cancer and Regulates Glucose Metabolism. *Cel Oncol (Dordr)* 43, 617–629. doi:10.1007/s13402-020-00508-6

Shulman, R. G., and Rothman, D. L. (2017). The Glycogen Shunt Maintains Glycolytic Homeostasis and the Warburg Effect in Cancer. *Trends Cancer* 3, 761–767. doi:10.1016/j.trecan.2017.09.007

Sinha, T., Panigrahi, C., Das, D., and Chandra, Panda. A. (2021). *Circular RNA Translation, a Path to Hidden Proteome*. Wiley Interdiscip Rev RNA, e1685.

Smiles, W. J., and Camera, D. M. (2018). The Guardian of the Genome P53 Regulates Exercise-Induced Mitochondrial Plasticity beyond Organelle Biogenesis. *Acta Physiol. (Oxf)* 222, 3. doi:10.1111/apha.13004

Storkus, W. J., Maurer, D., Lin, Y., Ding, F., Bose, A., Lowe, D., et al. (2021). Dendritic Cell Vaccines Targeting Tumor Blood Vessel Antigens in Combination with Dasatinib Induce Therapeutic Immune Responses in Patients with Checkpoint-Refractory Advanced Melanoma. *J. Immunother. Cancer* 9, 11. doi:10.1136/jitc-2021-003675

Strycharz, J., Drzewoski, J., Szemraj, J., and Sliwinska, A. (2017). Erratum to "Is P53 Involved in Tissue-specific Insulin Resistance Formation?". *Oxid Med. Cel Longev* 2017, 8036902. doi:10.1155/2017/8036902

Su, H., Huang, J., Weng, S., Zhang, B., Zhang, T., and Xu, Y. (2021). Glutathione Synthesis Primes Monocytes Metabolic and Epigenetic Pathway for β-glucan-trained Immunity. *Redox Biol.* 48, 102206. doi:10.1016/j.redox.2021.102206

Sun, L., Suo, C., Li, S. T., Zhang, H., and Gao, P. (2018). Metabolic Reprogramming for Cancer Cells and Their Microenvironment: Beyond the Warburg Effect. *Biochim. Biophys. Acta Rev. Cancer* 1870, 51–66. doi:10.1016/j.bbcan.2018.06.005

Sun, X., and Feinberg, M. W. (2021). Vascular Endothelial Senescence: Pathobiological Insights, Emerging Long Noncoding RNA Targets, Challenges and Therapeutic Opportunities. *Front. Physiol.* 12, 693067. doi:10.3389/fphys.2021.693067

Tan, V. P., and Miyamoto, S. (2015). HK2/hexokinase-II Integrates Glycolysis and Autophagy to Confer Cellular protection. *Autophagy* 11, 963–964. doi:10.1080/15548627.2015.1042195

Tang, Y.-C., Hsiao, J.-R., Jiang, S.-S., Chang, J.-Y., Chu, P.-Y., Liu, K.-J., et al. (2021). c-MYC-directed NRF2 Drives Malignant Progression of Head and Neck Cancer via Glucose-6-Phosphate Dehydrogenase and Transketolase Activation. *Theranostics* 11, 5232–5247. doi:10.7150/thno.53417

Tao, L. M., Gong, Y. F., Yang, H. M., Pei, J. H., Zhao, X. J., and Liu, S. S. (2020). LINC00662 Promotes Glycolysis and Cell Survival by Regulating miR- 375/HIF-1α axis in Ovarian Cancer. *J. Biol. Regul. Homeost Agents* 34 (3), 467–477. doi:10.23812/19-300-A-18

Tekade, R. K., and Sun, X. (2017). The Warburg Effect and Glucose-Derived Cancer Theranostics. *Drug Discov. Today* 22, 1637–1653. doi:10.1016/j.drudis.2017.08.003

Teng, Y., Zhang, Y., Qu, K., Yang, X., Fu, J., Chen, W., et al. (2015). MicroRNA-29B (Mir-29b) Regulates the Warburg Effect in Ovarian Cancer by Targeting AKT2 and AKT3. *Oncotarget* 6, 40799–40814. doi:10.18632/oncotarget.5695

Tyagi, K., Mandal, S., and Roy, A. (2021). Recent Advancements in Therapeutic Targeting of the Warburg Effect in Refractory Ovarian Cancer: A Promise towards Disease Remission. *Biochim. Biophys. Acta Rev. Cancer* 1876, 188563. doi:10.1016/j.bbcan.2021.188563

Tymon-Rosario, J., Adjei, N. N., Roque, D. M., and Santin, A. D. (2021). Microtubule-Interfering Drugs: Current and Future Roles in Epithelial Ovarian Cancer Treatment. *Cancers (Basel)* 13, 24. doi:10.3390/cancers13246239

Upadhyay, M., Samal, J., Kandpal, M., Singh, O. V., and Vivekanandan, P. (2013). The Warburg Effect: Insights from the Past Decade. *Pharmacol. Ther.* 137, 318–330. doi:10.1016/j.pharmthera.2012.11.003

Vafadar, A., Shabaninejad, Z., Movahedpour, A., Fallahi, F., Taghavipour, M., Ghasemi, Y., et al. (2020). Quercetin and Cancer: New Insights into its Therapeutic Effects on Ovarian Cancer Cells. *Cell Biosci* 10, 32. doi:10.1186/s13578-020-00397-0

van Niekerk, G., and Engelbrecht, A. M. (2018). Role of PKM2 in Directing the Metabolic Fate of Glucose in Cancer: a Potential Therapeutic Target. *Cel Oncol (Dordr)* 41, 343–351. doi:10.1007/s13402-018-0383-7

Vergote, I., Gonzalez-Martin, A., Ray-Coquard, I., Harter, P., Colombo, N., Pujol, P., et al. (2021). European Experts Consensus: BRCA/homologous Recombination Deficiency Testing in First-Line Ovarian Cancer. *Ann. Oncol.* 33 (3), 276–287. doi:10.1016/j.annonc.2021.11.013

Wang, D., Li, C., Zhu, Y., Song, Y., Lu, S., Sun, H., et al. (2021). TEPP-46-Based AIE Fluorescent Probe for Detection and Bioimaging of PKM2 in Living Cells. *Anal. Chem.* 93, 12682–12689. doi:10.1021/acs.analchem.1c02529

Ward, P. S., and Thompson, C. B. (2012). Metabolic Reprogramming: a Cancer Hallmark Even Warburg Did Not Anticipate. *Cancer Cell* 21 (3), 297–308. doi:10.1016/j.ccr.2012.02.014

Werner, H., Sarfstein, R., LeRoith, D., and Bruchim, I. (2016). Insulin-like Growth Factor 1 Signaling Axis Meets P53 Genome Protection Pathways. *Front. Oncol.* 6, 159. doi:10.3389/fonc.2016.00159

Wu, M., Yang, L.-Z., and Chen, L.-L. (2021). Long Noncoding RNA and Protein Abundance in lncRNPs. *RNA* 27, 1427–1440. doi:10.1261/rna.078971.121

Xia, Q., Jia, J., Hu, C., Lu, J., Li, J., Xu, H., et al. (2021). Tumor-associated Macrophages Promote PD-L1 Expression in Tumor Cells by Regulating PKM2 Nuclear Translocation in Pancreatic Ductal Adenocarcinoma. *Oncogene*. doi:10.1038/s41388-021-02133-5

Xie, H., Wang, W., Qi, W., Jin, W., and Xia, B. (2021). Targeting DNA Repair Response Promotes Immunotherapy in Ovarian Cancer: Rationale and Clinical Application. *Front. Immunol.* 12, 661115. doi:10.3389/fimmu.2021.661115

Xie W, W., Liu, L. U., He, C., Zhao, M., Ni, R., Zhang, Z., et al. (2021). Circ_0002711 Knockdown Suppresses Cell Growth and Aerobic Glycolysis by Modulating miR-1244/ROCK1 axis in Ovarian Cancer. *J. Biosci.* 46, 136. doi:10.1007/s12038-020-00136-0

Xu, D., Liang, J., Lin, J., and Yu, C. (2019). PKM2: A Potential Regulator of Rheumatoid Arthritis via Glycolytic and Non-glycolytic Pathways. *Front. Immunol.* 10, 2919. doi:10.3389/fimmu.2019.02919

Xu, H., Sun, X., Huang, Y., Si, Q., and Li, M. (2020). Long Non-coding RNA NEAT1 M-odifies C-ell P-roliferation, colony F-ormation, A-poptosis, M-igration and I-nvasion via the miR-4500/BZW1 axis in O-varian C-ancer. *Mol. Med. Rep.* 22, 3347–3357. doi:10.3892/mmr.2020.11408

Xu, S., and Herschman, H. R. (2019). A Tumor Agnostic Therapeutic Strategy for Hexokinase 1-Null/Hexokinase 2-Positive Cancers. *Cancer Res.* 79, 5907–5914. doi:10.1158/0008-5472.CAN-19-1789

Xu, S., Jia, G., Zhang, H., Wang, L., Cong, Y., Lv, M., et al. (2021). LncRNA HOXB-AS3 Promotes Growth, Invasion and Migration of Epithelial Ovarian Cancer by Altering Glycolysis. *Life Sci.* 264, 118636. doi:10.1016/j.lfs.2020.118636

Xu, X. L., Deng, S. L., Lian, Z. X., and Yu, K. (2021). Resveratrol Targets a Variety of Oncogenic and Oncosuppressive Signaling for Ovarian Cancer Prevention and Treatment. *Antioxidants (Basel)* 10, 1718. doi:10.3390/antiox10111718

Yalan, S., Yanfang, L., He, C., and Yujie, T. (2020). Circular RNA circRHOBTB3 Inhibits Ovarian Cancer Progression through PI3K/AKT Signaling Pathway. *Panminerva Med.* doi:10.23736/s0031-0808.20.03957-9

Yang, C., Wang, R., and Hardy, P. (2021). Potential of miRNA-Based Nanotherapeutics for Uveal Melanoma. *Cancers (Basel)* 13, 5192. doi:10.3390/cancers13205192

Yang, G. J., Wu, J., Leung, C. H., Ma, D. L., and Chen, J. (2021). A Review on the Emerging Roles of Pyruvate Kinase M2 in Anti-leukemia Therapy. *Int. J. Biol. Macromol* 193, 1499–1506. doi:10.1016/j.ijbiomac.2021.10.213

Yang, J., Ren, B., Yang, G., Wang, H., Chen, G., You, L., et al. (2020). The Enhancement of Glycolysis Regulates Pancreatic Cancer Metastasis. *Cel Mol Life Sci* 77, 305–321. doi:10.1007/s00018-019-03278-z

Yellen, G. (2018). Fueling Thought: Management of Glycolysis and Oxidative Phosphorylation in Neuronal Metabolism. *J. Cel Biol* 217, 2235–2246. doi:10.1083/jcb.201803152

Yeung, S. J., Pan, J., and Lee, M. H. (2008). Roles of P53, MYC and HIF-1 in Regulating Glycolysis - the Seventh Hallmark of Cancer. *Cel Mol Life Sci* 65, 3981–3999. doi:10.1007/s00018-008-8224-x

Yin, X., Choudhury, M., Kang, J. H., Schaefbauer, K. J., Jung, M. Y., Andrianifahanana, M., et al. (2019). Hexokinase 2 Couples Glycolysis with the Profibrotic Actions of TGF-β. *Sci. Signal.* 12, 612. doi:10.1126/scisignal.aax4067

Youssef, A., Haskali, M. B., and Gorringe, K. L. (2021). The Protein Landscape of Mucinous Ovarian Cancer: Towards a Theranostic. *Cancers (Basel)* 13, 596. doi:10.3390/cancers13225596

Yu, H., Yang, W., Huang, J., Miao, X., Wang, B., Ren, X., et al. (2021). GPR120 Induces Regulatory Dendritic Cells by Inhibiting HK2-dependent Glycolysis to Alleviate Fulminant Hepatic Failure. *Cel Death Dis* 13, 1. doi:10.1038/s41419-021-04394-0

Yuan, Q., Zhang, J., Liu, Y., Chen, H., Liu, H., Wang, J., et al. (2021). MyD88 in Myofibroblasts Regulates Aerobic Glycolysis-Driven Hepatocarcinogenesis via ERK-dependent PKM2 Nuclear Relocalization and Activation. *J. Pathol.* 256 (4), 414–426. doi:10.1002/path.5856

Yue, J., Jin, S., Gu, S., Sun, R., and Liang, Q. (2019). High Concentration Magnesium Inhibits Extracellular Matrix Calcification and Protects Articular Cartilage via Erk/autophagy Pathway. *J. Cel Physiol* 234, 23190–23201. doi:10.1002/jcp.28885

Yue, J., Jin, S., Li, Y., Zhang, L., Jiang, W., Yang, C., et al. (2016). Magnesium Inhibits the Calcification of the Extracellular Matrix in Tendon-Derived Stem Cells via the ATP-P2r and Mitochondrial Pathways. *Biochem. Biophys. Res. Commun.* 478, 314–322. doi:10.1016/j.bbrc.2016.06.108

Zeng, X., Yuan, X., Cai, Q., Tang, C., and Gao, J. (2021). Circular RNA as an Epigenetic Regulator in Chronic Liver Diseases. *Cells* 339, 1945. doi:10.3390/cells10081945

Zhang, J., Zhang, J., Wei, Y., Li, Q., and Wang, Q. (2019). ACTL6A Regulates Follicle-Stimulating Hormone-Driven Glycolysis in Ovarian Cancer Cells via PGK1. *Cel Death Dis* 10, 811. doi:10.1038/s41419-019-2050-y

Zhang, S., Pei, M., Li, Z., Li, H., Liu, Y., and Li, J. (2018). Double-negative Feedback Interaction between DNA Methyltransferase 3A and microRNA-145 in the Warburg Effect of Ovarian Cancer Cells. *Cancer Sci.* 109, 2734–2745. doi:10.1111/cas.13734

Zhao, L., Ji, G., Le, X., Wang, C., Xu, L., Feng, M., et al. (2017). Long Noncoding RNA LINC00092 Acts in Cancer-Associated Fibroblasts to Drive Glycolysis and Progression of Ovarian Cancer. *Cancer Res.* 77, 1369–1382. doi:10.1158/0008-5472.CAN-16-1615

Zhao, Q., Li, J., Wu, B., Shang, Y., Huang, X., Dong, H., et al. (2020). Smart Biomimetic Nanocomposites Mediate Mitochondrial Outcome through Aerobic Glycolysis Reprogramming: A Promising Treatment for Lymphoma. *ACS Appl. Mater. Inter.* 12, 22687–22701. doi:10.1021/acsami.0c05763

Zhao, S., Heng, N., Weldegebriall Sahlu, B., Wang, H., and Zhu, H. (2021). Long Noncoding RNAs: Recent Insights into Their Role in Male Infertility and Their Potential as Biomarkers and Therapeutic Targets. *Int. J. Mol. Sci.* 22, 24. doi:10.3390/ijms222413579

Zheng, X., Zhou, Y., Chen, W., Chen, L., Lu, J., He, F., et al. (2018). Ginsenoside 20(S)-Rg3 Prevents PKM2-Targeting miR-324-5p from H19 Sponging to Antagonize the Warburg Effect in Ovarian Cancer Cells. *Cell Physiol Biochem* 51, 1340–1353. doi:10.1159/000495552

Zhong, J., Kang, Q., Cao, Y., He, B., Zhao, P., Gou, Y., et al. (2021). BMP4 Augments the Survival of Hepatocellular Carcinoma (HCC) Cells under Hypoxia and Hypoglycemia Conditions by Promoting the Glycolysis Pathway. *Am. J. Cancer Res.* 11, 793–811.

Zhong, J. T., and Zhou, S. H. (2017). Warburg Effect, Hexokinase-II, and Radioresistance of Laryngeal Carcinoma. *Oncotarget* 8, 14133–14146. doi:10.18632/oncotarget.13044

Zhou, Y., Zheng, X., Lu, J., Chen, W., Li, X., and Zhao, L. (2018). Ginsenoside 20(S)-Rg3 Inhibits the Warburg Effect via Modulating DNMT3A/ MiR-532-3p/ HK2 Pathway in Ovarian Cancer Cells. *Cel Physiol Biochem* 45, 2548–2559. doi:10.1159/000488273

Zuo, J., Tang, J., Lu, M., Zhou, Z., Li, Y., Tian, H., et al. (2021). Glycolysis Rate-Limiting Enzymes: Novel Potential Regulators of Rheumatoid Arthritis Pathogenesis. *Front. Immunol.* 12, 779787. doi:10.3389/fimmu.2021.779787

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

frontiers | Frontiers in Pharmacology

ORIGINAL RESEARCH
published: 05 May 2022
doi: 10.3389/fphar.2022.855351

GM-CSF-miRNA-Jak2/Stat3 Signaling Mediates Chemotherapy-Induced Cancer Cell Stemness in Gastric Cancer

Xue Xiang[1,2,3†], *Hai-zhong Ma*[1,3†], *Ya-qiong Chen*[1,3†], *Dong-zhi Zhang*[1,3], *Shi-xu Ma*[1,3], *Hong-jing Wang*[1,3], *De-ming Liu*[1,2,3], *Yuan Yuan*[1,3] *and Hui Cai*[1,3,4*]

[1]*Gansu General Surgery Clinical Medical Center, Gansu Provincial Hospital, Lanzhou, China,* [2]*Department of Clinical Medicine, Ningxia Medical University, Yinchuan, China,* [3]*Key Laboratory of Molecular Diagnostics and Precision Medicine for Surgical Oncology in Gansu Province, Gansu Provincial Hospital, Gansu, China,* [4]*NHC Key Laboratory of Diagnosis and Therapy of Gastrointestinal Tumor, Gansu Provincial Hospital, Lanzhou, China*

Chemotherapy serves as the first choice in clinic to treat advanced gastric cancer. However, emerging evidence indicated the induction of drug resistance and cancer stem cells occasionally by chemotherapy, which seriously limit the therapeutic effects, but the regulatory mechanism remains unclear. Here we treated two human gastric cancer cell lines SGC7901 and BGC823 with 5-Fluorouracil (5-Fu) or Cisplatin (DDP) *in vitro*. The survived cells showed significant increase of drug resistance, cell stemness and cytokine *GM-CSF* expression and secretion. As such, *GM-CSF* was applied to stimulate gastric cancer cells, followed by the subpopulation of *CD133*$^{+}$ CSC analysis, sphere formation assay and stemness genes expression analysis. As a result, CSCs showed induction by *GM-CSF* treatment. A gastric cancer animal model further indicated that the gastric cancer cells significantly promoted tumor growth after *GM-CSF* treatment *in vivo*. High-throughput miRNA and mRNA sequencing analyses identified a subset of miRNAs and mRNAs under regulation of both 5-Fu and *GM-CSF* in gastric cancer cells, including upregulation of *miR-877-3p* and downregulation of *SOCS2*. Targeted overexpression or knockdown of *miR-877-3p* in gastric cancer cells revealed the oncogenic function of *miR-877-3p* in regulating gastric cancer by suppressing target gene *SOCS2. Jak2/Stat3* signaling pathway, as a downstream target of *SOCS2*, showed activation *in vitro* and *in vivo* after treatment with *miR-877-3p* or *GM-CSF*. Our findings not only revealed a novel mechanism through which chemotherapy induced CSCs in gastric cancer via *GM-CSF-miRNA-Jak2/Stat3* signaling, but also provided an experimental evidence for appropriate dose reduction of adjuvant chemotherapy in treatment of cancer patients.

Keywords: gastric cancer, cancer stem cells, GM-CSF, miR-877–3p, chemotherapy

OPEN ACCESS

Edited by:
Zhengquan Yu,
China Agricultural University, China

Reviewed by:
Zhaoyuan Hou,
Shanghai Jiao Tong University, China
Cong Lv,
China Agricultural University, China

****Correspondence:***
Hui Cai
caialonteam@163.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *15 January 2022*
Accepted: *14 March 2022*
Published: *05 May 2022*

Citation:
Xiang X, Ma H-z, Chen Y-q, Zhang D-z, Ma S-x, Wang H-j, Liu D-m, Yuan Y and Cai H (2022) GM-CSF-miRNA-Jak2/Stat3 Signaling Mediates Chemotherapy-Induced Cancer Cell Stemness in Gastric Cancer. Front. Pharmacol. 13:855351. doi: 10.3389/fphar.2022.855351

INTRODUCTION

Gastric cancer (GC), as the fourth leading cause of cancer death all over the world (Sung et al., 2021), shows good clinical outcomes to chemotherapy including 5-Fluoride (5-FU) and Cisplatin (DDP) (Seo et al., 2019). However, chemo-resistance is commonly observed in patients with GC after chemotherapy (Choi et al., 2002). Chemotherapy-induced resistance was reported to be related to the

acquisition of stem cell-like properties in cancer cells (Li and Clevers, 2010; Xu et al., 2015). This type of cell is called cancer stem cells (CSCs), which are characterized by self-renewal, differentiation, strong tumor-regenerative ability and resistance to therapy. CSCs are believed to play important roles in tumor invasion, cancer metastasis and cancer recurrence (Alison et al., 2012; Schulenburg et al., 2015). Since the first identification of CSCs in myeloid leukemia in 1997, numerous studies have identified CSCs in multiple types of solid cancer tumors including breast cancer, brain cancer, prostate cancer and GC (Bonnet and Dick, 1997; Al-Hajj et al., 2003; Ricci-Vitiani et al., 2007; Takaishi et al., 2009). Although the regulation of chemo-resistance and cancer relapse by CSCs has been well demonstrated, the molecular mechanisms remain unclear.

Tissue damage and inflammatory response caused by chemotherapy are believed as one of the main causes of chemo-resistance. In addition to kill cancer cells, chemotherapy causes the abundance changes of a variety of inflammatory factors in the microenvironment, affecting the chemotherapeutic outcomes (Edwardson et al., 2019). For example, granulocyte-macrophage colony-stimulating factor (*GM-CSF*), as a monomeric cytokine involving in the immune modulation and hematopoiesis, can be induced by chemotherapy (Hong, 2016; O'Shaughnessy et al., 1994). *GM-CSF* is mostly secreted by activated monocytes, macrophages, T cells, B cells, fibroblasts, mast cells, vascular endothelial cells, and a variety of cancer cells (Shi et al., 2006), regulating proliferation and maturation of immune cells including dendritic cells, granulocytes and macrophages (McLeish et al., 1998; Pei et al., 1999; Ju et al., 2016). Emerging evidence indicates *GM-CSF* acting as a tumor-driver in some cases by promoting tumor growth and progression in multiple cancer types, such as meningiomas, gliomas, skin cancer, head and neck cancer, lung cancer, and so on (Pei et al., 1999; Obermueller et al., 2004; Gutschalk et al., 2006; Uemura et al., 2006; Hong, 2016; Sielska et al., 2020).

MicroRNAs (miRNAs) are a class of highly conserved small non-coding RNA with 18–24 nucleotides in length. Typically, miRNAs bind to the 3′-untranslated region (3′-UTR) of target mRNAs, directing the formation of miRNA-mRNA silencing complexes and leading to degradation or translational inhibition of the targeted mRNAs (Bartel, 2009; Su et al., 2015). MiRNAs play an important role in regulating cancer cell stemness, tumor regeneration, metastasis and chemo-resistance during the development and progression of cancer (Sun et al., 2014; Rupaimoole and Slack, 2017) via targeting various signaling pathways including *Wnt, Akt, Jak/Stat*, et al. (Gomes et al., 2016; Matsui, 2016; Mihanfar et al., 2019). For example, *miR-106a-3p* induced apatinib resistance in gastric cancer cells by targeting the Cytokine signaling (SOCS) system and activating *Jak2/Stat3* signaling (Guo et al., 2019). Activation of the *Jak2/Stat3* signaling promotes cell proliferation and cell stemness in cancer (Yu et al., 2014; Park et al., 2019). SOCS proteins function as negative regulators of cytokine-triggered cell signaling. In gastric cancer, *Jak/Stat* signal pathway is frequently deregulated by the SOCS family and miRNAs (Zhou et al., 2015; Guo et al., 2019).

In the current study, we demonstrated the increased level of *GM-CSF* both inside and outside of the survived gastric cancer cells after treatment with 5-FU or DDP, which was associated with promoted drug resistance and cell stemness. In order to determine the relationship between the increased *GM-CSF* level and promoted cell stemness after chemotherapy in GC, exogenous *GM-CSF* was applied to the culture medium of GC cells, followed by the analysis of $CD133^+$ CSC subpopulation, indicating positive regulation of cancer cell stemness by *GM-CSF* stimulation *in vitro*. A GC animal model further demonstrated increased growth of tumors derived from the *GM-CSF*-treated GC cells *in vivo*. To further reveal the regulatory mechanism, high-throughput miRNA and mRNA sequencing analyses were applied to the GC cells before and after chemotherapy or *GM-CSF* treatment. As a result, a subset of miRNAs was identified with deregulation upon treatment with 5-FU or *GM-CSF*, including upregulation of *miR-877-3p* and downregulation of *SOCS2*. Functional assays demonstrated that *miR-877-3p* is capable to promote GC cell proliferation and cell stemness. *SOCS2* was identified as a key direct target gene of *miR-877-3p* in GC, where *miR-877-3p* suppressed the expression of *SOCS2* and promoted cancer cell stemness and chemoresistance subsequently by activating *Jak2/Stat3* signaling. The current study is the first to demonstrate a mechanism through which *GM-CSF-miRNA-Jak/Stat* signaling mediates chemotherapy-induced cell stemness and drug resistance in gastric cancer.

MATERIALS AND METHODS

Animals. Six-week-old immune-deficient female nude mice were purchased from the SiPeiFu Animal Company (Beijing, China) for *in vivo* assays. 2×10^6 SGC7901 cells with or without *GM-CSF* stimulation were transplanted per mouse by subcutaneous injection to establish the animal model with gastric cancer. All animal studies were performed following the relevant guidelines, regulations and protocols approved by our Institutional Animal Care and Use Committee.

Cells. Human gastric cancer cell lines SGC7901 and BGC823 were purchased from the cell bank of the Chinese Academy of Sciences at Shanghai, China, maintained in our lab, and cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 1% penicillin-streptomycin and 10% fetal bovine serum (Gibco, United States). All of these cells were cultured at 37°C with 5% CO_2 in a humidified environment.

RNA Extraction, miRNA and mRNA sequencing, Bioinformatics analysis. Total RNA was extracted using Trizol reagent (Invitrogen, Thermo Fisher Scientific) following the manufacturer's instructions. The quantity of the total RNA was accessed by NanoDrop One spectrophotometer (Thermo Fisher Scientific), and the integrity of the RNA was assessed by Bioanalyzer 2,100 (Agilent, CA, United States) with RIN number >7.0, and confirmed by electrophoresis with denaturing agarose gel. After quality check, approximately 1 μg of total RNA was used to prepare small RNA library according to protocol of TruSeq Small RNA Sample Prep Kits (Illumina, San Diego,

United States), and approximately 1 μg of total RNA was used for mRNA library. In two libraries, we performed the single-end sequencing (1 × 50 bp) on an Illumina Hiseq2500 and paired-end sequencing (2 × 150 bp) on an illumine Novaseq™ 6000 LC-Bio Technology Company, Ltd., (Hangzhou, China) following the vendor's recommended protocol. Differentially expressed miRNAs based on normalized deep-sequencing counts were analyzed using Student's t-test. The screening criteria were a fold change >−2 and $p < 0.01$. The differentially expressed mRNAs were selected with log2 (fold change) > 1 or log2 (fold change) <−1 and with statistical significance (p-value < 0.05) by the edgeR package. After quality control, bioinformatics analyses (Heatmaps and Venn diagram) were performed with the online OmicStudio tools at http://www.omicstudio.cn/tool.

Plasmids, oligos, and transfection. *miR-877-3p* mimics, *anti-miR-877-3p* inhibitors, and corresponding negative controls were synthesized by RiboBio Co., Ltd. (Guangzhou, China). Firefly luciferase reporter plasmids carrying either wild type or mutated *SOCS2* 3′UTR were constructed by Genomeditech company (Shanghai, China). Oligo transfection was performed using lipofectamine 2000 (Invitrogen, United States) following the manufacturer's instructions. A final concentration of 30 nM of miRNA mimic or negative control was used in all *in vitro* assays.

First strand cDNA preparation and Real-Time PCR. Total RNAs were extracted by using Trizol reagent (Invitrogen, Thermo Fisher Scientific). The method of adding a poly A tail to small RNAs was used for reverse transcription of miRNAs. Prime script™ RT Reagent kit with gDNA Eraser (Takara, Japan) was used for reverse transcription of mRNAs. Power Up SYBR Green Master Mix (Applied Biosystem, Thermo Fisher Scientific) and Applied Biosystems QuantStudio 6 (Applied Biosystem, Thermo Fisher Scientific) were used for real-time PCR assays. GAPDH and 5s rRNA were used for mRNA and miRNA normalization. GAPDH forward: 5'-GGAGCGAGATCCCTCCAAAAT-3'; reverse: 5'-GGCTGTTGTCATACTTCTCATGG-3'; 5s forward: 5'-AGTACTTGGATGGGAGACCG-3'; miR-877-3p forward: 5'-UCCUCUUCUCCCUCCUCCCAG-3'.

Quantitative analysis of *GM-CSF*. Secreted *GM-CSF* in the supernatant of SGC7901 or BGC823 cells before or after treatment with 5-FU or DDP was quantified using sandwich ELISA following the manufacturer's instructions (Multi Sciences, Hangzhou, China).

Western Blot. Cells were lysed in RIPA buffer (Beyotime, China), and protein concentration was measured using a BCA Assay Kit (Beyotime, China). 50μg protein lysates were prepared and resolved by 8–12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS/PAGE) and transferred onto an Immuno-Blot Polyvinylidene difluoride (PVDF) membrane (Millipore, United States). The membranes were then blocked with 5% non-fat milk in TBST for 1 h at room temperature and subsequently incubated with the primary antibodies in 1:1,000 dilution overnight at 4°C. After washing with TBST three times, then the membrane was incubated with the secondary antibody for 1 h at room temperature. Protein bands were visualized using the Minichemi chemiluminescence Imaging System (Beijing Sage Creation Science Co., Ltd., China). The following antibodies were used for Western blot: anti-*SOCS2* (2779T, Cell Signaling Technology), anti-*JAK2* (3230T, Cell Signaling Technology), anti-*p-JAK2* (4406T, Cell Signaling Technology), anti-STAT3 (9139T, Cell Signaling Technology), anti-*p-STAT3* (9145T, Cell Signaling Technology), anti-*OCT4* (2750S, Cell Signaling Technology), anti-*NANOG* (4903S, Cell Signaling Technology), anti-*GAPDH* (sc-47724, Santa Cruz), anti-*KLF4* (sc-393462, Santa Cruz), anti-*h-TERT* (sc-377511, Santa Cruz), anti-*GM-CSF* (sc-32753, Santa Cruz) and anti-*β-tubulin* (ab18207, Abcam). Secondary antibodies (1:10,000) were HRP-linked anti-rabbit IgG (7074S, Cell Signaling Technology) and HRP-linked anti-mouse IgG (7076S, Cell Signaling Technology).

Cell proliferation assay. For proliferation assay, 3,000 cells per well were seeded into 96-well culture plates in triplicate. and incubated for 2 days at 37°C in a humidified incubator with 5% CO_2. Every 24 h interval, each well was added with 10 μL CCK-8 solution (SB-CCK8, Sharebio, Shanghai, China), then cultured for 3 h at cell culturing condition followed by measurement of OD value at 450 nm wavelength (SpectraMax M5, MolecularDevices, United States).

Colony formation assay. Cancer cells were plated into a 6-well plate at a density of 2,000 cells/well, and after 7-14 days culture until visible colonies were formed. Then, colonies were washed with PBS and fixed with 4% paraformaldehyde. Finally, the visible colonies were stained with 0.5% crystal violet for 20 min. All experiments have three repetitions.

Sphere formation assay. After GC cells were transfected with miRNA-877-3p (mimic, negative control and inhibitor) for 24h, 2,000 GC cells per well were seeded into a 6-well ultra-low attachment cell culture plate (Corning, United States), and cultured with 20 ng/ml of bFGF (R&D Systems, United States), 20 ng/mL EGF (Sigma, United States), and 1×B27 supplement (Invitrogen, United States) in stem cell medium DMEM/F12. The number and sizes of tumorsphere in each well were determined after incubation for 10 days.

Luciferase reporter assay. pGL-3 luciferase reporter plasmids carrying either wild type or mutated SOCS2 3'UTR and Renilla luciferase plasmid (pRL-TK) were co-transfected into 293T cells with miR-877-3p mimic or negative control in a 24-well plate. After 18-h transfection. Luciferase activities were determined with the Dual-Luciferase Reporter Assay kit (Promega, USA).

Statistical analysis. Quantitative data are expressed as mean ±SEM unless otherwise stated. Statistical significance was determined using Student's t-test followed by least-significant difference (LSD). The data were considered to be significant when the $P < 0.05$.

RESULTS

Induction of drug resistance and *GM-CSF* expression/secretion by chemotherapy in gastric cancer. In view of observation GM-CSF is overexpressed in tumor cells after radiotherapy and induced tumor migration (Vilalta et al., 2014; Vilalta et al., 2018). GM-CSF combined with chemoradiation could trigger abscopal effect (Benna et al., 2020). Highly expressed granulocyte colony-stimulating factor (G-CSF) and granulocyte colony-

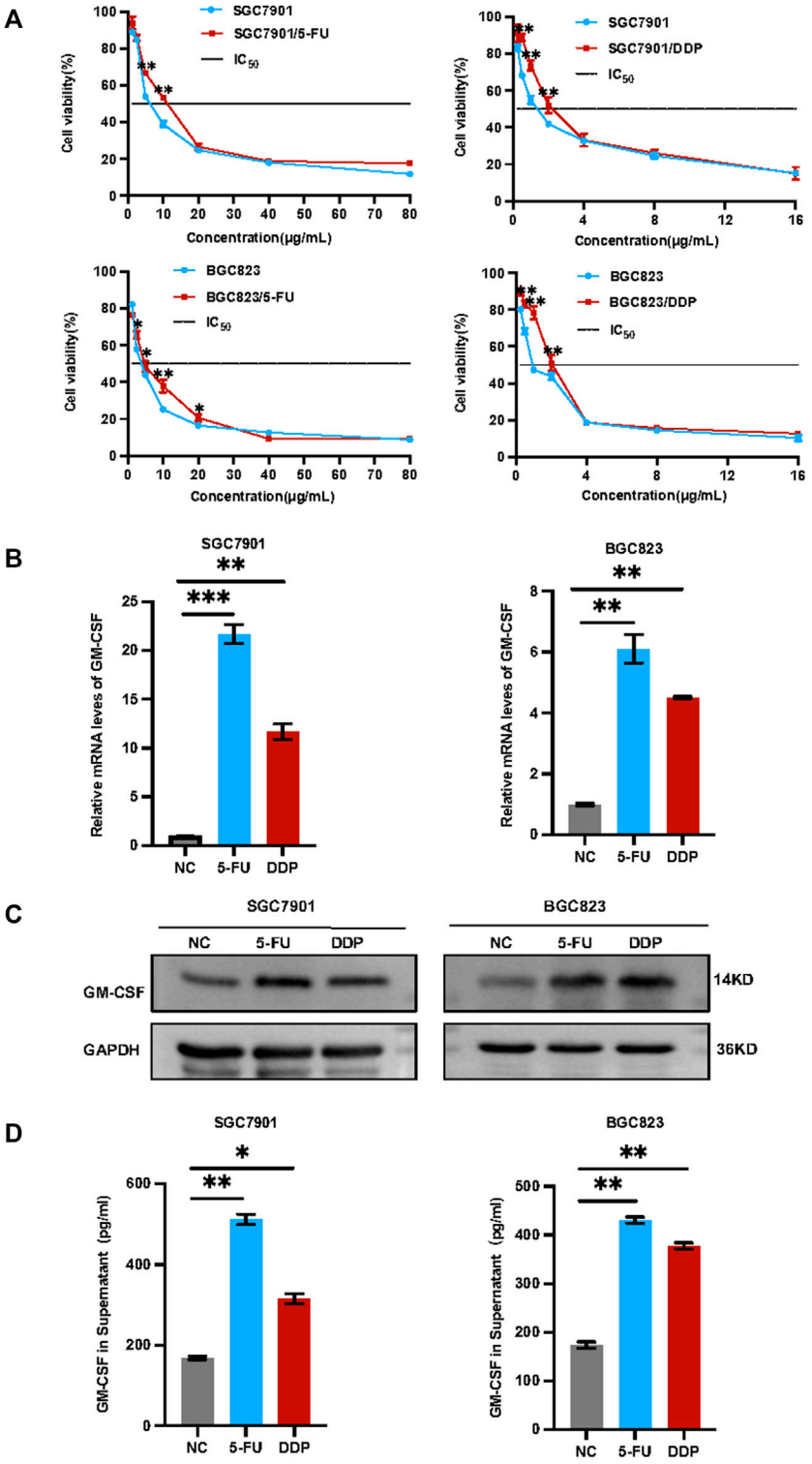


FIGURE 1 | Induction of drug resistance and *GM-CSF* expression/secretion in the survived gastric cancer cells after chemotherapy. **(A)**: IC_{50} analysis of the survived SGC7901 and BGC823 cells after treatment with 5-FU or DDP for 72 h. **(B)**: QRT-PCR analysis of the *GM-CSF* mRNA levels in the survived SGC7901 and BGC823 cells. **(C)**: Western blot analysis of the GM-CSF protein levels in the survived SGC7901 and BGC823 cells. **(D)**: ELISA analysis of the *GM-CSF* levels in supernatants of the survived SGC7901 and BGC823 cells. Data are presented as the mean ± SEM ($N = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

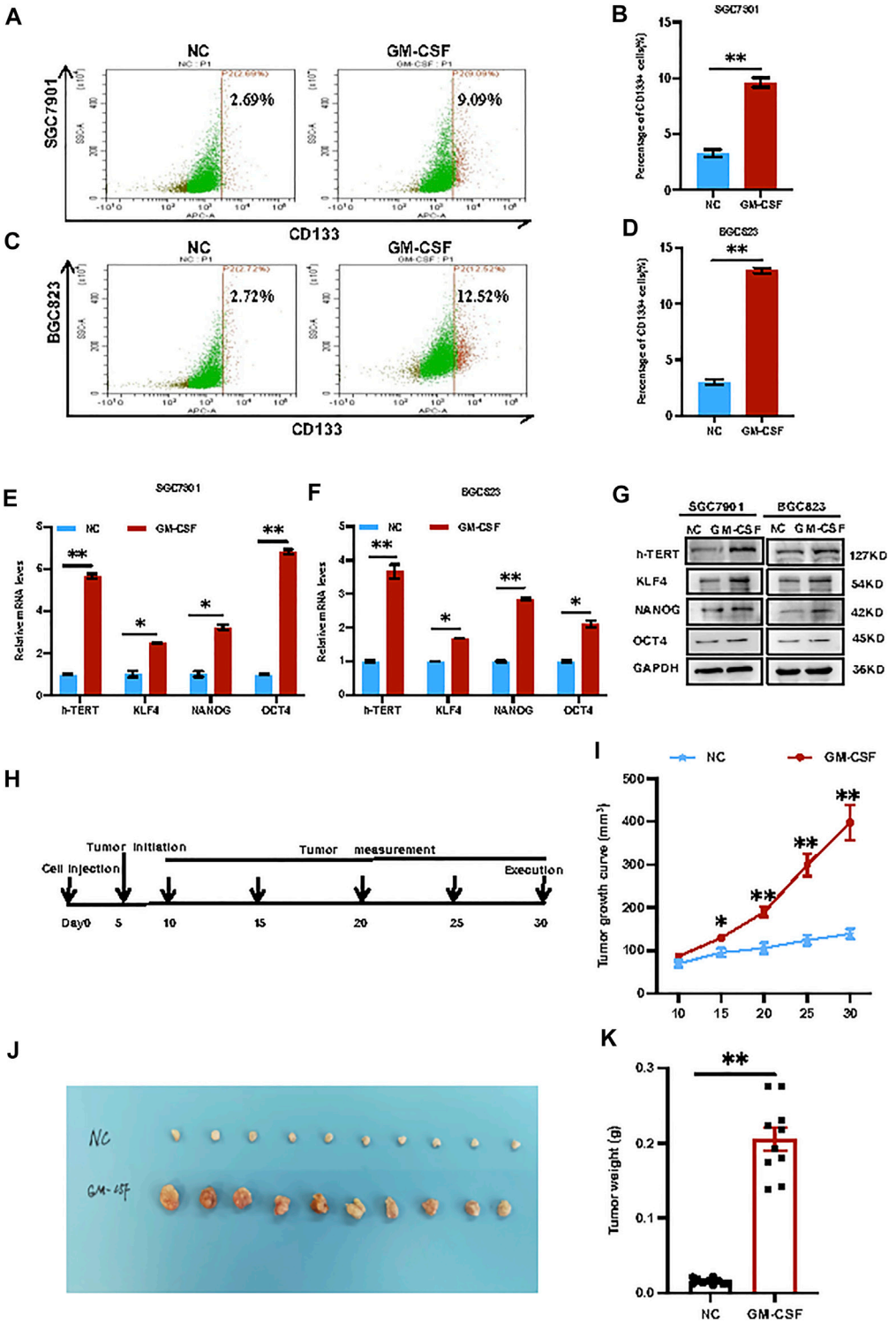


FIGURE 2 | *GM-CSF* treatment promoted cancer cell stemness *in vitro* and tumorigenesis *in vivo*. **(A,C)**: Flow cytometry analysis of *CD133*$^+$ CSCs in SGC7901 **(A)** and BGC823 **(C)** cells before and after stimulation with exogenous GM-CSF in the cell culture medium. B and D: Quantitative analysis of A **(B)** and C **(D)**. **(E,F)**: QRT-PCR analysis of the stemness genes expression (*h-Tert*, *Klf4*, *Nanog* and *Oct4*) in SGC7901 **(E)** and BGC823 **(F)** cells with or without treatment with *GM-CSF*. **(G)**: Western blot analysis of the stemness genes expression in SGC7901 and BGC823 cells with or without treatment with *GM-CSF*. **(H)**: A gastric cancer xenograft model by transplantation of SGC7901 cells with or without stimulation with *GM-CSF* into nude mice (n = 10 in each group). **(I)**: Tumor growth curves in **(H)**. **(J)**: Tumor images in **(H)**. **(K)**: Tumor weight in **(H)**. Data are presented as the mean ± SEM (N = 3 for *in vitro* assays, and N = 10 for *in vivo* assay). *p < 0.05, **p < 0.01, ***p < 0.001.

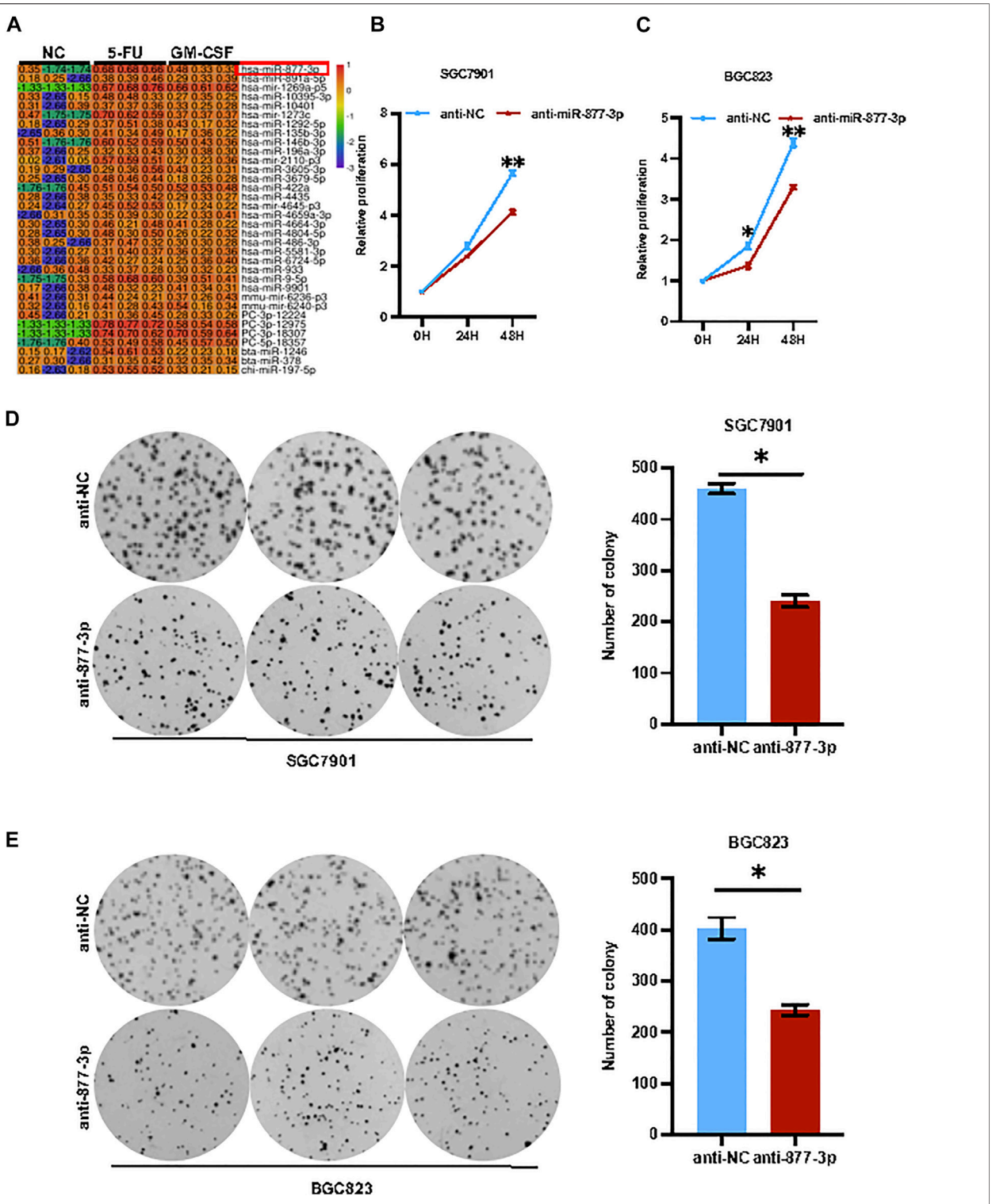


FIGURE 3 | Knockdown of *miR-877-3p* suppressed gastric cancer cell proliferation. **(A)**: High-throughput miRNA sequencing analyses on SGC7901 cells with or without stimulation with 5-FU or *GM-CSF* identified a group of deregulated miRNAs, including *miR-877-3p*. **(B,C)**: Knockdown of *miR-877-3p* in SGC7901 **(B)** and BGC823 **(C)** cells suppressed cell proliferation assayed by CCK8. **(D,E)**: Knockdown of *miR-877-3p* in SGC7901 **(D)** and BGC823 **(E)** cells suppressed the cellular colony formation. Data are presented as the mean ± SEM ($N = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

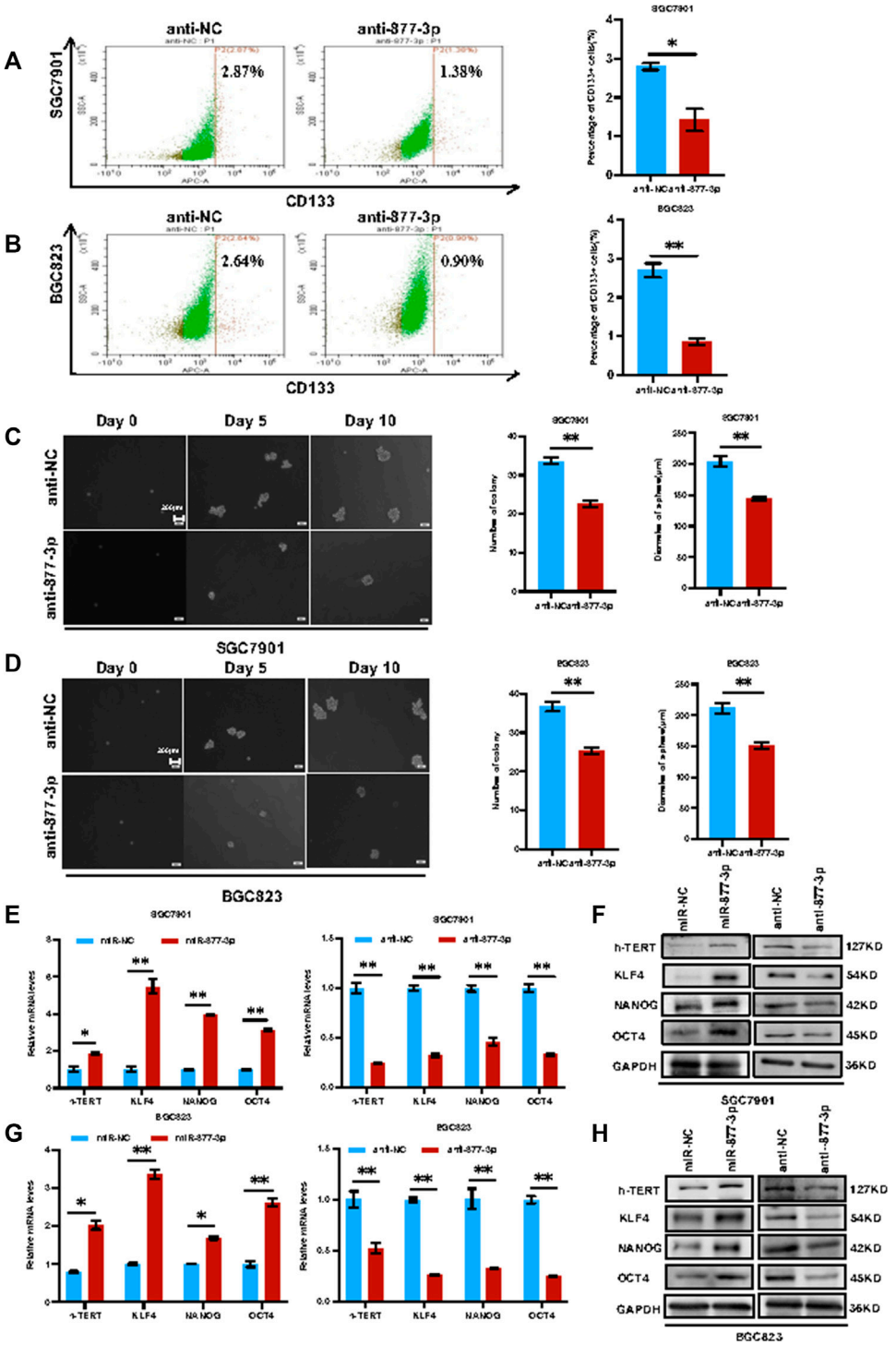


FIGURE 4 | Knockdown of *miR-877-3p* suppressed gastric cancer cell stemness. **(A,B)**: Knockdown of *miR-877-3p* in SGC7901 and BGC823 cells suppressed the subpopulation of $CD133^+$ CSCs. **(C,D)**: Knockdown of *miR-877-3p* in SGC7901 and BGC823 cells suppressed the sphere formation ability in the serum-free culture condition. **(E,F)**: QRT-PCR **(E)** and western blot **(F)** analyses of the stemness genes including *h-Tert*, *Klf4*, *Nanog* and *Oct4* in SGC7901 cells with or without overexpression or knockdown of *miR-877-3p*. **(G,H)**: QRT-PCR **(G)** and western blot **(H)** analyses of the stemness genes in BGC823 cells with or without overexpression or knockdown of *miR-877-3p*. Data are presented as the mean ± SEM ($N = 3$). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

stimulating factor receptor (G-CSFR) leads to poor survival in gastric cancer (Fan et al., 2018). Tumor-derived GM-CSF promotes gastrointestinal tumorigenesis (Wang et al., 2014), we herein applied *in vitro* and *in vivo* assays to validate the phenotypes and determine the regulatory mechanism. Human gastric cancer cells SGC7901 and BGC823 were treated with a low concentration of 5-FU or DDP for 72 h *in vitro*. Survived cells were collected for further analysis including IC_{50}, cell stemness, as well as *GM-CSF* levels. As shown in **Figure 1A**, both survived SGC7901 and BGC823 cells showed increased IC_{50} and drug resistance, associated with increased *GM-CSF* levels at both mRNA and protein levels in cells (**Figures 1B,C**) and in secretion in the supernatant (**Figure 1D**).

***GM-CSF* treatment promoted cancer cell stemness *in vitro* and tumorigenesis *in vivo*.** SGC7901 and BGC823 cells were stimulated with exogenous GM-CSF by adding into the cell culture medium, followed by the *CD133*$^+$ CSC subpopulation analysis. As a result, The *CD133*$^+$ CSC subpopulation increased from 2.69% to 9.09% in SGC7901 cells (**Figures 2A,B**), and from 2.72% to 12.52.% in BGC823 cells (**Figures 2C,D**) after stimulation, respectively. In addition, a group of well-defined stemness genes including *h-Tert, Klf4, Nanog* and *Oct4* was examined by quantitative RT-PCR and western blot analyses in the 2 GC cell lines before or after treatment with *GM-CSF*. In consistent with the results in **Figures 2A–D**, these stemness genes showed induction in expression at both mRNA and protein levels by *GM-CSF* stimulation (**Figures 2E–G**).

In order to further determine the effects of *GM-CSF* on tumorigenesis *in vivo*, a gastric cancer xenograft model was established by transplantation of SGC7901 cells with or without stimulation with *GM-CSF* into immunodeficient female nude mice through via subcutaneous injection, followed by continuous tracking of the tumor growth (**Figure 2H**). The tumor growth curves (**Figure 2I**), tumor images (**Figure 2J**) and tumor weight (**Figure 2K**) indicated significant promotion of tumor growth by *GM-CSF* stimulation.

miR-877-3p mediated chemotherapy and *GM-CSF* induced tumor progression in gastric cancer. To identify the key genes regulating chemotherapy-induced or GM-CSF-induced tumor progression in gastric cancer, SGC7901 cells with or without stimulation with 5-FU or *GM-CSF* were applied for a high-throughput miRNA sequencing analysis. As a result, a subset of miRNAs was identified with a differential expression upon treatment with 5-FU and *GM-CSF*, respectively (**Figure 3A**). Some miRNAs, such as *miR-9-5p, miR-196a and miR-422a,* have been well documented to regulate tumorigenesis and cancer stem cells in gastric cancer (Pan et al., 2017; He et al., 2018; Liu et al., 2020), while the function of *miR-877-3p* remains unclear in GC. As shown in **Supplementary Figure S1**, quantitative real-time PCR analysis validated of miR-877-3p overexpression in both SGC7901 and BGC823 cells were treated with 5-FU and DDP respectively. Therefore, we focused on *miR-877-3p* to determine the relationship between upregulation of *miR-877-3p* and chemotherapy-induced drug resistance and cell stemness. Overexpression or knockdown of *miR-877-3p* was applied to gastric cancer cells (**Supplementary Figures S2, S3**), followed by CCK8 cell proliferation and colony formation assay. As shown in **Figures 3B–E**, knockdown of *miR-877-3p* suppressed cell proliferation and colony formation in both SGC7901 and BGC823 cells, respectively. Whereas overexpression of *miR-877-3p* dramatically increased cell proliferation and colony formation in both SGC7901 and BGC823 cells (**Supplementary Figure S6**).

Overexpression of *miR-877-3p* promoted the cell stemness in both SGC7901 and BGC823 cells. After overexpression or knockdown of *miR-877-3p* in both SGC7901 and BGC823 cells, the changes of the CD133$^+$ CSC percentage were determined by flow cytometry analysis. As shown in **Figure 4A**, knockdown of *miR-877-3p* in SGC7901 cells decreased CD133$^+$ CSC subpopulation. Similar results were obtained from BGC823cells (**Figure 4B**). In addition, As shown in **Figures 4C,D**, sphere formation assays were performed to further determine the stemness changes after knockdown of *miR-877-3*p in both SGC7901 and BGC823 cells. Quantitative analysis indicated that knockdown of *miR-877-3p* decreased both sphere number and sphere size. Whereas overexpression of *miR-877-3p* dramatically increased CD133$^+$ CSC subpopulation and sphere formation in both SGC7901 and BGC823 cells (**Supplementary Figure S7**). Moreover, a group of well-defined stemness genes including *h-Tert, Klf4, Nanog* and *Oct4* was examined in both SGC7901 and BGC823 cells by quantitative RT-PCR and western blot analyses. The results showed that overexpression or knockdown of *miR-877-3p* remarkably increased or decreased the expression of *h-Tert, Klf4, Nanog* and *Oct4* at both mRNA (**Figures 4E,G**) and protein levels (**Figures 4F, 4H**).

***miR-877-3p* activated *Jak2/Stat3* signaling through targeting *SOCS2*.** In order to determine the molecular mechanism(s) by which *miR-877-3p* promotes gastric cancer development and progression, RNA-seq was applied to the SGC7901 cells with or without stimulation with 5-FU or *GM-CSF*, deriving 176 downregulated genes by 5-FU treatment and 207 downregulated genes by *GM-CSF* treatment (**Figures 5A,B**). Bioinformatic analysis was using TargetScan Human8.0 (http://www.targetscan.org/vert_80/) predicted 5,091 potential target genes of *miR-877-3p*. From these three groups of genes, 32 genes were overlapped including *SOCS2* (**Figure 5C**). Quantitative real-time PCR analysis validated downregulation of *SOCS2* at the mRNA levels by overexpression of *miR-877-3p* in both SGC7901 and BGC823 cells (**Supplementary Figure S4**). Upregulation of *SOCS2* was shown after knockdown of miR-877–3p in both SGC7901 and BGC823 cells (**Supplementary Figure S5**). Western blot analysis further demonstrated downregulation or upregulation of *SOCS2* at the protein levels by overexpression or knockdown of *miR-877-3p* in both SGC7901 and BGC823 cells (**Figures 5D,E**). In order to demonstrate the direct interaction between *SOCS2* and *miR-877-3p*, luciferase (Luc) reporter constructs carrying either wide type (WT) or *miR-877-3p*-binding sites-mutated (MU) 3′UTR of *SOCS2* were co-transfected with *miR-877-3p* mimics into 293T cells (**Figure 5G**). As a

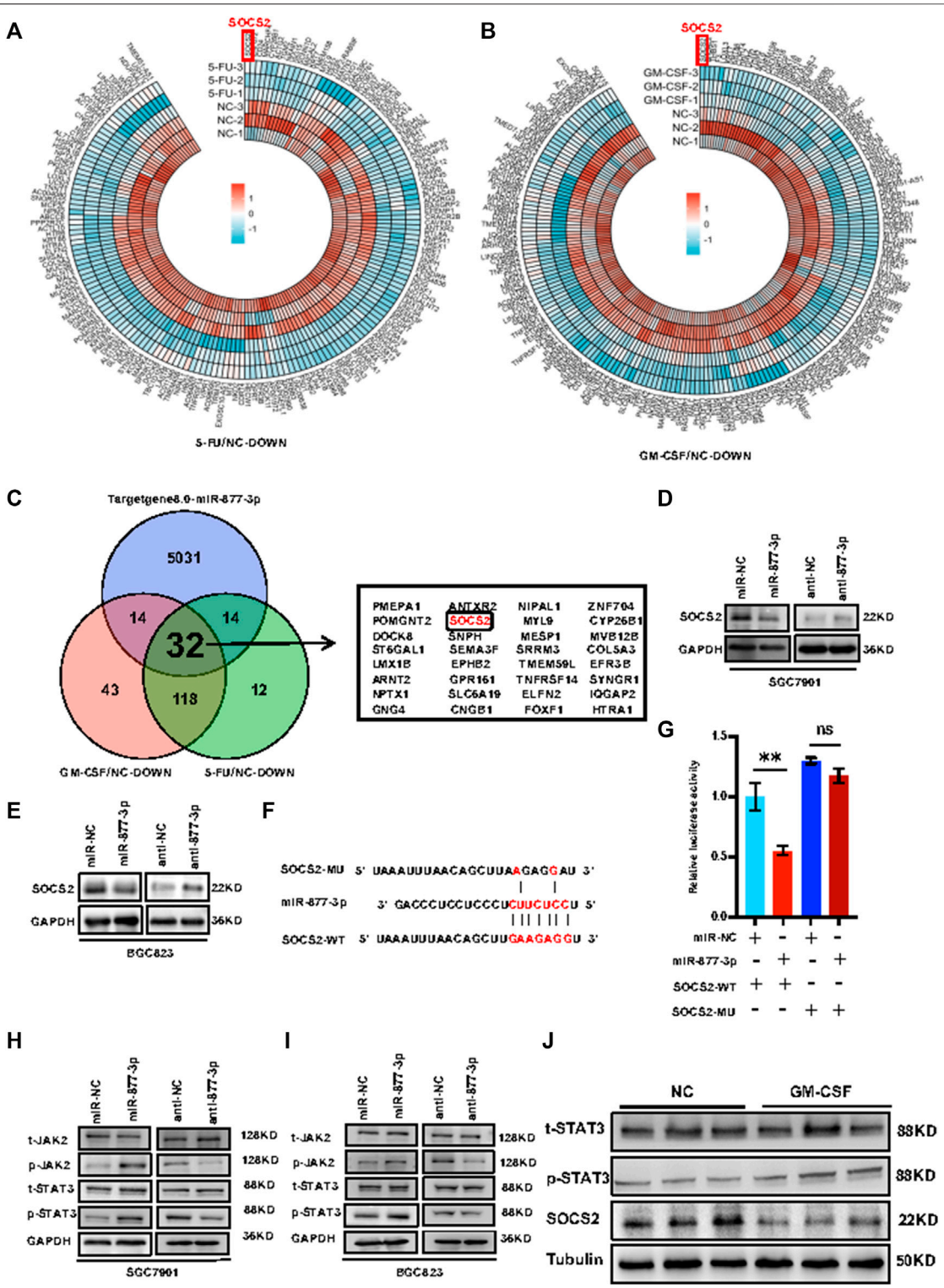


FIGURE 5 | *miR-877-3p* activated *Jak2/Stat3* signaling through targeting *SOCS2* in gastric cancer. **(A,B)**: RNA-seq analysis of the SGC7901 cells with or without stimulation with 5-FU **(A)** or *GM-CSF* **(B)** identified a list of differentially expressed downregulated genes. **(C)**: 32 genes were overlapped from the 176 downregulated genes by 5-FU treatment, 207 downregulated genes by *GM-CSF* treatment, and 5,091 potential target genes of *miR-877-3p* predicted by TargetScan Human8.0, including *SOCS2*. **(D,E)**: Western blot demonstrated inhibition of *SOCS2* by *miR-877-3p* overexpression and promotion of SOCS2 by *miR-877-3p* knockdown in both SGC7901 and BGC823 cells. **(F)**: Sequence alignment of wide type (WT) or *miR-877-3p*-binding sites-mutated (MU) 3′UTR of *SOCS2*. **(G)**: luciferase reporter assay demonstrated inhibition of WT-*SOCS2*-3′UTR by *miR-877-3p*, but not MU-*SOCS2*-3′UTR. **(H,I)**: Western blot demonstrated positive or negative regulation of *p-Jak2* and *p-Stat3* by overexpression or knockdown of *miR-877-3p* in both SGC7901 and BGC823 cells. **(J)**: Western blot demonstrated downregulation of *SOCS2* and activation of *Jak2/Stat3* signaling by *GM-CSF* treatment in the tumors from the mice model. Data are presented as the mean ± SEM ($N = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

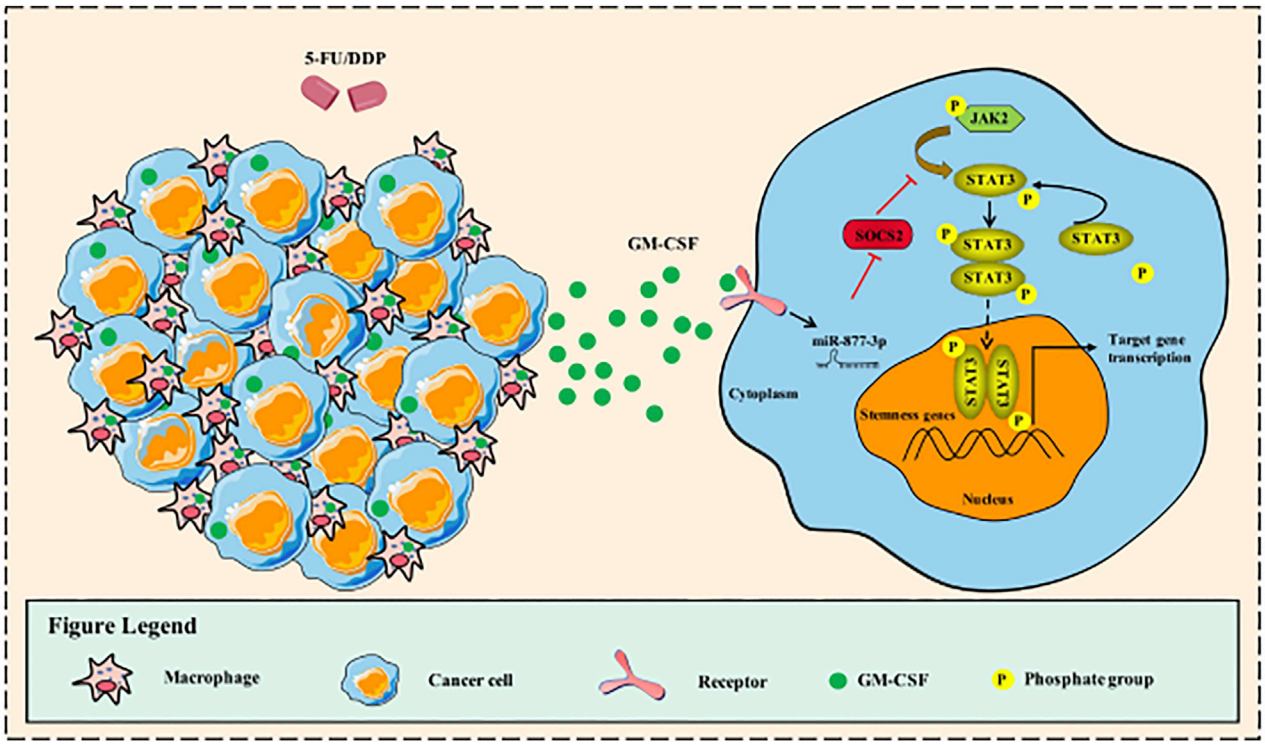


FIGURE 6 | Working Model. Schematic representation of the mechanism through which *GM-CSF* increased the expression of *miR-877-3p* in gastric cancer cells, which suppressed the expression of *SOCS2* as a target gene. *SOCS2*, as a suppressor gene of *Jak2/Stat3* signaling, mediated the chemotherapy-induced cancer cell stemness and drug resistance.

result, WT-*SOCS2*-Luc was inhibited by *miR-877-3p*, but MU-*SOCS2*-Luc was not, supporting the target interaction between *SOCS2* 3′-UTR and *miR-877-3p* via sequence complementarity (**Figures 5F,G**). In view of the well-defined tumor-suppressing function of *SOCS2* by inhibiting *Jak2/Stat3* signaling (Uen et al., 2018; Ren et al., 2019), we detected the effects of *miR-877-3p* on *Jak2/Stat3* signaling in GC. As shown in **Figures 5H,I**, *p-Stat3* and *p-Jak2* were induced by overexpression of *miR-877-3p*, and suppressed by knockdown of *miR-877-3p* in both SGC7901 and BGC823 cells, which was further validated by western blot analysis on the tumor samples derived from the mouse model (**Figure 5J**).

DISCUSSION

Since *GM-CSF* is able to induce pluripotent stem cells to differentiate into mature granulocytes, macrophages and T cells in bone marrow, it has been used in clinic to protect cancer patients against chemotherapy- or radiotherapy-induced neutropenia. However, emerging evidence found that application of *GM-CSF* therapy occasionally promoted tumor progression (Uemura et al., 2004; Metcalf, 2010), indicating complexity of the *GM-CSF*-based cancer therapy. Herein, we experimentally demonstrated a mechanism through which chemotherapy or *GM-CSF*-based therapy of gastric cancer may induce cancer cell stemness and drug resistance.

Activation of *Jak/Stat3* signaling pathway plays a critical role in promoting tumorigenesis, epithelial and mesenchymal transition (EMT), chemo-resistance, and cancer cell stemness (Jin, 2020). In gastric cancer, overexpression of *p-Stat3* increased sphere formation from $CD44^+$ CSCs (Hajimoradi et al., 2016). In the current study, we are the first to identify *miR-877-3p* with upregulation in the chemo survived gastric cancer cells, which was mediated by *GM-CSF* induction but in turn suppressed *SOCS2* and activated *Jak/Stat3* signaling. This is in consistence with the literature about the oncogenic function of miR-877-3p in Pancreatic Cancer by interacting with STARD13 (Xu and Zheng, 2020). In addition to *Jak/Stat3*, *PI3k/Akt* and *Erk* signaling pathways have been reported to have interacted with *GM-CSF* in regulating tumor cell proliferation and migration (Kawaguchi et al., 2004; Carlson et al., 2011). Although we did not analyze in the current study whether these two pathways are involved in regulating the *GM-CSF*-induced cell stemness and drug resistance, our high-throughput RNA sequencing data analyses suggested activation of *PI3k/Akt* signaling after *GM-CSF* treatment in gastric cancer.

In conclusion, CSCs are believed to be the main source of cancer initiation, relapse, and drug resistance. Therapeutic strategies targeting CSCs hold great promise in the fight against cancer. The current study demonstrated a novel mechanism regulating chemotherapy-induced CSCs and drug resistance in gastric cancer.

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 below: National Center for Biotechnology Information (NCBI) BioProject database under accession number PRJNA811393.

ETHICS STATEMENT

The animal study was reviewed and approved by All animal studies were performed following the relevant guidelines, regulations and protocols approved by our Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

HC and YY designed the research and wrote the paper. XX, H-ZM, Y-qC, D-zZ, S-xM and H-jW performed the *in vitro* and *in vivo* experiments. XX and D-mL did data analysis. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from the Central to guide local scientific and Technological Development (ZYYDDFFZZJ-1), Gansu Provincial Youth Science and Technology Fund Program (21JR7RA642), Gansu Provincial Hospital project (2019–206), Lanzhou talent innovation and Entrepreneurship Project (2016-RC-56), Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (21GSSYC-2).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.855351/full#supplementary-material

REFERENCES

Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J., and Clarke, M. F. (2003). Prospective Identification of Tumorigenic Breast Cancer Cells. *Proc. Natl. Acad. Sci. USA.* 100 (7), 3983–3988. doi:10.1073/pnas.0530291100

Alison, M. R., Lin, W. R., Lim, S. M., and Nicholson, L. J. (2012). Cancer Stem Cells: in the Line of Fire. *Cancer Treat. Rev.* 38 (6), 589–598. doi:10.1016/j.ctrv.2012.03.003

Bartel, D. P. (2009). MicroRNAs: Target Recognition and Regulatory Functions. *Cell* 136 (2), 215–233. doi:10.1016/j.cell.2009.01.002

Benna, M., Guy, J. B., Bosacki, C., Jmour, O., Ben Mrad, M., Ogorodniitchouk, O., et al. (2020). Chemoradiation and Granulocyte-Colony or Granulocyte Macrophage-Colony Stimulating Factors (G-CSF or GM-CSF): Time to Think Out of the Box? *Br. J. Radiol.* 93 (1109), 20190147.

Bonnet, D., and Dick, J. E. (1997). Human Acute Myeloid Leukemia Is Organized as a Hierarchy that Originates from a Primitive Hematopoietic Cell. *Nat. Med.* 3 (7), 730–737. doi:10.1038/nm0797-730

Carlson, S. M., Chouinard, C. R., Labadorf, A., Lam, C. J., Schmelzle, K., Fraenkel, E., et al. (2011). Large-scale Discovery of ERK2 Substrates Identifies ERK-Mediated Transcriptional Regulation by ETV3. *Sci. Signal.* 4 (196), rs11. doi:10.1126/scisignal.2002010

Choi, J. H., Lim, H. Y., Joo, H. J., Kim, H. S., Yi, J. W., Kim, H. C., et al. (2002). Expression of Multidrug Resistance-Associated protein1,P-Glycoprotein, and Thymidylate Synthase in Gastric Cancer Patients Treated with 5-fluorouracil and Doxorubicin-Based Adjuvant Chemotherapy after Curative Resection. *Br. J. Cancer* 86 (10), 1578–1585. doi:10.1038/sj.bjc.6600305

Edwardson, D. W., Parissenti, A. M., and Kovala, A. T. (2019). Chemotherapy and Inflammatory Cytokine Signalling in Cancer Cells and the Tumour Microenvironment. *Adv. Exp. Med. Biol.* 1152, 173–215. doi:10.1007/978-3-030-20301-6_9

Fan, Z., Li, Y., Zhao, Q., Fan, L., Tan, B, Zuo, J., et al. (2018). Highly Expressed Granulocyte Colony-Stimulating Factor (G-CSF) and Granulocyte Colony-Stimulating Factor Receptor (G-CSFR) in Human Gastric Cancer Leads to Poor Survival. *Med. Sci. Monit.* 24, 1701–1711.

Gomes, B. C., Rueff, J., and Rodrigues, A. S. (2016). MicroRNAs and Cancer Drug Resistance. *Methods Mol. Biol.* 1395, 137–162. doi:10.1007/978-1-4939-3347-1_9

Guo, W., Li, W., Yuan, L., Mei, X., and Hu, W. (2019). MicroRNA-106a-3p Induces Apatinib Resistance and Activates Janus-Activated Kinase 2 (JAK2)/Signal Transducer and Activator of Transcription 3 (STAT3) by Targeting the SOCS System in Gastric Cancer. *Med. Sci. Monit.* 25, 10122–10128. doi:10.12659/MSM.919610

Gutschalk, C. M., Herold-Mende, C. C., Fusenig, N. E., and Mueller, M. M. (2006). Granulocyte colony-stimulating Factor and Granulocyte-Macrophage colony-stimulating Factor Promote Malignant Growth of Cells from Head and Neck Squamous Cell Carcinomas *In Vivo*. *Cancer Res.* 66 (16), 8026–8036. doi:10.1158/0008-5472.CAN-06-0158

Hajimoradi, M., Mohammad Hassan, Z., Ebrahimi, M., Soleimani, M., Bakhshi, M., Firouzi, J., et al. (2016). STAT3 Is Overactivated in Gastric Cancer Stem-like Cells. *Cell J* 17 (4), 617–628. doi:10.22074/cellj.2016.3834

He, Z., Li, Z., Zhang, X., Yin, K., Wang, W., and Xu, Z. (2018). MiR-422a Regulates Cellular Metabolism and Malignancy by Targeting Pyruvate Dehydrogenase Kinase 2 in Gastric Cancer. *Cell Death Dis* 9 (5), 505. doi:10.1038/s41419-018-0564-3

Hong, I. S. (2016). Stimulatory versus Suppressive Effects of GM-CSF on Tumor Progression in Multiple Cancer Types. *Exp. Mol. Med.* 48 (7), e242. doi:10.1038/emm.2016.64

Jin, W. (2020). Role of JAK/STAT3 Signaling in the Regulation of Metastasis, the Transition of Cancer Stem Cells, and Chemoresistance of Cancer by Epithelial-Mesenchymal Transition. *Cells* 9 (1), 217. doi:10.3390/cells9010217

Ju, H., Xing, W., Yang, J., Zheng, Y., Jia, X., Zhang, B., et al. (2016). An Effective Cytokine Adjuvant Vaccine Induces Autologous T-Cell Response against colon Cancer in an Animal Model. *BMC Immunol.* 17 (1), 31. doi:10.1186/s12865-016-0172-x

Kawaguchi, M., Kokubu, F., Odaka, M., Watanabe, S., Suzuki, S., Ieki, K., et al. (2004). Induction of Granulocyte-Macrophage colony-stimulating Factor by a New Cytokine, ML-1 (IL-17F), via Raf I-MEK-ERK Pathway. *J. Allergy Clin. Immunol.* 114 (2), 444–450. doi:10.1016/j.jaci.2004.03.047

Li, L., and Clevers, H. (2010). Coexistence of Quiescent and Active Adult Stem Cells in Mammals. *Science* 327 (5965), 542–545. doi:10.1126/science.1180794

Liu, T., Liu, Y., Wei, C., Yang, Z., Chang, W., and Zhang, X. (2020). LncRNA HULC Promotes the Progression of Gastric Cancer by Regulating miR-9-5p/MYH9 axis. *Biomed. Pharmacother.* 121, 109607. doi:10.1016/j.biopha.2019.109607

Lu, J., Wang, Y. H., Yoon, C., Huang, X. Y., Xu, Y., Xie, J. W., et al. (2020). Circular RNA Circ-RanGAP1 Regulates VEGFA Expression by Targeting miR-877-3p to Facilitate Gastric Cancer Invasion and Metastasis. *Cancer Lett.* 471, 38–48. doi:10.1016/j.canlet.2019.11.038

Matsui, W. H. (2016). Cancer Stem Cell Signaling Pathways. *Medicine (Baltimore)* 95 (1 Suppl. 1), S8–S19. doi:10.1097/MD.0000000000004765

McLeish, K. R., Knall, C., Ward, R. A., Gerwins, P., Coxon, P. Y., Klein, J. B., et al. (1998). Activation of Mitogen-Activated Protein Kinase Cascades during Priming of Human Neutrophils by TNF-Alpha and GM-CSF. *J. Leukoc. Biol.* 64 (4), 537–545. doi:10.1002/jlb.64.4.537

Metcalf, D. (2010). The colony-stimulating Factors and Cancer. *Nat. Rev. Cancer* 10 (6), 425–434. doi:10.1038/nrc2843

Mihanfar, A., Fattahi, A., and Nejabati, H. R. (2019). MicroRNA-mediated Drug Resistance in Ovarian Cancer. *J. Cel Physiol* 234 (4), 3180–3191. doi:10.1002/jcp.26060

O'Shaughnessy, J. A., Denicoff, A. M., Venzon, D. J., Danforth, D., Pierce, L. J., Frame, J. N., et al. (1994). A Dose Intensity Study of FLAC (5-fluorouracil, Leucovorin, Doxorubicin, Cyclophosphamide) Chemotherapy and Escherichia Coli-Derived Granulocyte-Macrophage colony-stimulating Factor (GM-CSF) in Advanced Breast Cancer Patients. *Ann. Oncol.* 5 (8), 709–716. doi:10.1093/oxfordjournals.annonc.a058975

Obermueller, E., Vosseler, S., Fusenig, N. E., and Mueller, M. M. (2004). Cooperative Autocrine and Paracrine Functions of Granulocyte colony-stimulating Factor and Granulocyte-Macrophage colony-stimulating Factor in the Progression of Skin Carcinoma Cells. *Cancer Res.* 64 (21), 7801–7812. doi:10.1158/0008-5472.CAN-03-3301

Pan, Y., Shu, X., Sun, L., Yu, L., Sun, L., Yang, Z., et al. (2017). miR-196a-5p M-odulates G-astric C-ancer S-tem C-ell C-haracteristics by T-argeting Smad4. *Int. J. Oncol.* 50 (6), 1965–1976. doi:10.3892/ijo.2017.3965

Park, S. Y., Lee, C. J., Choi, J. H., Kim, J. H., Kim, J. W., Kim, J. Y., et al. (2019). The JAK2/STAT3/CCND2 Axis Promotes Colorectal Cancer Stem Cell Persistence and Radioresistance. *J. Exp. Clin. Cancer Res.* 38 (1), 399. doi:10.1186/s13046-019-1405-7

Pei, X. H., Nakanishi, Y., Takayama, K., Bai, F., and Hara, N. (1999). Granulocyte, Granulocyte-Macrophage, and Macrophage colony-stimulating Factors Can Stimulate the Invasive Capacity of Human Lung Cancer Cells. *Br. J. Cancer* 79 (1), 40–46. doi:10.1038/sj.bjc.6690009

Ren, W., Wu, S., Wu, Y., Liu, T., Zhao, X., and Li, Y. (2019). MicroRNA-196a/-196b Regulate the Progression of Hepatocellular Carcinoma through Modulating the JAK/STAT Pathway via Targeting SOCS2. *Cel Death Dis* 10 (5), 333. doi:10.1038/s41419-019-1530-4

Ricci-Vitiani, L., Lombardi, D. G., Pilozzi, E., Biffoni, M., Todaro, M., Peschle, C., et al. (2007). Identification and Expansion of Human colon-cancer-initiating Cells. *Nature* 445 (7123), 111–115. doi:10.1038/nature05384

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 (3), 203–222. doi:10.1038/nrd.2016.246

Schulenburg, A., Blatt, K., Cerny-Reiterer, S., Sadovnik, I., Herrmann, H., Marian, B., et al. (2015). Cancer Stem Cells in Basic Science and in Translational Oncology: Can We Translate into Clinical Application? *J. Hematol. Oncol.* 8, 16. doi:10.1186/s13045-015-0113-9

Seo, S., Ryu, M. H., Park, Y. S., Ahn, J. Y., Park, Y., Park, S. R., et al. (2019). Loss of HER2 Positivity after Anti-HER2 Chemotherapy in HER2-Positive Gastric Cancer Patients: Results of the GASTric Cancer HER2 Reassessment Study 3 (GASTHER3). *Gastric Cancer* 22 (3), 527–535. doi:10.1007/s10120-018-0891-1

Shi, Y., Liu, C. H., Roberts, A. I., Das, J., Xu, G., Ren, G., et al. (2006). Granulocyte-macrophage colony-stimulating Factor (GM-CSF) and T-Cell Responses: what We Do and Don't Know. *Cell Res* 16 (2), 126–133. doi:10.1038/sj.cr.7310017

Sielska, M., Przanowski, P., Pasierbińska, M., Wojnicki, K., Poleszak, K., Wojtas, B., et al. (2020). Tumour-derived CSF2/granulocyte Macrophage colony Stimulating Factor Controls Myeloid Cell Accumulation and Progression of Gliomas. *Br. J. Cancer* 123 (3), 438–448. doi:10.1038/s41416-020-0862-2

Su, Z., Yang, Z., Xu, Y., Chen, Y., and Yu, Q. (2015). MicroRNAs in Apoptosis, Autophagy and Necroptosis. *Oncotarget* 6 (11), 8474–8490. doi:10.18632/oncotarget.3523

Sun, X., Jiao, X., Pestell, T. G., Fan, C., Qin, S., Mirabelli, E., et al. (2014). MicroRNAs and Cancer Stem Cells: the Sword and the Shield. *Oncogene* 33 (42), 4967–4977. doi:10.1038/onc.2013.492

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 (3), 209–249. doi:10.3322/caac.21660

Takaishi, S., Okumura, T., Tu, S., Wang, S. S., Shibata, W., Vigneshwaran, R., et al. (2009). Identification of Gastric Cancer Stem Cells Using the Cell Surface Marker CD44. *Stem Cells* 27 (5), 1006–1020. doi:10.1002/stem.30

Uemura, Y., Kobayashi, M., Nakata, H., Harada, R., Kubota, T., and Taguchi, H. (2004). Effect of Serum Deprivation on Constitutive Production of Granulocyte-colony Stimulating Factor and Granulocyte Macrophage-colony Stimulating Factor in Lung Cancer Cells. *Int. J. Cancer* 109 (6), 826–832. doi:10.1002/ijc.20023

Uemura, Y., Kobayashi, M., Nakata, H., Kubota, T., Bandobashi, K., Saito, T., et al. (2006). Effects of GM-CSF and M-CSF on Tumor Progression of Lung Cancer: Roles of MEK1/ERK and AKT/PKB Pathways. *Int. J. Mol. Med.* 18 (2), 365–373. doi:10.3892/ijmm.18.2.365

Uen, Y. H., Fang, C. L., Lin, C. C., Hseu, Y. C., Hung, S. T., Sun, D. P., et al. (2018). Ceramide Synthase 6 Predicts the Prognosis of Human Gastric Cancer: It Functions as an Oncoprotein by Dysregulating the SOCS2/JAK2/STAT3 Pathway. *Mol. Carcinog* 57 (12), 1675–1689. doi:10.1002/mc.22888

Vilalta, M., Rafat, M., Giaccia, A. J., and Graves, E. E. (2014). Recruitment of Circulating Breast Cancer Cells is Stimulated by Radiotherapy. *Cell Rep.* 8 (2), 402–409.

Vilalta, M., Brune, J., Rafat, M., Soto, L., and Graves, E. E. (2018). The Role of Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) in Radiation-Induced Tumor Cell Migration. *Clin. Exp. Metastasis* 35 (4), 247–254.

Wang, Y., Han, G., Wang, K., Liu, G., Wang, R., Xiao, H., et al. (2014). Tumor-derived GM-CSF Promotes Inflammatory colon Carcinogenesis via Stimulating Epithelial Release of VEGF. *Cancer Res.* 74 (3), 716–726. doi:10.1158/0008-5472.CAN-13-1459

Xu, X., and Zheng, S. (2020). MiR-887-3p Negatively Regulates STARD13 and Promotes Pancreatic Cancer Progression. *Cancer Manag. Res.* 12, 6137–6147. doi:10.2147/CMAR.S260542

Xu, Z. Y., Tang, J. N., Xie, H. X., Du, Y. A., Huang, L., Yu, P. F., et al. (2015). 5-Fluorouracil Chemotherapy of Gastric Cancer Generates Residual Cells with Properties of Cancer Stem Cells. *Int. J. Biol. Sci.* 11 (3), 284–294. doi:10.7150/ijbs.10248

Yu, H., Lee, H., Herrmann, A., Buettner, R., and Jove, R. (2014). Revisiting STAT3 Signalling in Cancer: New and Unexpected Biological Functions. *Nat. Rev. Cancer* 14 (11), 736–746. doi:10.1038/nrc3818

Zhou, X., Xia, Y., Li, L., and Zhang, G. (2015). MiR-101 Inhibits Cell Growth and Tumorigenesis of *Helicobacter pylori* Related Gastric Cancer by Repression of SOCS2. *Cancer Biol. Ther.* 16 (1), 160–169. doi:10.4161/15384047.2014.987523

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.

frontiers | Frontiers in Pharmacology

REVIEW
published: 01 June 2022
doi: 10.3389/fphar.2022.908882

Adenosine Kinase on Deoxyribonucleic Acid Methylation: Adenosine Receptor-Independent Pathway in *Cancer* Therapy

Hao-Yun Luo[1†], *Hai-Ying Shen*[2,3†], *R. Serene Perkins*[4,5] *and Ya-Xu Wang*[1*]

[1]Department of Gastrointestinal and Anorectal Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing Medical University, Chongqing, China, [2]Department of Neuroscience, Legacy Research Institute, Portland, OR, United States, [3]Integrative Physiology and Neuroscience, Washington State University, Vancouver, WA, United States, [4]Legacy Tumor Bank, Legacy Research Institute, Portland, OR, United States, [5]Mid-Columbia Medical Center, The Dalles, OR, United States

Methylation is an important mechanism contributing to cancer pathology. Methylation of tumor suppressor genes and oncogenes has been closely associated with tumor occurrence and development. New insights regarding the potential role of the adenosine receptor-independent pathway in the epigenetic modulation of DNA methylation offer the possibility of new interventional strategies for cancer therapy. Targeting DNA methylation of cancer-related genes is a promising therapeutic strategy; drugs like 5-Aza-2′-deoxycytidine (5-AZA-CdR, decitabine) effectively reverse DNA methylation and cancer cell growth. However, current anti-methylation (or methylation modifiers) are associated with severe side effects; thus, there is an urgent need for safer and more specific inhibitors of DNA methylation (or DNA methylation modifiers). The adenosine signaling pathway is reported to be involved in cancer pathology and participates in the development of tumors by altering DNA methylation. Most recently, an adenosine metabolic clearance enzyme, adenosine kinase (ADK), has been shown to influence methylation on tumor suppressor genes and tumor development and progression. This review article focuses on recent updates on ADK and its two isoforms, and its actions in adenosine receptor-independent pathways, including methylation modification and epigenetic changes in cancer pathology.

Keywords: DNA methylation, adenosine, receptor-independent pathway, adenosine kinase, ADK isoforms, ADK inhibitor, cancer therapy

OPEN ACCESS

Edited by:
Ke-Wu Zeng,
Peking University, China

Reviewed by:
Mariachiara Zuccarini,
University of Studies G. d'Annunzio Chieti and Pescara, Italy
Elena De Marchi,
University of Ferrara, Italy

***Correspondence:**
Ya-Xu Wang
300897@hospital.cqmu.edu.cn

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *31 March 2022*
Accepted: *16 May 2022*
Published: *01 June 2022*

Citation:
Luo H-Y, Shen H-Y, Perkins RS and Wang Y-X (2022) Adenosine Kinase on Deoxyribonucleic Acid Methylation: Adenosine Receptor-Independent Pathway in Cancer Therapy. Front. Pharmacol. 13:908882. doi: 10.3389/fphar.2022.908882

1 INTRODUCTION

The relationship between cancer and DNA methylation was first described by Feinberg and Vogelstein, who revealed that changes in DNA methylation promote the development of invasive colorectal cancer (Feinberg and Vogelstein, 1983). This led to the hypothesis that epigenetic silencing of tumor suppressors promotes carcinogenesis, as well as the finding that reversing this silencing suppresses tumor growth and may prevent tumorigenesis (Feinberg and Vogelstein, 1983). Aberrant DNA methylation has been confirmed to influence the development of numerous human cancers (Nejman et al., 2014; Sun et al., 2014). DNA hypermethylation in cancer cells has been studied most extensively as targeting promoter regions, especially the tumor

suppressor genes. The promoter region of tumor suppressor genes is structurally rich in CpG and focal hypermethylation often occurs in its promoter region (López-Moyado et al., 2019), which leads to gene silencing, genomic instability, cell apoptosis, altered DNA repair, and cell cycle control (Wu and Bekaii-Saab, 2012). Hypermethylation inactivates the transcription of tumor suppressor genes, but it does not change the sequence of the gene itself. The methylation process and status can potentially be reversed and regulated.

DNA methylation utilizes methyl from S-adenosylmethionine (SAM). DNA methyltransferase (DNMT) catalyzes DNA methylation by transferring the methyl group from SAM to a target adenine or cytosine at a specific DNA site (Zhao et al., 2015), SAM is thus irreversibly converted to S- adenosylhomocysteine (SAH). SAH is then converted into adenosine and homocysteine (Hcy) by S-adenosylhomocysteine hydrolase (SAHH). Studies showed that increased downstream adenosine product can reversely influent the SAH to Hcy and transmethylation. Blockade of an adenosine metabolic enzyme, adenosine kinase (ADK) results in reduced adenosine removal and causes adenosine accumulation, and also elevates SAH level (Boison et al., 2002); the increased SAH, as a potent inhibitor of all DNMT, allows reversal of aberrant DNA methylation and expression of antioncogene (James et al., 2002).

Of note, adenosine, as an essential biological molecule of life, plays an important role in various aspects of cancer pathology, such as tumor immunity, tissue ischemia, hypoxia, revascularization, and apoptosis (Fishman et al., 2009a; Antonioli et al., 2013). Adenosine can conduct its manipulatory effects via the G protein-coupled four subtypes of adenosine receptors, i.e., adenosine A_1, A_{2A}, A_2B, and A_3 receptors (A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R) (Fredholm et al., 2005; Jacobson, 2009). The activation of adenosine receptors is primarily determined by the availability of extracellular levels of adenosine. In addition to the aforementioned receptor-*dependent* actions, adenosine also yields receptor-*independent* actions, which rely on metabolic and intracellular levels and the metabolism of adenosine (Boison and Yegutkin, 2019). ADK plays a crucial role in the regulation of both extracellular and intracellular adenosine levels (Jacobson and Reitman, 2020) and adenosine receptor-dependent and independent pathways, in coordination with other adenosine metabolizing enzymes (Boison and Yegutkin, 2019). We will briefly review adenosine metabolism with a focus on the relationship between receptor-independent pathways of adenosine and DNA methylation in cancer.

2 DEOXYRIBONUCLEIC ACID HYPERMETHYLATION IN TUMOR-SUPPRESSOR GENES

DNA methylation, one of the most abundant epigenetic modifications modulates gene expression and affects cellular processes of metabolism, survival, proliferation, and apoptosis, among others. (Weber et al., 2007; Baylin et al., 2001). Methylation occurs on cytosines within dinucleotide CpG islands (CGIs) which are rich in CpG and usually located at the promoter regions of genes (Oates et al., 2006). It is commonly associated with a transcriptionally repressed status. However, methylation-dependent transcriptional changes can result in both gain and loss of function depending on the gene region affected (Weber et al., 2007). DNA methylation consists of two functionally overlapped aspects: *de novo* and maintenance methylation. A new DNA methylation commonly yields 5-methylcytosine (5-mC), which is established by transferring the methyl group from S-adenosylmethionine (SAM) to cytosine at a CpG site by DNA methyltransferases DNMT3A and DNMT3B (Egger et al., 2006; Hung and Shen, 2003). DNMT3A and DNMT3B mediate *de novo* DNA methylation that does not require a DNA template with preexisting methylation (Okano et al., 1999) whereas DNMT1 contributes to maintaining methylation that involves replicating methylation patterns into a newly-synthesized DNA strand (Goyal et al., 2006). On the other hand, a demethylation system also exists, which includes ten-eleven translocation methylcytosine dioxygenases (TETs) and thymine DNA glycosylase (TDG)-base excision repair (BER) (Pan et al., 2017). The TETs catalyze the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, and its downstream oxidation products: 5-formylcytosine and 5-carboxylcytosine, are removed by TDG of BER (**Figure 1**). Both methylation and demethylation systems contribute to the dynamically balanced methylation status of the genome (Weber et al., 2007).

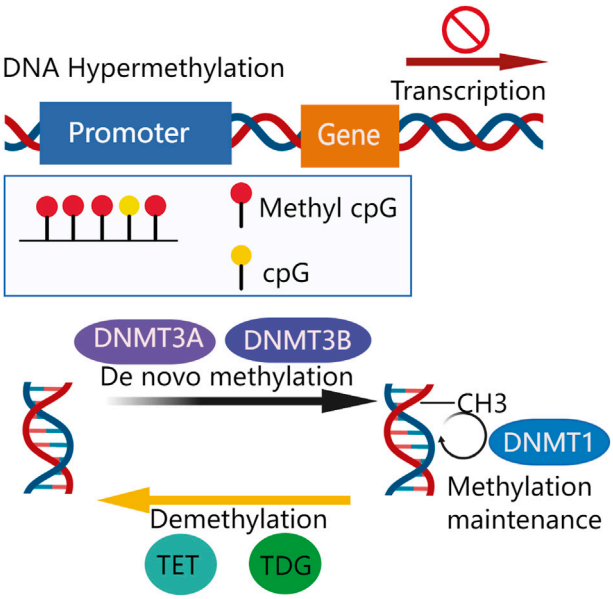


FIGURE 1 | Overview of DNA methylation in CpG of the gene promoter region.

De novo methylation is mediated by DNMT3A and DNMT3B to transfer methyl group (-CH3). Methylation is maintained by DNMT1. Demethylation of DNA is mediated by TET, TDG, and BER. A certain extent of promoter CpG island methylation impairs transcription, silencing gene expression.

Alternation in DNA methylation patterns is of importance in cancer pathology without affecting genome editing (Feinberg and Tycko, 2004) while DNA both hypermethylation and hypomethylation are seen in cancers (Das and Singal, 2004; Franco et al., 2008; Sinčić and Herceg, 2011). Cancer-associated methylome alterations are attributable to expressional changes of DNMTs (Morey et al., 2006; Gao et al., 2013; Micevic et al., 2017), which can result in increased genomic instability, expression of oncogenes, and/or decreased expression of tumor suppressor genes (Zhang et al., 2017; Valencia and Kadoch, 2019). Specifically, hypomethylation commonly occurs in oncogenes during cancer development and has been extensively reviewed (Mendizabal et al., 2017); in contrast, DNA hypermethylation is mostly found in tumor suppressor genes (Su et al., 2018). In the present review, we will focus on hypermethylation of tumor suppressor genes and possible adenosine regulations.

Hypermethylation resulting in epigenetic silencing was first demonstrated in the studies of retinoblastoma patients, in which hypermethylation was discovered in the promoter of the retinoblastoma tumor-suppressor *(RB1)* gene (Greger et al., 1989). Since then, a large number of tumor-suppressor genes have been identified as being silenced by DNA hypermethylation in tumorigenesis of different cancers. In colorectal cancers: 1) a cytokinesis-related gene Septin9 was identified highly correlated with the occurrence and development of colorectal cancer (Tanaka et al., 2002) and DNA methylation is the main mechanism regulating Septin9 gene expression (Sellin et al., 2011; Connolly et al., 2011), which mediates cytokinesis failure, leading to aneuploidy, centrosome amplification, and multipolar mitosis, eventually cause cell division and carcinogenesis (Sun et al., 2019; Cortez et al., 2016). In addition, the methylation level of the Septin9 gene is also considered to have clinical guiding significance due to the correlation with malignancy (Sun et al., 2019; Bae et al., 2017) and the overall survival of patients (Yang et al., 2019). Methylation of Septin9 in peripheral blood is the first blood DNA methylation marker approved by the US Food and Drug Administration (FDA) for CRC screening (Church et al., 2014), and is now widely used as a colorectal cancer biomarker (Xie et al., 2018). 2) MLH1, as the homolog of MutL, the main protein of the mismatch repair (MMR) system (Gelsomino et al., 2016), is silenced due to the hypermethylation of its promoter (Liu et al., 2017), resulting in deficient mismatch repair (dMMR) (Yamamoto and Imai, 2015). The replication errors of microsatellites (MS) cannot be corrected and accumulate continuously, resulting in microsatellite instability (MSI). Significance correlations were found in MLH1 promoter methylation and gender, tumor position, tumor differentiation, MSI, MLH1 protein expression, and v-RAF murine sarcoma viral oncogene homolog B1(BRAF) mutation in CRC patients (Li et al., 2013). In gastric cancer: runt-related transcription factor 3 (RUNX3) is an important downstream target of transforming growth factor-beta (TGFb) superfamily signaling, CpG silencing in the promoter region of regulated genes by hypermethylation is thought to be one of the mechanisms leading to loss of gene function (Fan et al., 2011). Through the detection of plasma samples, RUNX3 methylation level was considered to be a risk factor for gastric cancer metastasis and a potential indicator of gastric cancer progression (Fan et al., 2011). In breast cancer, the following genes are described: 1) ataxia-telangiectasia mutation (ATM) gene, a tumor suppressor plays a crucial role in maintaining genome integrity by activating cell cycle checkpoints and promoting the repair of DNA double-strand breaks (Wengner et al., 2020). Hypermethylation in ATM gene promoter downregulates ATM mRNA expression and positively correlates with increased tumor size and advanced disease stages III and IV (Begam et al., 2017; Cao et al., 2018). 2) a DNA repair gene, breast cancer 1 (BRCA1) - when a pathogenic mutation occurs, resulting in homologous recombination deficiency, the damaged DNA is difficult to repair, and it has been proved to easily lead to malignant tumors such as triple-negative breast cancer (TNBC) (Sharma, 2016). By comprehensively comparing the molecular biological characteristics of TNBC patients with BRCA1 hypermethylation and BRCA1 mutation, Dominik Glodzik et al. found the frequency of BRCA1 promoter hypermethylation correlates with clinicopathological variables, molecular subtypes, and patient outcomes in the early-stage of TNBC. This study indicated hypermethylation of the BRCA1 promoter region as a potential biomarker of early TNBC occurrence (Glodzik et al., 2020).

Together, the evidence indicates that DNA hypermethylation in the promoter region of tumor suppressors plays a crucial role in tumorigenesis, which is an epigenetic hallmark of various types of cancer. **Table 1** lists representative tumor suppression genes with hypermethylation in their promoters. Indeed, the demethylation treatment strategy was proposed after discovering abnormal hypermethylation in tumors and researchers started the attempt to reverse hypermethylation (Issa, 2007). 5-Aza-2′-deoxycytidine (5-AZA-CdR, decitabine) (Karahoca and Momparler, 2013) was shown to have the ability to reverse DNA methylation, activate tumor suppressor genes, and promote apoptosis (Flohr and Breull, 1975), with possible mechanisms relied on the inhibition of DNMT1 (Chen et al., 2019). In a xenograft mouse model bearing the colon cancer line, HCT116, the 5-AZA-CdR was shown to demethylate the *CDH13 gene*, restoring its expression, resulting in a suppression of tumor growth (Ren and Huo, 2012). However, related experiments confirmed that gene re-expression in response to 5-AZA-CdR was transient and re-silenced upon drug removal (Bender et al., 1998; Egger et al., 2007). Besides, studies have also pointed out that 5-AZA-CdR treatment has always been interpreted with caution since the 5-AZA-CdR treatment can non-selectively affect the entire genome (Christman, 2002; Sigalotti et al., 2014). The non-selective demethylation yielded from 5-AZA-CdR may trigger serious adverse reactions, which limit its clinical use. Thus, methylation inhibitors with fewer side effects and higher selectivity on cancer cells are of interest for development.

3 ADENOSINE REGULATIONS IN CANCER

Adenosine is an endogenous purine nucleoside and an intermediary metabolite in DNA methylation. Adenosine

TABLE 1 | Promoter hypermethylated genes in cancers.

Cancer Type	Gene	Detection	Hypermethylation Indication	References
Colorectal cancer	Septin9	Peripheral blood assays	Tumor malignancy Affect overall survival of patients	(Sun et al., 2019; Yang et al., 2019)
	MLH1	Immunohistochemistry (indirect)	Tumor differentiation and position BRAF mutation	Li et al. (2013)
Gastric cancer	RUNX3	Peripheral blood assays	Tumor differentiation Risk factors for the carcinogenesis of chronic atrophic gastritis with *H. pylori* infection Tumor malignancy	Fan et al. (2011)
Lung cancer	SHOX2	Bronchial aspirates Peripheral blood assays	Early detection of lung cancer with high sensitivity and specificity	Kneip et al. (2011)
Breast *Cancer*	APC	Peripheral blood assays	Better sensitivity than traditional tumor markers for early detection of breast cancer	(Van der Auwera et al., 2009; Swellam et al., 2015; Debouki-Joudi et al., 2017)
	BRCA1	Peripheral blood assays	Biomarkers of early TNBC occurrence	(Sharma, 2016; Winter et al., 2016)
Prostate *Cancer*	CDH13	Peripheral blood assays	Increased risk of death Independent predictor of a poor prognosis	Wang et al. (2014)

accumulation has been observed in tumor tissues, which is associated with tumor growth, invasion, metastasis, and immune evasion in tumor pathology (Mastelic-Gavillet et al., 2019; Borodovsky et al., 2020; Wang et al., 2021). Adenosine has immunosuppressive effects on intratumoral immune populations (Stagg and Smyth, 2010). It can bind cell surface receptors and is secreted in a paracrine or autocrine manner or reverse regulate DNA methylation through substrate accumulation, thus exerting its biological effect. Major pathways regarding adenosine production, metabolic removal, and transportation across the cell membranes have been extensively reviewed otherwise (Boison and Yegutkin, 2019), we briefly summarize them as follows.

Adenosine production and transportation in cancer tissues are similar to physiological conditions; extracellular ATP and ADP can rapidly metabolize to adenosine monophosphate (AMP) majorly through two steps of dephosphorylation: 1) The first step, ATP and ADP are both converted to AMP by ecto-nucleoside triphosphate diphosphohydrolase-1 (CD39); then 2) AMP can generate adenosine by the final dephosphorylation reaction catalyzed by the enzyme ecto-5′-nucleotidase (CD73) (Fishman et al., 2009b) - this called CD39/CD73 pathway. Alternatively, cyclic ADP ribose hydrolase (CD38) can convert adenosine diphosphate ribose (ADPR) to AMP, this process can be regulated by ecto-nucleotide pyrophosphatase/phosphodiesterase 1, NPP1 (CD203a) (Gazzoli et al., 2002; Häusler et al., 2011). Afterward, CD73 converts AMP into adenosine–called CD38/CD203a pathway. In adenosine transportation across membranes, equilibrative nucleoside transporter (ENT) and concentrative nucleoside transporters (CNTs) play important roles (Song et al., 2017); Adenosine removal differs between intracellular and extracellular. Extracellular adenosine is converted to inosine by adenosine deaminase (ADA), which is widely expressed in the plasma as well as on the cell membrane. Inosine is then derivatized (removed from ribose) by purine nucleoside phosphorylase (PNP), which converts it to hypoxanthine. It is worth noting

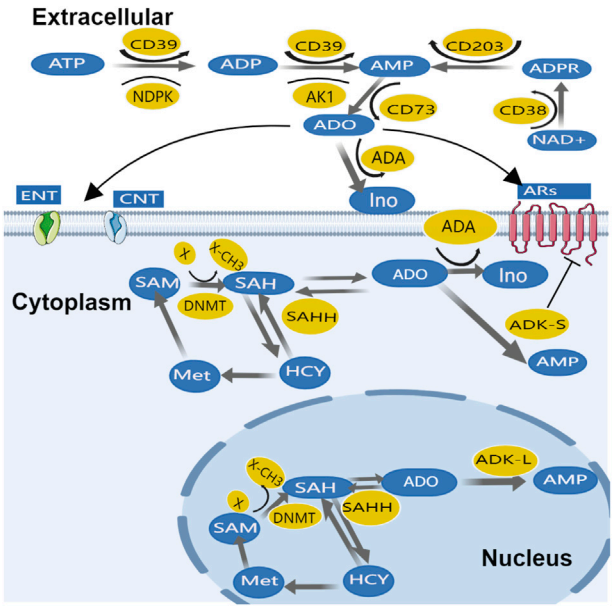


FIGURE 2 | Major pathways of adenosine production, metabolism, and transport.

that ADA not only metabolizes adenosine, it also allosterically modulates ARs, resulting in a positive effect of amplifying downstream signals (Borea et al., 2018) including 1) enhanced AR1 sensitivity to adenosine (SU Xiaoyang, 2018); 2) interaction of ADA-CD26 complex in T cells with ADA-anchored protein in dendritic cells enhanced T cell proliferation (Pacheco et al., 2005), etc.

While the metabolism of intracellular adenosine is mainly dominated by ADK. The major adenosine removal enzyme ADK has two isoforms with distinguished subcellular expression patterns; while ADK short isoform (ADK-S) is expressed dominantly in cytosolic space, ADK long isoform (ADK-L) is

solely located in the nuclei (Cui et al., 2009; Fedele et al., 2005). Intracellular adenosine is mainly removed by ADK-S, which converts adenosine to AMP (Boison and Yegutkin, 2019). Adenosine can also be directly inactivated on the cell surface by adenosine deaminase (ADA). In addition, adenosine metabolism also depends on adenosine phosphoribosyltransferase (APRT) to catalyze adenine reaction with ribose 1-phosphate to generate phosphate and adenosine in the nucleus. However, when energy consumption increases and/or energy supply is compromised, ATP is converted into AMP by adenylate kinase-1 (AK1) and nucleotide diphosphate kinase (NDPK), and then dephosphorylated into adenosine by 5-nucleotidase (Eltzschig et al., 2012). This process promotes extracellular ATP regeneration through a reversible phosphonate transfer reaction (Boison, 2013). The nucleoside transporters and adenosine removal enzymes maintain a dynamic balance between extracellular and intracellular adenosine (**Figure 2**). Due to mitochondria being the main source of ATP, mitochondrial bioenergy is related to adenosine homeostasis (Ashar et al., 2017).

Moreover, adenosine metabolism is a part of the transmethylation pathway, in which DNA can be methylated by DNMTs while SAM donates methyl group (-CH3) via a methyltransferase (MT) - catalyzed transmethylation reaction (**Figure 2**). Then, the SAM converted SAH is hydrolyzed to adenosine and Hcy by SAHH. Interestingly, the nuclear form of ADK-L drives methyl flux, enhancing DNA and histone methylation (Yegutkin, 2014).

Extracellular adenosine turnover is mediated by AR, ENT, and CNT. Factors that mediate adenosine production and removal include the enzymes CD39, CD73, ADK, and ADA. Additionally, intracellular adenosine metabolism depends on the cytoplasmic form of ADK-S and ADA. In the nucleus adenosine is part of the transmethylation pathway in which DNA is methylated by DNMT. ADK-L participates in driving the methyl groups through the transmethylation pathway affecting DNA and histone methylations. For the sake of clarity, only the most important enzymes are mentioned.

4 ADENOSINE RECEPTOR-DEPENDENT PATHWAY IN CANCER

Substantial evidence indicates that adenosine mediates its physiological effects (Borea et al., 2018) as well as its pathophysiological actions in cancer (Fishman et al., 2009a; Franco et al., 2021) through the activation of four adenosine receptors (ARs), i.e., A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R. Activation of ARs by specific ligands, agonists, or antagonists will regulate the occurrence and development of tumors through a series of signaling pathways (Borea et al., 2018; Franco et al., 2021). A_1R has been studied mainly in glioblastoma (Synowitz et al., 2006; Fishman et al., 2009a), where A_1R activation on microglia/macrophages in the tumor suppresses not only the production of cytokines such as interleukin-1β but also stromal metalloproteinase (MMP) (Tsutsui et al., 2004). Based on that, A_1R is thought to have the effect of inhibiting tumor growth (Synowitz et al., 2006). Besides, what cannot be ignored is the important role of ARs in tumor immunity. In the tumor microenvironment, adenosine suppresses antitumor immunity, essentially through $A_{2A}R$ and $A_{2B}R$ (Buisseret et al., 2018). In particular, the $A_{2A}R$, due to the high concentration of Ado in the tumor microenvironment, activates Gs-coupled A2AR and leads to an increase in cAMP, thereby inhibiting the activation of tumor lymphocytes (Fishman et al., 2009a; Merighi et al., 2019). Therefore, selective antagonism of $A_{2A}R$ can reduce cAMP levels, thereby enabling lymphocytes to effectively fight tumor cells (Franco et al., 2021). So far, a large number of clinical trials on $A_{2A}R/A_{2B}R$ antagonists are also in progress (Franco et al., 2021). On the other hand, adenosine was observed to increase HIF1α protein accumulation under hypoxia situations through cell surface A_3R interaction in various tumors (Merighi et al., 2005), and HIF1α plays an important role in tumor VEGF expression and angiogenesis (Merighi et al., 2005). Based on the relationship between tumor, hypoxia, and adenosine concentrations, A_3R antagonists are considered to have a potential role in cancer therapy (Franco et al., 2021). Adenosine receptor-dependent pathway in cancer was already described in detail by Pier Andrea (Borea et al., 2018).

5 ADENOSINE RECEPTOR-INDEPENDENT PATHWAY WITH DEOXYRIBONUCLEIC ACID METHYLATION IN CANCER

As an ATP metabolite, adenosine is released by all cell types and is shown to accumulate in tumor cells, which is associated with increased angiogenesis, high metabolism rate, and compromised hypoxia of the microenvironment (Losenkova et al., 2020). Accumulation of adenosine in the tumor microenvironment (TME) (de Lera Ruiz et al., 2014) has been proven to play an important role in tumor immunity, high concentrations of adenosine inhibit tumor immune effects (Ohta and Sitkovsky, 2001; Ohta et al., 2006; Ohta, 2016) and facilitate angiogenesis (van de Veen et al., 2020), which offers the possibility of targeting adenosine in cancer pathology and manipulation of adenosine actions represents a potential anti-cancer strategy. Meanwhile, solid tumors can maintain adenosine gradients - the adenosine levels in the tumor center are higher than in the peripheral area of the tumor (Ohta et al., 2006). High levels of adenosine are shown to hinder tumor growth and proliferation. For instance, peripheral tumor cells located in the parenchyma and stroma have been shown to have high proliferative and invasive abilities (Seetulsingh-Goorah, 2006) and their proliferation can be suppressed by adenosine (Seetulsingh-Goorah, 2006; Schiedel et al., 2013). Based on that, Sanna S. Virtanen *et al.* found adenosine with relatively high (10 μmol/L for the former and 50 μmol/L for the latter) concentrations showed the ability to inhibit tumor invasion and migration (Schiedel et al., 2013). Besides, incubation of human prostate carcinoma cell line PC-3 cells triggered a concentration-dependent increase in cAMP levels with increasing adenosine concentrations. However, in the presence of $A_{2B}R$-selective antagonists, no changes in

cAMP levels were observed (Schiedel et al., 2013). In addition, in a study on glioblastoma, Helena Marcelino *et al.* found that proliferation/viability of glioblastoma cells was significantly reduced after 30 μM doses of adenosine for three consecutive days. At the same time, the cocktail of adenosine receptor antagonists (Fredholm et al., 2001) was administered, but the tumor suppressor effect was not affected (Marcelino et al., 2021).

The above described discrepant effects of adenosine on pro- and anti-tumor cell growth suggest a possible involvement of multiple mechanisms. In other words, its inhibitory effect on proliferation is proposed beyond receptor-mediated adenosine activity (Virtanen et al., 2014), though the underlying mechanisms remain unclear. Possible metabolic contributors that determine high-adenosine level mediated inhibition may include extracellular adenosine deaminase activity, subsequent cellular uptake, interconversion of transported nucleosides, simultaneous inhibition of multiple protein kinases (Virtanen et al., 2014), as well as ADK actions. However, the potential involvement of multiple pathways in adenosine production, transportation, and metabolism, suggests the complexity of adenosine's effect on tumor pathology.

Importantly, the metabolism of adenosine also affects the methylation process. When SAM/SAH is an important source of adenosine, it can reverse regulate DNA methylation through the substrate accumulation effect (Kloor and Osswald, 2004; Viré et al., 2006). Kai X *et al.*, by observing the effects of different concentrations of adenosine (0, 1.5, 3.0, 4.5 mmol/L) and treatment time (24, 48, 72, 96 h) on the proliferation, apoptosis, and HMLH1 expression of human colorectal cancer cell SW480, found that after treating colorectal cancer cells with different concentrations of adenosine, the hypermethylation of tumor suppressor genes hMLH1 was reversed and inhibited the proliferation of tumor cells. This kind of positive effect increased with the addition of exogenous adenosine concentration and treatment time (Xie et al., 2014). Meanwhile, Li Q *et al.* found that after treating human colorectal cancer cells SW480 with adenosine (3.0 mmol/L) for 72 h, the activity of methyltransferase (DNMT1 and DNMT3A) in these cells was inhibited, and similar to the above finding the hypermethylation of tumor suppressor genes RECK was reversed (Li et al., 2015). Like the aforementioned, alternations in DNA methylation patterns impact the occurrence and development of tumors (Klutstein et al., 2016). Studies regarding adenosine and DNA methylation status have also been reported in non-tumor disorders such as epilepsy, showing that inhibition of DNA methyltransferase activity during adenosine release is associated with restoration of global DNA methylation levels (Williams-Karnesky et al., 2013), this suggests that adenosine manipulation is a potential strategy in cancer manipulation via DNA methylation.

However, side effects such as flushing, dyspnea, chest pain, hypotension, bradycardia, etc. make the usage of exogenous adenosine less feasible for cancer treatment (Pritchard et al., 2010; Galagudza et al., 2012; Gul et al., 2020). A further question is whether systemic adenosine leads to a reversal of global methylation status or affects the site that should have been hypomethylated. Another concern is adenosine receptor-mediated action showed a cancer-promoting effect. Conversely, accumulating evidence supports ADK as a therapeutic target in cancer (Boison and Yegutkin, 2019; Murugan et al., 2021). The expression of ADK was shown to be upregulated in specific cancer types, including colorectal cancer (Giglioni et al., 2008), and breast cancer (Wang and Yang, 2014; Shamloo et al., 2019). Most recently, it has been found that a significantly enhanced expression of ADK in specimens of patients with glioma, both the tumor center and peritumoral tissue (de Groot et al., 2012). The general increase of purine metabolizing enzymes including ADK may allow accelerated purine metabolism to support the growth of cancer (Vannoni et al., 2004; Giglioni et al., 2008).

6 TARGETING ADENOSINE KINASE ON DEOXYRIBONUCLEIC ACID METHYLATION IN CANCER

The above described receptor-independent pathway mechanisms of adenosine play important roles in various types of cells with diverse functions (Boison et al., 2002). As an essential adenosine removal enzyme, inhibition of ADK can be more effective to decrease the cellular reuptake of adenosine and thereby increase the ambient concentration of extracellular adenosine (Newby et al., 1983; Davies et al., 1984). ADK inhibition was hypothesized to function as a site- and event-specific modulator for adenosine levels (Yamamoto and Imai, 2015; Cortez et al., 2016). This also provides a new direction for the treatment of tumors–targeting overexpression of ADK to regulate onsite adenosine level and DNA methylation, thereby affecting the proliferation and apoptosis of tumor cells. ADK-based adenosine intervention can avoid the aforementioned side effects of systemic adenosine administration (Liu et al., 2019) and pharmacokinetics limitation of the very short half-life in circulation (Hwang et al., 2016). ADK inhibitors have been revealed to have anti-inflammatory, antinociceptive, and anticonvulsant features (McGaraughty et al., 2005), and is being considered for the treatment of various diseases, including diabetes (Annes et al., 2012) and diseases of the nervous system (Chen et al., 2016).

ADK inhibitor development was initially based on 5-iodotubercidin (5-ITU), and 5′-amino-5′-deoxyadenosine (Cottam et al., 1993; Wiesner et al., 1999; Chen et al., 2016). Since then, several types of ADK inhibitors have been developed, which are classified as nucleoside and non-nucleoside ADK inhibitors (Boison, 2013). Nucleoside ADK inhibitors are adenosine derivatives that have hydroxylated ribose or cyclopentane rings, and additional purines or pyrimidine heterocyclic bases (Gomtsyan and Lee, 2004). The 5-aza group of the purine ring is replaced by a carbon connected to iodine. These compounds bind to enzymes to competitively inhibit adenosine (McGaraughty et al., 2005). In contrast, non-nucleoside ADK inhibitors lack ribose or cyclopentane rings, while some of them are constructed on pyrimidine or pyridyl pyrimidine nuclei. The non-nucleoside ADK inhibitors have been shown to relieve pain and inflammation in animal models (McGaraughty et al., 2005). Some ADK inhibitors are based on 6-(het)aryl-7-deazapurine pro-nucleotides that can inhibit

cell growth by strongly inhibiting ADK activity (Spácilová et al., 2010), however, the mechanism of this finding has not been further investigated. Helena Marcelino *et al.* tested the effect of two ADK inhibitors on tumor cells in experiments on glioblastoma, and the results suggested that both ITU (25 μM) and ABT702 (15 μM) affected cells proliferation/viability (Marcelino et al., 2021). Co-incubation of ITU (25 μM) and adenosine (30 μM) produced a strong and similar decrease in cell proliferation in both GBM cell lines compared to ITU alone, this suggests that only 25 mM ITU may be sufficient to generate the maximum accumulation of intracellular adenosine (Marcelino et al., 2021). Zhang LM *et al.* showed that 5-ITU with concentrations (1, 2, 4, 6, 8, 10 μmol/L) for 48 h could significantly inhibit proliferation and induced apoptosis in a colon cancer cell line HT-29 (Zhang and Xie, 2015). Compared to the inhibitory effect of each concentrations group on HT-29 cells, the 6 μmol/L group showed a better effect on HT-29 cells, and the tumor suppressor gene DLC-1 in HT-29 cells was up-regulated and its methylation level was decreased after being treated with 2, 4, and 6 μmol/L ITU, respectively, this effect increases with increasing concentration (Zhang and Xie, 2015). As discussed above, ADK may play a potential adenosine receptor-independent epigenetic function, however, current available ADK inhibitors have not yet been reported to have high selectivity to target ADK-L or ADK-S. To distinguish the role of ADK-S and ADK-L on the regulation of cytoplasmic or nuclear adenosine levels and their possible epigenetic functions, using genetic approaches may bring us the answer.

Targeted therapy is a new strategy for cancer treatment. The goal is to use gene therapy to suppress the endogenous expression of ADK, with or without selectively targeting its two isoforms, i.e., the nuclear ADK-L and cytosolic ADK-S (Chen, 2010). Previous studies identified two independent promoters driving the expression of ADK isoforms, suggesting that each of the two isoforms of ADK are independently regulated at the transcriptional level (Cui et al., 2011), and independent transcriptional regulation may in turn indicate distinct physiological functions of the two isoforms (Boison, 2013). Besides, distinguish expression locations of two isoforms indicate that ADK-L (vs. ADK-S) has a unique role in proliferation and differentiation - two main nuclear activities associated with cancer pathology (Cui et al., 2009; Kiese et al., 2016). In patients with grade II and III gliomas, both subtypes of ADK are increased in the tumor and peritumoral areas, in addition to the detection of tumor invasion in the peritumoral tissue suggesting that ADK is involved in glioma progression and ADK level elevations may be associated with epilepsy in glioma patients (Huang et al., 2015). Amir E *et al.* reported a high positive correlation between ADK-L expression and whole-genome methylation in HeLa cells, (Wahba et al., 2021). Most recently, Shen HY *et al.* revealed that the expression level of ADK-L in breast cancer tissue was elevated compared to adjacent tissues, while the ADK-S expression level had no significant change, by measuring the protein expression level (Shamloo et al., 2019). Selective knockout of ADK isoforms via CRISPR/Cas9-mediated approaches suppressed breast cancer cell migration and invasion, which with the elevation of a tumor-related enzyme, matrix metalloproteinases, and downregulation of cyclin D2 and THB1 (Shamloo et al., 2019). Williams Karnesky *et al.* transfected ADK deficient BHK-AK2 cells with ADK-L- or ADK-S-expressing plasmids (Williams-Karnesky et al., 2013). ADK-L receptors showed a 400% increase in overall DNA methylation compared to controls, while ADK-S receptors showed only a modest 50% increase in overall DNA methylation. While both isoforms of ADK are involved in the regulation of overall DNA methylation, the nuclear subtype is more effective in regulating DNA methylation (Williams-Karnesky et al., 2013). ADK-L affects epigenetic remodeling by regulating methyltransferase activity and is considered the preferred mechanism for adenosine clearance in the nuclei (Boison and Yegutkin, 2019). ADK-L is directly related to the S-adenosylmethionine-dependent transmethylation pathway, which drives DNA and histone methylation (Boison, 2013). ADK-S regulates extracellular adenosine concentration for the availability of ARs activation (Pignataro et al., 2007; Boison and Yegutkin, 2019).

These studies support the observed functional differences of ADK-L and ADK-S in cancer. While ADK-L and ADK-S control adenosine concentrations in the nucleus and cytoplasm/extracellular respectively, ADK-L may play a role in adenosine receptor-independent regulation of epigenetic functions, and ADK-S determines adenosine availability for activation of adenosine receptors (Pignataro et al., 2007; Williams-Karnesky et al., 2013). Additional experimental evidence is needed to evaluate this notion. Together, selective inhibition of ADK-L is indicated as a novel adenosine receptor-independent strategy to offer a new perspective on cancer therapy, which may achieve more precise cancer intervention than general ADK or ADK-S manipulation.

7 PROSPECT AND CHALLENGE

With the observations that ADK inhibitions with isoform- and site-selective manners enhance the beneficial effect of endogenous adenosine and avoid various side effects of systemic manipulation of adenosine and adenosine receptors, research on ADK has made considerable progress in recent years. The emergence of new molecular tools including genetic approaches has enabled deeper exploration of ADK function. Further characterization of the metabolism of adenosine in different subcellular contexts, including cytoplasm, nucleus, and extracellular space, is needed for potential targeted ADK therapy. Studies have shown that elevated adenosine levels are related to apoptosis in various cancers (Xie et al., 2014; Jafari et al., 2017), which may be attributed to nuclear ADK-L (vs. ADK-S). In addition, the ADK effects on epigenetics, especially DNA methylation, may also be through its direct interaction with other nuclear proteins (Wang et al., 2005; Mohannath et al., 2014) rather than its regulation on the adenosine level. We should always bear in mind the challenge that increased adenosine levels can: 1) inhibit immune and inflammatory responses; 2) stimulate

angiogenesis: epigenetic regulation of pro-angiogenic genes by ADK, and is thought to be another mechanism by which ADK is involved in cancer (Murugan et al., 2021). Knockdown of ADK decreases the methylation level of the VEGFR2 promoter region, which elevates intracellular adenosine and promotes proliferation, migration, and angiogenesis of human endothelial cells (Xu et al., 2017)—all aspects that may promote tumor growth. Last but not least, the downregulation of ADK found in hepatocellular carcinoma cells (YH, 2017) suggests the diversity of ADK changes across cancers. In summary, additional studies are needed to fully understand the role of adenosine in cancer pathology and to reveal the anticancer potential of ADK inhibition.

AUTHOR CONTRIBUTIONS

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

FUNDING

The project was supported by grants from the Good Samaritan Foundation of Legacy Health: No. 750390799 (H-YS) and the General Project of Chongqing Natural Science Foundation: No. cstc2021jcyj-msxmX0112 (Y-XW).

REFERENCES

Annes, J. P., Ryu, J. H., Lam, K., Carolan, P. J., Utz, K., Hollister-Lock, J., et al. (2012). Adenosine Kinase Inhibition Selectively Promotes Rodent and Porcine Islet β-cell Replication. *Proc. Natl. Acad. Sci. U. S. A.* 109 (10), 3915–3920. doi:10.1073/pnas.1201149109

Antonioli, L., Blandizzi, C., Pacher, P., and Haskó, G. (2013). Immunity, Inflammation and Cancer: a Leading Role for Adenosine. *Nat. Rev. Cancer* 13 (12), 842–857. doi:10.1038/nrc3613

Ashar, F. N., Zhang, Y., Longchamps, R. J., Lane, J., Moes, A., Grove, M. L., et al. (2017). Association of Mitochondrial DNA Copy Number with Cardiovascular Disease. *JAMA Cardiol.* 2 (11), 1247–1255. doi:10.1001/jamacardio.2017.3683

Bae, J. M., Kim, J. H., Kwak, Y., Lee, D. W., Cha, Y., Wen, X., et al. (2017). Distinct Clinical Outcomes of Two CIMP-Positive Colorectal Cancer Subtypes Based on a Revised CIMP Classification System. *Br. J. Cancer* 116 (8), 1012–1020. doi:10.1038/bjc.2017.52

Baylin, S. B., Esteller, M., Rountree, M. R., Bachman, K. E., Schuebel, K., and Herman, J. G. (2001). Aberrant Patterns of DNA Methylation, Chromatin Formation and Gene Expression in Cancer. *Hum. Mol. Genet.* 10 (7), 687–692. doi:10.1093/hmg/10.7.687

Begam, N., Jamil, K., and Raju, S. G. (2017). Promoter Hypermethylation of the ATM Gene as a Novel Biomarker for Breast Cancer. *Asian Pac J. Cancer Prev.* 18 (11), 3003–3009. doi:10.22034/APJCP.2017.18.11.3003

Bender, C. M., Pao, M. M., and Jones, P. A. (1998). Inhibition of DNA Methylation by 5-Aza-2'-Deoxycytidine Suppresses the Growth of Human Tumor Cell Lines. *Cancer Res.* 58 (1), 95–101.

Boison, D. (2013). Adenosine Kinase: Exploitation for Therapeutic Gain. *Pharmacol. Rev.* 65 (3), 906–943. doi:10.1124/pr.112.006361

Boison, D., Scheurer, L., Zumsteg, V., Rülicke, T., Litynski, P., Fowler, B., et al. (2002). Neonatal Hepatic Steatosis by Disruption of the Adenosine Kinase Gene. *Proc. Natl. Acad. Sci. U. S. A.* 99 (10), 6985–6990. doi:10.1073/pnas.092642899

Boison, D., and Yegutkin, G. G. (2019). Adenosine Metabolism: Emerging Concepts for Cancer Therapy. *Cancer Cell* 36 (6), 582–596. doi:10.1016/j.ccell.2019.10.007

Borea, P. A., Gessi, S., Merighi, S., Vincenzi, F., and Varani, K. (2018). Pharmacology of Adenosine Receptors: The State of the Art. *Physiol. Rev.* 98 (3), 1591–1625. doi:10.1152/physrev.00049.2017

Borodovsky, A., Barbon, C. M., Wang, Y., Ye, M., Prickett, L., Chandra, D., et al. (2020). Small Molecule AZD4635 Inhibitor of A2AR Signaling Rescues Immune Cell Function Including CD103+ Dendritic Cells Enhancing Anti-tumor Immunity. *J. Immunother. Cancer* 8 (2). doi:10.1136/jitc-2019-000417

Buisseret, L., Pommey, S., Allard, B., Garaud, S., Bergeron, M., Cousineau, I., et al. (2018). Clinical Significance of CD73 in Triple-Negative Breast Cancer: Multiplex Analysis of a Phase III Clinical Trial. *Ann. Oncol.* 29 (4), 1056–1062. doi:10.1093/annonc/mdx730

Cao, X., Tang, Q., Holland-Letz, T., Gündert, M., Cuk, K., Schott, S., et al. (2018). Evaluation of Promoter Methylation of RASSF1A and ATM in Peripheral Blood of Breast Cancer Patients and Healthy Control Individuals. *Int. J. Mol. Sci.* 19 (3). doi:10.3390/ijms19030900

Chen, J., Wu, L., Xu, H., and Cheng, S. (2019). 5-Aza-CdR Regulates RASSF1A by Inhibiting DNMT1 to Affect Colon Cancer Cell Proliferation, Migration and Apoptosis. *Cancer Manag. Res.* 11, 9517–9528. doi:10.2147/CMAR.S229726

Chen, L. (2010). A Global Comparison between Nuclear and Cytosolic Transcriptomes Reveals Differential Compartmentalization of Alternative Transcript Isoforms. *Nucleic Acids Res.* 38 (4), 1086–1097. doi:10.1093/nar/gkp1136

Chen, Y. L., Zhang, Y. N., Wang, Z. Z., Xu, W. G., Li, R. P., and Zhang, J. D. (2016). Effects of Adenosine Metabolism in Astrocytes on Central Nervous System Oxygen Toxicity. *Brain Res.* 1635, 180–189. doi:10.1016/j.brainres.2016.01.026

Christman, J. K. (2002). 5-Azacytidine and 5-Aza-2'-Deoxycytidine as Inhibitors of DNA Methylation: Mechanistic Studies and Their Implications for Cancer Therapy. *Oncogene* 21 (35), 5483–5495. doi:10.1038/sj.onc.1205699

Church, T. R., Wandell, M., Lofton-Day, C., Mongin, S. J., Burger, M., Payne, S. R., et al. (2014). Prospective Evaluation of Methylated SEPT9 in Plasma for Detection of Asymptomatic Colorectal Cancer. *Gut* 63 (2), 317–325. doi:10.1136/gutjnl-2012-304149

Connolly, D., Yang, Z., Castaldi, M., Simmons, N., Oktay, M. H., Coniglio, S., et al. (2011). Septin 9 Isoform Expression, Localization and Epigenetic Changes during Human and Mouse Breast Cancer Progression. *Breast Cancer Res.* 13 (4), R76. doi:10.1186/bcr2924

Cortez, B. A., Rezende-Teixeira, P., Redick, S., Doxsey, S., and Machado-Santelli, G. M. (2016). Multipolar Mitosis and Aneuploidy after Chrysotile Treatment: a Consequence of Abscission Failure and Cytokinesis Regression. *Oncotarget* 7 (8), 8979–8992. doi:10.18632/oncotarget.6924

Cottam, H. B., Wasson, D. B., Shih, H. C., Raychaudhuri, A., Di Pasquale, G., and Carson, D. A. (1993). New Adenosine Kinase Inhibitors with Oral Antiinflammatory Activity: Synthesis and Biological Evaluation. *J. Med. Chem.* 36 (22), 3424–3430. doi:10.1021/jm00074a024

Cui, X. A., Agarwal, T., Singh, B., and Gupta, R. S. (2011). Molecular Characterization of Chinese Hamster Cells Mutants Affected in Adenosine Kinase and Showing Novel Genetic and Biochemical Characteristics. *BMC Biochem.* 12, 22. doi:10.1186/1471-2091-12-22

Cui, X. A., Singh, B., Park, J., and Gupta, R. S. (2009). Subcellular Localization of Adenosine Kinase in Mammalian Cells: The Long Isoform of AdK Is Localized in the Nucleus. *Biochem. Biophys. Res. Commun.* 388 (1), 46–50. doi:10.1016/j.bbrc.2009.07.106

Das, P. M., and Singal, R. (2004). DNA Methylation and Cancer. *J. Clin. Oncol.* 22 (22), 4632–4642. doi:10.1200/JCO.2004.07.151

Davies, L. P., Jamieson, D. D., Baird-Lambert, J. A., and Kazlauskas, R. (1984). Halogenated Pyrrolopyrimidine Analogues of Adenosine from Marine Organisms: Pharmacological Activities and Potent Inhibition of Adenosine Kinase. *Biochem. Pharmacol.* 33 (3), 347–355. doi:10.1016/0006-2952(84)90225-9

de Groot, M., Iyer, A., Zurolo, E., Anink, J., Heimans, J. J., Boison, D., et al. (2012). Overexpression of ADK in Human Astrocytic Tumors and Peritumoral Tissue Is Related to Tumor-Associated Epilepsy. *Epilepsia* 53 (1), 58–66. doi:10.1111/j.1528-1167.2011.03306.x

de Lera Ruiz, M., Lim, Y. H., and Zheng, J. (2014). Adenosine A2A Receptor as a Drug Discovery Target. *J. Med. Chem.* 57 (9), 3623–3650. doi:10.1021/jm4011669

Debouki-Joudi, S., Trifa, F., Khabir, A., Sellami-Boudawara, T., Frikha, M., Daoud, J., et al. (2017). CpG Methylation of APC Promoter 1A in Sporadic and Familial Breast Cancer Patients. *Cancer Biomark.* 18 (2), 133–141. doi:10.3233/CBM-160005

Egger, G., Aparicio, A. M., Escobar, S. G., and Jones, P. A. (2007). Inhibition of Histone Deacetylation Does Not Block Resilencing of P16 after 5-Aza-2'-Deoxycytidine Treatment. *Cancer Res.* 67 (1), 346–353. doi:10.1158/0008-5472.CAN-06-2845

Egger, G., Jeong, S., Escobar, S. G., Cortez, C. C., Li, T. W., Saito, Y., et al. (2006). Identification of DNMT1 (DNA Methyltransferase 1) Hypomorphs in Somatic Knockouts Suggests an Essential Role for DNMT1 in Cell Survival. *Proc. Natl. Acad. Sci. U. S. A.* 103 (38), 14080–14085. doi:10.1073/pnas.0604602103

Eltzschig, H. K., Sitkovsky, M. V., and Robson, S. C. (2012). Purinergic Signaling during Inflammation. *N. Engl. J. Med.* 367 (24), 2322–2333. doi:10.1056/NEJMra1205750

Fan, X. Y., Hu, X. L., Han, T. M., Wang, N. N., Zhu, Y. M., Hu, W., et al. (2011). Association between RUNX3 Promoter Methylation and Gastric Cancer: a Meta-Analysis. *BMC Gastroenterol.* 11, 92. doi:10.1186/1471-230X-11-92

Fedele, D. E., Gouder, N., Güttinger, M., Gabernet, L., Scheurer, L., Rülicke, T., et al. (2005). Astrogliosis in Epilepsy Leads to Overexpression of Adenosine Kinase, Resulting in Seizure Aggravation. *Brain* 128 (Pt 10), 2383–2395. doi:10.1093/brain/awh555

Feinberg, A. P., and Tycko, B. (2004). The History of Cancer Epigenetics. *Nat. Rev. Cancer* 4 (2), 143–153. doi:10.1038/nrc1279

Feinberg, A. P., and Vogelstein, B. (1983). Hypomethylation Distinguishes Genes of Some Human Cancers from Their Normal Counterparts. *Nature* 301 (5895), 89–92. doi:10.1038/301089a0

Fishman, P., Bar-Yehuda, S., Synowitz, M., Powell, J. D., Klotz, K. N., Gessi, S., et al. (2009). Adenosine Receptors and Cancer. *Handb. Exp. Pharmacol.* (193), 399–441. doi:10.1007/978-3-540-89615-9_14

Flohr, H., and Breull, W. (1975). Effect of Etafenone on Total and Regional Myocardial Blood Flow. *Arzneimittelforschung* 25 (9), 1400–1403.

Franco, R., Rivas-Santisteban, R., Navarro, G., and Reyes-Resina, I. (2021). Adenosine Receptor Antagonists to Combat Cancer and to Boost Anti-cancer Chemotherapy and Immunotherapy. *Cells* 10 (11). doi:10.3390/cells10112831

Franco, R., Schoneveld, O., Georgakilas, A. G., and Panayiotidis, M. I. (2008). Oxidative Stress, DNA Methylation and Carcinogenesis. *Cancer Lett.* 266 (1), 6–11. doi:10.1016/j.canlet.2008.02.026

Fredholm, B. B., Chen, J. F., Cunha, R. A., Svenningsson, P., and Vaugeois, J. M. (2005). Adenosine and Brain Function. *Int. Rev. Neurobiol.* 63, 191–270. doi:10.1016/S0074-7742(05)63007-3

Fredholm, B. B., IJzerman, A. P., Jacobson, K. A., Klotz, K. N., and Linden, J. (2001). International Union of Pharmacology. XXV. Nomenclature and Classification of Adenosine Receptors. *Pharmacol. Rev.* 53 (4), 527–552.

Galagudza, M., Korolev, D., Postnov, V., Naumisheva, E., Grigorova, Y., Uskov, I., et al. (2012). Passive Targeting of Ischemic-Reperfused Myocardium with Adenosine-Loaded Silica Nanoparticles. *Int. J. Nanomedicine* 7, 1671–1678. doi:10.2147/IJN.S29511

Gao, J., Wang, L., Xu, J., Zheng, J., Man, X., Wu, H., et al. (2013). Aberrant DNA Methyltransferase Expression in Pancreatic Ductal Adenocarcinoma Development and Progression. *J. Exp. Clin. Cancer Res.* 32, 86. doi:10.1186/1756-9966-32-86

Gazzoli, I., Loda, M., Garber, J., Syngal, S., and Kolodner, R. D. (2002). A Hereditary Nonpolyposis Colorectal Carcinoma Case Associated with Hypermethylation of the MLH1 Gene in Normal Tissue and Loss of Heterozygosity of the Unmethylated Allele in the Resulting Microsatellite Instability-High Tumor. *Cancer Res.* 62 (14), 3925–3928.

Gelsomino, F., Barbolini, M., Spallanzani, A., Pugliese, G., and Cascinu, S. (2016). The Evolving Role of Microsatellite Instability in Colorectal Cancer: A Review. *Cancer Treat. Rev.* 51, 19–26. doi:10.1016/j.ctrv.2016.10.005

Giglioni, S., Leoncini, R., Aceto, E., Chessa, A., Civitelli, S., Bernini, A., et al. (2008). Adenosine Kinase Gene Expression in Human Colorectal Cancer. *Nucleosides Nucleotides Nucleic Acids* 27 (6), 750–754. doi:10.1080/15257770802145629

Glodzik, D., Bosch, A., Hartman, J., Aine, M., Vallon-Christersson, J., Reuterswärd, C., et al. (2020). Comprehensive Molecular Comparison of BRCA1 Hypermethylated and BRCA1 Mutated Triple Negative Breast Cancers. *Nat. Commun.* 11 (1), 3747. doi:10.1038/s41467-020-17537-2

Gomtsyan, A., and Lee, C. H. (2004). Nonnucleoside Inhibitors of Adenosine Kinase. *Curr. Pharm. Des.* 10 (10), 1093–1103. doi:10.2174/1381612043452703

Goyal, R., Reinhardt, R., and Jeltsch, A. (2006). Accuracy of DNA Methylation Pattern Preservation by the Dnmt1 Methyltransferase. *Nucleic Acids Res.* 34 (4), 1182–1188. doi:10.1093/nar/gkl002

Greger, V., Passarge, E., Höpping, W., Messmer, E., and Horsthemke, B. (1989). Epigenetic Changes May Contribute to the Formation and Spontaneous Regression of Retinoblastoma. *Hum. Genet.* 83 (2), 155–158. doi:10.1007/BF00286709

Gul, M., Sheikh, M., Chaudhry, A., Gerges, L., Al Halabi, H., Feldman, E., et al. (2020). Role of Cardiac Nuclear Stress Perfusion Exam after Computed Tomographic Coronary Angiogram for Evaluation of Obstructive Coronary Artery Disease in Patients with Chest Pain. *J. Thorac. Dis.* 12 (9), 5067–5077. doi:10.21037/jtd-2019-pitd-12

Häusler, S. F., Montalbán del Barrio, I., Strohschein, J., Chandran, P. A., Engel, J. B., Hönig, A., et al. (2011). Ectonucleotidases CD39 and CD73 on OvCA Cells Are Potent Adenosine-Generating Enzymes Responsible for Adenosine Receptor 2A-dependent Suppression of T Cell Function and NK Cell Cytotoxicity. *Cancer Immunol. Immunother.* 60 (10), 1405–1418. doi:10.1007/s00262-011-1040-4

Huang, J., He, Y., Chen, M., Du, J., Li, G., Li, S., et al. (2015). Adenosine Deaminase and Adenosine Kinase Expression in Human Glioma and Their Correlation with Glioma-associated E-pilepsy. *Mol. Med. Rep.* 12 (5), 6509–6516. doi:10.3892/mmr.2015.4285

Hung, M. S., and Shen, C. K. (2003). Eukaryotic Methyl-CpG-Binding Domain Proteins and Chromatin Modification. *Eukaryot. Cell* 2 (5), 841–846. doi:10.1128/ec.2.5.841-846.2003

Hwang, D., Lee, J. M., and Koo, B. K. (2016). Physiologic Assessment of Coronary Artery Disease: Focus on Fractional Flow Reserve. *Korean J. Radiol.* 17 (3), 307–320. doi:10.3348/kjr.2016.17.3.307

Issa, J. P. (2007). DNA Methylation as a Therapeutic Target in Cancer. *Clin. Cancer Res.* 13 (6), 1634–1637. doi:10.1158/1078-0432.CCR-06-2076

Jacobson, K. A., and Reitman, M. L. (2020). Adenosine-Related Mechanisms in Non-adenosine Receptor Drugs. *Cells* 9 (4). doi:10.3390/cells9040956

Jacobson, K. A. (2009). Introduction to Adenosine Receptors as Therapeutic Targets. *Handb. Exp. Pharmacol.* 193.

Jafari, S. M., Joshaghani, H. R., Panjehpour, M., Aghaei, M., and Zargar Balajam, N. (2017). Apoptosis and Cell Cycle Regulatory Effects of Adenosine by Modulation of GLI-1 and ERK1/2 Pathways in CD44+ and CD24- Breast Cancer Stem Cells. *Cell Prolif.* 50 (4). doi:10.1111/cpr.12345

James, S. J., Melnyk, S., Pogribna, M., Pogribny, I. P., and Caudill, M. A. (2002). Elevation in S-Adenosylhomocysteine and DNA Hypomethylation: Potential Epigenetic Mechanism for Homocysteine-Related Pathology. *J. Nutr.* 132 (8 Suppl. l), 2361S–2366S. doi:10.1093/jn/132.8.2361S

Karahoca, M., and Momparler, R. L. (2013). Pharmacokinetic and Pharmacodynamic Analysis of 5-Aza-2'-Deoxycytidine (Decitabine) in the Design of its Dose-Schedule for Cancer Therapy. *Clin. Epigenetics* 5 (1), 3. doi:10.1186/1868-7083-5-3

Kiese, K., Jablonski, J., Boison, D., and Kobow, K. (2016). Dynamic Regulation of the Adenosine Kinase Gene during Early Postnatal Brain Development and Maturation. *Front. Mol. Neurosci.* 9, 99. doi:10.3389/fnmol.2016.00099

Kloor, D., and Osswald, H. (2004). S-adenosylhomocysteine Hydrolase as a Target for Intracellular Adenosine Action. *Trends Pharmacol. Sci.* 25 (6), 294–297. doi:10.1016/j.tips.2004.04.004

Klutstein, M., Nejman, D., Greenfield, R., and Cedar, H. (2016). DNA Methylation in Cancer and Aging. *Cancer Res.* 76 (12), 3446–3450. doi:10.1158/0008-5472.CAN-15-3278

Kneip, C., Schmidt, B., Seegebarth, A., Weickmann, S., Fleischhacker, M., Liebenberg, V., et al. (2011). SHOX2 DNA Methylation Is a Biomarker for the Diagnosis of Lung Cancer in Plasma. *J. Thorac. Oncol.* 6 (10), 1632–1638. doi:10.1097/JTO.0b013e318220ef9a

Li, Q., Wang, Y.-X., Shen, H.-Y., Xie, K., Zhang, L.-M., and Zeng, L.-H. (2015). Adenosine Interferes with Gene Methylation and its Mechanism RECK Human Colorectal Cancer SW480 Cells. *J. Third Mil. Med. Univ.* 37 (1), 46–50.

Li, X., Yao, X., Wang, Y., Hu, F., Wang, F., Jiang, L., et al. (2013). MLH1 Promoter Methylation Frequency in Colorectal Cancer Patients and Related Clinicopathological and Molecular Features. *PloS One* 8 (3), e59064. doi:10.1371/journal.pone.0059064

Liu, L., Nishihara, R., Qian, Z. R., Tabung, F. K., Nevo, D., Zhang, X., et al. (2017). Association between Inflammatory Diet Pattern and Risk of Colorectal Carcinoma Subtypes Classified by Immune Responses to Tumor. *Gastroenterology* 153 (6), 1517–e14. doi:10.1053/j.gastro.2017.08.045

Liu, Y. J., Chen, J., Li, X., Zhou, X., Hu, Y. M., Chu, S. F., et al. (2019). Research Progress on Adenosine in Central Nervous System Diseases. *CNS Neurosci. Ther.* 25 (9), 899–910. doi:10.1111/cns.13190

López-Moyado, I. F., Tsagaratou, A., Yuita, H., Seo, H., Delatte, B., Heinz, S., et al. (2019). Paradoxical Association of TET Loss of Function with Genome-wide DNA Hypomethylation. *Proc. Natl. Acad. Sci. U. S. A.* 116 (34), 16933–16942. doi:10.1073/pnas.1903059116

Losenkova, K., Zuccarini, M., Karikoski, M., Laurila, J., Boison, D., Jalkanen, S., et al. (2020). Compartmentalization of Adenosine Metabolism in Cancer Cells and its Modulation during Acute Hypoxia. *J. Cell Sci.* 133 (10). doi:10.1242/jcs.241463

Marcelino, H., Carvalho, T. M. A., Tomás, J., Teles, F. I., Honório, A. C., Rosa, C. B., et al. (2021). Adenosine Inhibits Cell Proliferation Differently in Human Astrocytes and in Glioblastoma Cell Lines. *Neuroscience* 467, 122–133. doi:10.1016/j.neuroscience.2021.05.019

Mastelic-Gavillet, B., Navarro Rodrigo, B., Décombaz, L., Wang, H., Ercolano, G., Ahmed, R., et al. (2019). Adenosine Mediates Functional and Metabolic Suppression of Peripheral and Tumor-Infiltrating CD8+ T Cells. *J. Immunother. Cancer* 7 (1), 257. doi:10.1186/s40425-019-0719-5

McGaraughty, S., Cowart, M., Jarvis, M. F., and Berman, R. F. (2005). Anticonvulsant and Antinociceptive Actions of Novel Adenosine Kinase Inhibitors. *Curr. Top. Med. Chem.* 5 (1), 43–58. doi:10.2174/1568026053386845

Mendizabal, I., Zeng, J., Keller, T. E., and Yi, S. V. (2017). Body-hypomethylated Human Genes Harbor Extensive Intragenic Transcriptional Activity and Are Prone to Cancer-Associated Dysregulation. *Nucleic Acids Res.* 45 (8), 4390–4400. doi:10.1093/nar/gkx020

Merighi, S., Battistello, E., Giacomelli, L., Varani, K., Vincenzi, F., Borea, P. A., et al. (2019). Targeting A3 and A2A Adenosine Receptors in the Fight against Cancer. *Expert Opin. Ther. Targets* 23 (8), 669–678. doi:10.1080/14728222.2019.1630380

Merighi, S., Benini, A., Mirandola, P., Gessi, S., Varani, K., Leung, E., et al. (2005). A3 Adenosine Receptors Modulate Hypoxia-Inducible Factor-1alpha Expression in Human A375 Melanoma Cells. *Neoplasia* 7 (10), 894–903. doi:10.1593/neo.05334

Micevic, G., Theodosakis, N., and Bosenberg, M. (2017). Aberrant DNA Methylation in Melanoma: Biomarker and Therapeutic Opportunities. *Clin. Epigenetics* 9, 34. doi:10.1186/s13148-017-0332-8

Mohannath, G., Jackel, J. N., Lee, Y. H., Buchmann, R. C., Wang, H., Patil, V., et al. (2014). A Complex Containing SNF1-Related Kinase (SnRK1) and Adenosine Kinase in Arabidopsis. *PLoS One* 9 (1), e87592. doi:10.1371/journal.pone.0087592

Morey, S. R., Smiraglia, D. J., James, S. R., Yu, J., Moser, M. T., Foster, B. A., et al. (2006). DNA Methylation Pathway Alterations in an Autochthonous Murine Model of Prostate Cancer. *Cancer Res.* 66 (24), 11659–11667. doi:10.1158/0008-5472.CAN-06-1937

Murugan, M., Fedele, D., Millner, D., Alharfoush, E., Vegunta, G., and Boison, D. (2021). Adenosine Kinase: An Epigenetic Modulator in Development and Disease. *Neurochem. Int.* 147, 105054. doi:10.1016/j.neuint.2021.105054

Nejman, D., Straussman, R., Steinfeld, I., Ruvolo, M., Roberts, D., Yakhini, Z., et al. (2014). Molecular Rules Governing De Novo Methylation in Cancer. *Cancer Res.* 74 (5), 1475–1483. doi:10.1158/0008-5472.CAN-13-3042

Newby, A. C., Holmquist, C. A., Illingworth, J., and Pearson, J. D. (1983). The Control of Adenosine Concentration in Polymorphonuclear Leucocytes, Cultured Heart Cells and Isolated Perfused Heart from the Rat. *Biochem. J.* 214 (2), 317–323. doi:10.1042/bj2140317

Oates, N. A., van Vliet, J., Duffy, D. L., Kroes, H. Y., Martin, N. G., Boomsma, D. I., et al. (2006). Increased DNA Methylation at the AXIN1 Gene in a Monozygotic Twin from a Pair Discordant for a Caudal Duplication Anomaly. *Am. J. Hum. Genet.* 79 (1), 155–162. doi:10.1086/505031

Ohta, A. (2016). A Metabolic Immune Checkpoint: Adenosine in Tumor Microenvironment. *Front. Immunol.* 7, 109. doi:10.3389/fimmu.2016.00109

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. U. S. A.* 103 (35), 13132–13137. doi:10.1073/pnas.0605251103

Ohta, A., and Sitkovsky, M. (2001). Role of G-Protein-Coupled Adenosine Receptors in Downregulation of Inflammation and Protection from Tissue Damage. *Nature* 414 (6866), 916–920. doi:10.1038/414916a

Okano, M., Bell, D. W., Haber, D. A., and Li, E. (1999). DNA Methyltransferases Dnmt3a and Dnmt3b Are Essential for De Novo Methylation and Mammalian Development. *Cell* 99 (3), 247–257. doi:10.1016/s0092-8674(00)81656-6

Pacheco, R., Martinez-Navio, J. M., Lejeune, M., Climent, N., Oliva, H., Gatell, J. M., et al. (2005). CD26, Adenosine Deaminase, and Adenosine Receptors Mediate Costimulatory Signals in the Immunological Synapse. *Proc. Natl. Acad. Sci. U. S. A.* 102 (27), 9583–9588. doi:10.1073/pnas.0501050102

Pan, F., Wingo, T. S., Zhao, Z., Gao, R., Makishima, H., Qu, G., et al. (2017). Tet2 Loss Leads to Hypermutagenicity in Haematopoietic Stem/progenitor Cells. *Nat. Commun.* 8, 15102. doi:10.1038/ncomms15102

Pignataro, G., Simon, R. P., and Boison, D. (2007). Transgenic Overexpression of Adenosine Kinase Aggravates Cell Death in Ischemia. *J. Cereb. Blood Flow. Metab.* 27 (1), 1–5. doi:10.1038/sj.jcbfm.9600334

Pritchard, E. M., Szybala, C., Boison, D., and Kaplan, D. L. (2010). Silk Fibroin Encapsulated Powder Reservoirs for Sustained Release of Adenosine. *J. Control Release* 144 (2), 159–167. doi:10.1016/j.jconrel.2010.01.035

Ren, J. Z., and Huo, J. R. (2012). 5-aza-2'-deoxycytidine-induced Inhibition of CDH13 Expression and its Inhibitory Effect on Methylation Status in Human Colon Cancer Cells *In Vitro* and on Growth of Xenograft in Nude Mice. *Zhonghua Zhong Liu Za Zhi* 34 (1), 6–10.

Schiedel, A. C., Lacher, S. K., Linnemann, C., Knolle, P. A., and Müller, C. E. (2013). Antiproliferative Effects of Selective Adenosine Receptor Agonists and Antagonists on Human Lymphocytes: Evidence for Receptor-independent Mechanisms. *Purinergic Signal* 9 (3), 351–365. doi:10.1007/s11302-013-9354-7

Seetulsingh-Goorah, S. P. (2006). Mechanisms of Adenosine-Induced Cytotoxicity and Their Clinical and Physiological Implications. *Biofactors* 27 (1-4), 213–230. doi:10.1002/biof.5520270119

Sellin, M. E., Holmfeldt, P., Stenmark, S., and Gullberg, M. (2011). Microtubules Support a Disk-like Septin Arrangement at the Plasma Membrane of Mammalian Cells. *Mol. Biol. Cell* 22 (23), 4588–4601. doi:10.1091/mbc.E11-09-0754

Shamloo, B., Kumar, N., Owen, R. H., Reemmer, J., Ost, J., Perkins, R. S., et al. (2019). Dysregulation of Adenosine Kinase Isoforms in Breast Cancer. *Oncotarget* 10 (68), 7238–7250. doi:10.18632/oncotarget.27364

Sharma, P. (2016). Biology and Management of Patients with Triple-Negative Breast Cancer. *Oncologist* 21 (9), 1050–1062. doi:10.1634/theoncologist.2016-0067

Sigalotti, L., Fratta, E., Coral, S., and Maio, M. (2014). Epigenetic Drugs as Immunomodulators for Combination Therapies in Solid Tumors. *Pharmacol. Ther.* 142 (3), 339–350. doi:10.1016/j.pharmthera.2013.12.015

Sinčić, N., and Herceg, Z. (2011). DNA Methylation and Cancer: Ghosts and Angels above the Genes. *Curr. Opin. Oncol.* 23 (1), 69–76. doi:10.1097/CCO.0b013e3283412eb4

Song, A., Zhang, Y., Han, L., Yegutkin, G. G., Liu, H., Sun, K., et al. (2017). Erythrocytes Retain Hypoxic Adenosine Response for Faster Acclimatization upon Re-ascent. *Nat. Commun.* 8, 14108. doi:10.1038/ncomms14108

Spácilová, P., Naus, P., Pohl, R., Votruba, I., Snásel, J., Zábranská, H., et al. (2010). CycloSal-phosphate Pronucleotides of Cytostatic 6-(Het)aryl-7-Deazapurine Ribonucleosides: Synthesis, Cytostatic Activity, and Inhibition of Adenosine Kinases. *ChemMedChem* 5 (8), 1386–1396. doi:10.1002/cmdc.201000192

Stagg, J., and Smyth, M. J. (2010). Extracellular Adenosine Triphosphate and Adenosine in Cancer. *Oncogene* 29 (39), 5346–5358. doi:10.1038/onc.2010.292

Su, J., Huang, Y. H., Cui, X., Wang, X., Zhang, X., Lei, Y., et al. (2018). Homeobox Oncogene Activation by Pan-Cancer DNA Hypermethylation. *Genome Biol.* 19 (1), 108. doi:10.1186/s13059-018-1492-3

Su Xiaoyang, L. S. (2018). YAN Yusheng. Res. Prog. metabolic Pathw. adenosine Regul. pro Inflamm. cytokines Cell Inj. *J. Clin. Thorac. Cardiovasc. Surg.* 25 (12).

Sun, D., Luo, M., Jeong, M., Rodriguez, B., Xia, Z., Hannah, R., et al. (2014). Epigenomic Profiling of Young and Aged HSCs Reveals Concerted Changes during Aging that Reinforce Self-Renewal. *Cell stem Cell* 14 (5), 673–688. doi:10.1016/j.stem.2014.03.002

Sun, J., Fei, F., Zhang, M., Li, Y., Zhang, X., Zhu, S., et al. (2019). The Role of mSEPT9 in Screening, Diagnosis, and Recurrence Monitoring of Colorectal Cancer. *BMC Cancer* 19 (1), 450. doi:10.1186/s12885-019-5663-8

Swellam, M., Abdelmaksoud, M. D., Sayed Mahmoud, M., Ramadan, A., Abdel-Moneem, W., and Hefny, M. M. (2015). Aberrant Methylation of APC and RARβ2 Genes in Breast Cancer Patients. *IUBMB Life* 67 (1), 61–68. doi:10.1002/iub.1346

Synowitz, M., Glass, R., Färber, K., Markovic, D., Kronenberg, G., Herrmann, K., et al. (2006). A1 Adenosine Receptors in Microglia Control Glioblastoma-Host Interaction. *Cancer Res.* 66 (17), 8550–8557. doi:10.1158/0008-5472.CAN-06-0365

Tanaka, M., Kijima, H., Itoh, J., Matsuda, T., and Tanaka, T. (2002). Impaired Expression of a Human Septin Family Gene Bradeion Inhibits the Growth and Tumorigenesis of Colorectal Cancer *In Vitro* and *In Vivo*. *Cancer Gene Ther.* 9 (6), 483–488. doi:10.1038/sj.cgt.7700460

Tsutsui, S., Schnermann, J., Noorbakhsh, F., Henry, S., Yong, V. W., Winston, B. W., et al. (2004). A1 Adenosine Receptor Upregulation and Activation Attenuates Neuroinflammation and Demyelination in a Model of Multiple Sclerosis. *J. Neurosci.* 24 (6), 1521–1529. doi:10.1523/JNEUROSCI.4271-03.2004

Valencia, A. M., and Kadoch, C. (2019). Chromatin Regulatory Mechanisms and Therapeutic Opportunities in Cancer. *Nat. Cell Biol.* 21 (2), 152–161. doi:10.1038/s41556-018-0258-1

van de Veen, W., Globinska, A., Jansen, K., Straumann, A., Kubo, T., Verschoor, D., et al. (2020). A Novel Proangiogenic B Cell Subset Is Increased in Cancer and Chronic Inflammation. *Sci. Adv.* 6 (20), eaaz3559. doi:10.1126/sciadv.aaz3559

Van der Auwera, I., Bovie, C., Svensson, C., Limame, R., Trinh, X. B., van Dam, P., et al. (2009). Quantitative Assessment of DNA Hypermethylation in the Inflammatory and Non-inflammatory Breast Cancer Phenotypes. *Cancer Biol. Ther.* 8 (23), 2252–2259. doi:10.4161/cbt.8.23.10133

Vannoni, D., Bernini, A., Carlucci, F., Civitelli, S., Di Pietro, M. C., Leoncini, R., et al. (2004). Enzyme Activities Controlling Adenosine Levels in Normal and Neoplastic Tissues. *Med. Oncol.* 21 (2), 187–195. doi:10.1385/MO:21:2:187

Viré, E., Brenner, C., Deplus, R., Blanchon, L., Fraga, M., Didelot, C., et al. (2006). The Polycomb Group Protein EZH2 Directly Controls DNA Methylation. *Nature* 439 (7078), 871–874. doi:10.1038/nature04431

Virtanen, S. S., Kukkonen-Macchi, A., Vainio, M., Elima, K., Härkönen, P. L., Jalkanen, S., et al. (2014). Adenosine Inhibits Tumor Cell Invasion via Receptor-independent Mechanisms. *Mol. Cancer Res.* 12 (12), 1863–1874. doi:10.1158/1541-7786.MCR-14-0302-T

Wahba, A. E., Fedele, D., Gebril, H., AlHarfoush, E., Toti, K. S., Jacobson, K. A., et al. (2021). Adenosine Kinase Expression Determines DNA Methylation in Cancer Cell Lines. *ACS Pharmacol. Transl. Sci.* 4 (2), 680–686. doi:10.1021/acsptsci.1c00008

Wang, H., Buckley, K. J., Yang, X., Buchmann, R. C., and Bisaro, D. M. (2005). Adenosine Kinase Inhibition and Suppression of RNA Silencing by Geminivirus AL2 and L2 Proteins. *J. Virol.* 79 (12), 7410–7418. doi:10.1128/JVI.79.12.7410-7418.2005

Wang, J., Wang, Y., Chu, Y., Li, Z., Yu, X., Huang, Z., et al. (2021). Tumor-derived Adenosine Promotes Macrophage Proliferation in Human Hepatocellular Carcinoma. *J. hepatology* 74 (3), 627–637. doi:10.1016/j.jhep.2020.10.021

Wang, L., Lin, Y. L., Li, B., Wang, Y. Z., Li, W. P., and Ma, J. G. (2014). Aberrant Promoter Methylation of the Cadherin 13 Gene in Serum and its Relationship with Clinicopathological Features of Prostate Cancer. *J. Int. Med. Res.* 42 (5), 1085–1092. doi:10.1177/0300060514540631

Wang, L. F., and Yang, S. Y. (2014). Expression And Clinical Significance of Adenosine Kinase in Breast *Cancer*. 6, 475–477.

Weber, M., Hellmann, I., Stadler, M. B., Ramos, L., Pääbo, S., Rebhan, M., et al. (2007). Distribution, Silencing Potential and Evolutionary Impact of Promoter DNA Methylation in the Human Genome. *Nat. Genet.* 39 (4), 457–466. doi:10.1038/ng1990

Wengner, A. M., Scholz, A., and Haendler, B. (2020). Targeting DNA Damage Response in Prostate and Breast Cancer. *Int. J. Mol. Sci.* 21 (21). doi:10.3390/ijms21218273

Wiesner, J. B., Ugarkar, B. G., Castellino, A. J., Barankiewicz, J., Dumas, D. P., Gruber, H. E., et al. (1999). Adenosine Kinase Inhibitors as a Novel Approach to Anticonvulsant Therapy. *J. Pharmacol. Exp. Ther.* 289 (3), 1669–1677.

Williams-Karnesky, R. L., Sandau, U. S., Lusardi, T. A., Lytle, N. K., Farrell, J. M., Pritchard, E. M., et al. (2013). Epigenetic Changes Induced by Adenosine Augmentation Therapy Prevent Epileptogenesis. *J. Clin. Invest.* 123 (8), 3552–3563. doi:10.1172/JCI65636

Winter, C., Nilsson, M. P., Olsson, E., George, A. M., Chen, Y., Kvist, A., et al. (2016). Targeted Sequencing of BRCA1 and BRCA2 across a Large Unselected Breast Cancer Cohort Suggests that One-Third of Mutations Are Somatic. *Ann. Oncol.* 27 (8), 1532–1538. doi:10.1093/annonc/mdw209

Wu, C., and Bekaii-Saab, T. (2012). CpG Island Methylation, Microsatellite Instability, and BRAF Mutations and Their Clinical Application in the Treatment of Colon Cancer. *Chemother. Res. Pract.* 2012, 359041. doi:10.1155/2012/359041

Xie, K., Wang, Y.-X., Shen, H.-Y., Zhang, L.-M., Zeng, L.-H., and Shu, N.-B. (2014). Effect of Adenosine on hMLH1 Gene of Human Colorectal Cancer Cells. *Nat. Med. J. China* 2014 (94), 16. doi:10.3760/cma.j.issn.0376-2491.2014.16.016

Xie, L., Jiang, X., Li, Q., Sun, Z., Quan, W., Duan, Y., et al. (2018). Diagnostic Value of Methylated Septin9 for Colorectal Cancer Detection. *Front. Oncol.* 8, 247. doi:10.3389/fonc.2018.00247

Xu, Y., Wang, Y., Yan, S., Zhou, Y., Yang, Q., Pan, Y., et al. (2017). Intracellular Adenosine Regulates Epigenetic Programming in Endothelial Cells to Promote Angiogenesis. *EMBO Mol. Med.* 9 (9), 1263–1278. doi:10.15252/emmm.201607066

Yamamoto, H., and Imai, K. (2015). Microsatellite Instability: an Update. *Arch. Toxicol.* 89 (6), 899–921. doi:10.1007/s00204-015-1474-0

Yang, X., Xu, Z. J., Chen, X., Zeng, S. S., Qian, L., Wei, J., et al. (2019). Clinical Value of Preoperative Methylated Septin 9 in Chinese Colorectal Cancer Patients. *World J. Gastroenterol.* 25 (17), 2099–2109. doi:10.3748/wjg.v25.i17.2099

Yegutkin, G. G. (2014). Enzymes Involved in Metabolism of Extracellular Nucleotides and Nucleosides: Functional Implications and Measurement of Activities. *Crit. Rev. Biochem. Mol. Biol.* 49 (6), 473–497. doi:10.3109/10409238.2014.953627

Yh, L. (2017). Expression and Clinical Significance of Adenosine Kinase in Liver Cancer. *J. Hubei Coll. Med.* 36 (6), 504–507.

Zhang, L. M, Wang, Y.-X., Xie, K., Zeng, L.-H., and Li, Q. (2015). The Demethylation of DLC-1 Genes in HT-29 Cells by 5-iodonogenic Tuberculosis Journal of the Third Military. *Medical University*, 37(2), 106–110.

Zhang, Y., Zhang, L., Li, R., Chang, D. W., Ye, Y., Minna, J. D., et al. (2017). Genetic Variations in Cancer-Related Significantly Mutated Genes and Lung Cancer Susceptibility. *Ann. Oncol.* 28 (7), 1625–1630. doi:10.1093/annonc/mdx161

Zhao, Y., Chen, F., Li, Q., Wang, L., and Fan, C. (2015). Isothermal Amplification of Nucleic Acids. *Chem. Rev.* 115 (22), 12491–12545. doi:10.1021/acs.chemrev.5b00428

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.

frontiers | Frontiers in Pharmacology

TYPE Mini Review
PUBLISHED 15 July 2022
DOI 10.3389/fphar.2022.935553

OPEN ACCESS

EDITED BY
Ke-Wu Zeng,
Peking University, China

REVIEWED BY
Linchong Sun,
Guangdong Academy of Medical Sciences, China
Nicholas A. Graham,
University of Southern California, United States

*CORRESPONDENCE
Xing Zhao,
zhaoxing_dl@126.com

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 04 May 2022
ACCEPTED 29 June 2022
PUBLISHED 15 July 2022

CITATION
Sun N and Zhao X (2022), Argininosuccinate synthase 1, arginine deprivation therapy and cancer management.
Front. Pharmacol. 13:935553.
doi: 10.3389/fphar.2022.935553

Argininosuccinate synthase 1, arginine deprivation therapy and cancer management

Naihui Sun[1] and Xing Zhao[2]*

[1]Department of Anesthesiology, The First Affiliated Hospital of China Medical University, Shenyang, China, [2]Department of Pediatrics, The First Affiliated Hospital of China Medical University, Shenyang, China

Metabolic reprogramming is an emerging hallmark of tumor cells. In order to survive in the nutrient-deprived environment, tumor cells rewire their metabolic phenotype to provide sufficient energy and build biomass to sustain their transformed state and promote malignant behaviors. Amino acids are the main compositions of protein, which provide key intermediate substrates for the activation of signaling pathways. Considering that cells can synthesize arginine *via* argininosuccinate synthase 1 (ASS1), arginine is regarded as a non-essential amino acid, making arginine depletion as a promising therapeutic strategy for ASS1-silencing tumors. In this review, we summarize the current knowledge of expression pattern of ASS1 and related signaling pathways in cancer and its potential role as a novel therapeutic target in cancer. Besides, we outline how ASS1 affects metabolic regulation and tumor progression and further discuss the role of ASS1 in arginine deprivation therapy. Finally, we review approaches to target ASS1 for cancer therapies.

KEYWORDS

metabolic reprogramming, arginine, amino acid, resistance, prognosis

Introduction

A key characteristics of tumor metabolism is the capability to hijack and remodel existing metabolic pathways to obtain sufficient nutrients from a nutrient-deprived environment and use these nutrients to sustain cell survival and build cellular material (Pavlova and Thompson, 2016). Amino acids serve as the primary compositions of protein, which provide important intermediate substrates for the activation of signaling pathways. Therefore, therapies of amino acid depletion that impair amino acid utilization *via* targeting key enzymes engaged in amino acid metabolism have been extensively studied (Tabe et al., 2019). Arginine is utilized by various metabolic pathways to mediate a series of cellular processes including protein synthesis and production of nitric oxide (NO), creatine phosphate, agmatine, polyamines, ornithine, and citrulline (Tong and Barbul, 2004) (Figure 1). Given that cells can synthesize arginine from citrulline and aspartate *via* argininosuccinate synthase 1 (ASS1) and argininosuccinate lyase (ASL), arginine is considered as a non-essential amino acid (Chen et al., 2021). Thus, arginine depletion may be a promising therapeutic strategy for cancer management.

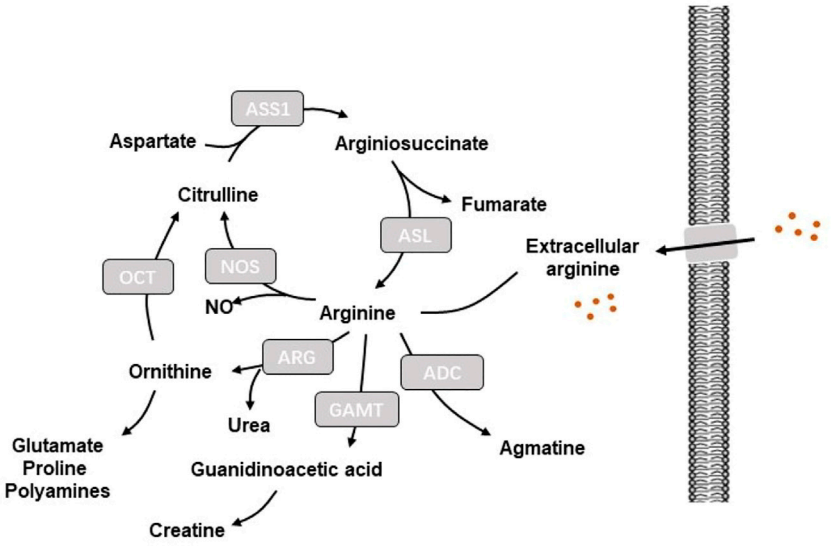


FIGURE 1
Illustration of arginine metabolism in cells. ADC, arginine decarboxylase; ADI, arginine deiminase; ARG, arginase; ASL, arginine-succinate lyase; ASS1, arginine-succinate synthetase 1; GAMT, guanidinoacetate-N-methyltransferase; NO: nitric oxide; NOS: nitric oxide synthase; OCT, Ornithine carbamoyl transferase.

ASS1 is the enzyme that catalyzes the conversion of nitrogen from ammonia and aspartate from glutamine to form argininosuccinate. The somatic silence of ASS1 expression is commonly observed in a wide range of tumors, such as mesothelioma, non-small-cell lung cancer, myxofibrosarcomas (Huang et al., 2013; Szlosarek et al., 2017; Giatromanolaki et al., 2021). Moreover, low ASS1 expression levels in tumor tissues are associated with unfavorable clinical outcomes in a wide variety of malignancies. ASS1 loss not only confers tumor cells with lack of tumor suppressor functions but also endows tumor cells to be more reliant on arginine supplement. As illustrated in previous review, ASS1 and arginine metabolism represent compelling molecular targets for cancer management. In this review, we summarize the current knowledge of expression pattern of ASS1 and related signaling pathways in cancer and the potential role as a novel therapeutic target in cancer. Besides, we outline how ASS1 affects metabolic regulation and tumor progression and further discuss the role of ASS1 in arginine deprivation therapy. Finally, we review approaches to target ASS1 for cancer therapies.

ASS1 regulation and signaling networks

ASS1 was initially identified in the liver, and functions as a rate-limiting enzyme for arginine metabolism. Dysregulated promoter methylation is regarded as a key feature of tumors *via* downregulating tumor suppressor genes (Kulis and Esteller, 2010). It is worth noticing that ASS1 silencing is resulted from the epigenetic silencing of the ASS1 promoter *via* methylation of the CpG islands, which has been observed in multiple tumor types (Syed et al., 2013). For instance, ASS1 promoter is frequently hypermethylated in myxofibrosarcoma, resulting in the aberrant loss of ASS1 expression to mediate tumor aggressiveness (Huang et al., 2013). Methylation landscape in cisplatin-resistant bladder cancer has shown that ASS1 is hypermethylated, leading to downregulated expression (Yeon et al., 2018). Aberrant methylation in the promoter of ASS1 makes ovarian tumor cells more resistant to platinum-induced cell death (Nicholson et al., 2009). Tumoral expression levels of ASS1 are also regulated when encountering external factors from the tumor microenvironment to metabolically benefit tumor cell survival. Under acidic and hypoxic conditions, it has been demonstrated that hypoxia inducible factor alpha (HIF1α) binds to ASS1 and downregulates the expression levels of ASS1, providing tumor cells with a metabolic advantage for survival (Silberman et al., 2019). Besides, arginine and glutamine starvation therapies can downregulate HIF1α to upregulate ASS1 expression (Long et al., 2017). Transcription factor c-Myc could directly bind to the promoter of ASS1, mediating arginine deiminase resistance in melanoma cells (Long et al., 2013). Methyltransferase 14 (METTL14), a RNA N6-adenosine methyltransferase, participates in tumor development *via* modulating RNA function (Chen et al., 2020). ASS1 is a target of METTL14-mediated N6-methyladenosine modification (Miao et al., 2022). Specifically, METTL14 upregulation increases mRNA m6A modification of ASS1 and suppresses ASS1 transcriptional expression. Additionally, miRNAs are also essential for the expression of ASS1. In renal cancer cell, miR-34a-5p directly

binds to the 3′ untranslated region of ASS1 to reduce its protein expression, while ASS1P3 serves as a competing endogenous RNA for miR-34a-5p to modulate ASS1 expression (Wang et al., 2019). Similarly, lncRNA 00312 attenuates tumor proliferation and invasion by functioning as a competitive endogenous RNA binding to miR34a-5p, making miR34a-5p unable binding to ASS1 to reduce ASS1 expression (Zeng et al., 2020). Considering that post-translational modification is essential for the stability of protein, it mediates diverse cellular processes. For instance, the TRAF2 E3 ubiquitin ligase binds to ASS1, leading to increased ubiquitination and degradation of ASS1 to reduce arginine biosynthesis. The diverse regulation pattern of ASS1 transcriptional and protein expression makes it a promising therapeutic target, and it is necessary to investigate the underlying mechanisms engaged in the expression of ASS1.

ASS1 and metabolic adaptation

Arginine is a precursor for a wide variety of molecules engaged in the regulation of tumor initiation and development (Zhang et al., 2021). Tumoral downregulation of ASS1 confers tumor cells to be more dependent on extracellular arginine since ASS1-negative cells fail to mediate arginine biosynthesis for tumor survival. The reliance on extracellular arginine has been regarded as arginine auxotrophy, which has been exploited as an"'Achilles' heel for cancer management. Thus, ASS1 has been established as a key indicator of arginine auxotrophy. Network analysis of the metabolomics revealed that ASS1-negative glioblastoma cells exhibit altered arginine and citrulline metabolism (Mörén et al., 2018). In ASS1-negative glioblastoma cells, levels of alanine and glutamate are reduced, whereas levels of α-ketoglutarate and pyruvate are increased, indicating that ASS1-negative glioblastoma cells are converting less pyruvate to alanine. Multiple pathways for citrulline production are upregulated, and degradation of arginine in ASS negative cells is decreased. In addition, ASS1 functions as an indicator for glutamine-deprivation response. ASS1 inhibition leads to increased sensitivity to both arginine and glutamine deprivation, whereas ASS1 overexpression increases resistance to both arginine and glutamine deprivation (Long et al., 2017). Depletion of extracellular arginine in arginine-auxotrophic cancer cells causes mitochondrial distress and transcriptional reprogramming. Mechanistically, arginine starvation induces asparagine synthetase (ASNS), depleting these cancer cells of aspartate, and disrupting their malate-aspartate shuttle (Cheng et al., 2018). A metabolite profiling of arginine depletion by pharmacological inhibition exhibits elevated serine biosynthesis, glutamine anaplerosis, oxidative phosphorylation, and impaired aerobic glycolysis (Kremer et al., 2017).

Pyrimidines play key role in mediating tumor cell survival and proliferation through providing the nucleic acids and other precursors for cell membrane synthesis (Mollick and Laín, 2020; Siddiqui and Ceppi, 2020). During the synthesis process, the pyrimidine ring structure is formed through a multi-step pathway with glutamine and aspartate as main precursors, which is conversed to dihydroorotate by the three activities of the multifunctional enzyme carbamoyl-phosphate synthase 2, aspartate transcarbamylase, dihydroorotase complex (CAD) (Del Caño-Ochoa et al., 2019; Li et al., 2021). It has been found that tumoral ASS1 expression determines aspartate availability for pyrimidine synthesis (Rabinovich et al., 2015). Intracellular aspartate functions as a substrate for both ASS1 and the enzymatic complex CAD. Tumoral ASS1 loss increases cytosolic aspartate availability for CAD for the synthesis of pyrimidine nucleotides to promote proliferation (Rabinovich et al., 2015). In renal cellular carcinoma, loss of ASS1 and ASL makes aspartate flux towards pyrimidine synthesis to support tumor proliferation (Khare et al., 2021).

The reaction catalyzed by ASS1 is essential for the citrulline-NO cycle. NO is a crucial regulator of multiple cellular processes, such as tumor cell proliferation and angiogenesis (Somasundaram et al., 2019). Decreased levels of NO metabolites and nitric oxide synthase expression have been observed in renal cellular carcinomas and tumor cells lacking ASS1 and ASL (Khare et al., 2021). Combined ASS1 and ASL downregulation significantly reduces aspartate level to impair NO production *via* decreased substrate availability or enzymatic activity. Therefore, ASS1 downregulation influences NO metabolism and promotes tumor cell survival and proliferation *via* mitigation of cytotoxic effects of NO accumulation. Under glucose deprivation, ASS1 expression is induced by c-MYC, therefore promoting tumor cell survival by upregulating NO production and activating the gluconeogenic enzymes *via* S-nitrosylation (Keshet et al., 2020). This metabolic rewiring leads to enhanced gluconeogenesis to increase serine, glycine and purine synthesis. In this ASS1-expressed tumors, purine synthesis inhibition is effective and sensitizes these tumors to immune checkpoint inhibition therapy.

ASS1 loss is associated with polyamine metabolic reprogramming. Results from transcriptomic and metabolomic profiling illustrates that ASS1-lacked cells exhibit reduced accumulation of acetylated polyamine metabolites and therefore a compensatory elevation in the expression of polyamine biosynthetic enzymes (Locke et al., 2016).

Different role of ASS1 in tumor progression

Numerous studies have shown that tumoral ASS1 functions as a tumor suppressor to sustain the anti-tumor function in a wide variety of tumors (Huang et al., 2013; Allen et al., 2014). Downregulation of ASS1 has been found to play a tumor suppressor role in multiple malignancies. ASS1 exerts its role mainly through its arginine metabolism-dependent mechanisms. It is gradually recognized

that ASS1 play different role according to different tumor types. Renal tumors exhibit downregulated ASS1, and loss of ASS1 redirects aspartate towards pyrimidine synthesis and regulates NO production to support enhanced proliferation, uncovering promising metabolic vulnerabilities in renal cellular carcinoma. Besides, it has also been demonstrated that ASS1 may function as a tumor suppressor via metabolism–independent mechanism. In hepatic cell carcinoma cells, ASS1 induces cell death by upregulating ER stress response, independent of arginine metabolism. Specifically, ASS1 overexpression effectively inhibits tumor growth by activating PERK/eIF2α/ATF4/CHOP axis in Huh7 and SNU475 cells, indicating upregulating tumoral ASS1 expression as a promising strategy in tumors with low ASS1 expression (Kim et al., 2021). In renal cellular carcinoma, androgen receptor could reduce ASS1 expression to promote SW-839 and OSRC-3 cell proliferation *via* ASS1P3. Thus, this androgen receptor-induced ASS1 downregulation as a therapeutic target for treatment.

In contrast to its tumor-inhibiting effects, ASS1 has a pro-tumor role in tumor proliferation and metastasis, and the mechanisms seem to vary and differ depending on the specific type of tumor. Based on the differential transcriptional expression analyses of colorectal cancer (CRC) tumors, urea cycle enzymes including ASS1 has been identified to be transcriptionally upregulated in KRAS-mutant primary CRC. Bateman et al. elucidated that ASS1 inhibition impairs CRC survival and proliferation. Metabolomic profiling has pointed that ASS1 inhibition reduces the levels of oncogenic metabolite fumarate, resulting in impaired glycolytic phenotype and reduced CRC progression (Bateman et al., 2017). Snail is a master regulator and transcriptional repressor of epithelial-mesenchymal transition. In colorectal cancer, Snail mediates tumor cell metastasis by preventing non-coding RNA LOC113230-induced degradation of ASS1. Snail regulates arginine biosynthesis by suppressing LOC113230-induced LRPPRC/TRAF2/ASS1 axis (Jia et al., 2021). In gastric cancer, ASS1 knockdown leads to impaired tumor cell invasion by promoting autophagy-lysosome machinery to degrade Snail and Twist (Tsai et al., 2018).

ASS1 and resistance to chemotherapy

Numerous studies have reported that ASS1 loss is correlated with the development of chemotherapeutic resistance in tumors. For instance, epigenetic silencing of ASS1 confers ovarian tumor cells resistance to platinum chemotherapy. Thus, the expression level of ASS1 has also been regarded as a predictor of clinical outcome in ovarian cancer patients treated with platinum-based chemotherapy (Nicholson et al., 2009). In hepatocellular carcinoma, ASS1 loss has been found to be associated with resistance to cisplatin. Moreover, ASS1 overexpression effectively improves the anti-tumor effect of chemotherapy by activating the PERK/eIF2α/ATF4/CHOP axis. Treatment with decitabine, a hypomethylating agent to increase ASS1 promoter activity, can effectively reduce cisplatin resistance in SNU449 and Huh7 cells. Apoptosis level indicated by cleaved PARP and caspase-3 is significantly upregulated in SNU449 and Huh7 cells after combination treatment with cisplatin and decitabine (Kim et al., 2021).

ASS1 and arginine deprivation therapy

Tumors with ASS1 loss fail to mediate the arginine biosynthesis, making these cells to be more reliant on extracellular arginine for tumor survival. Thus, arginine depletion therapy may be a promising therapeutic strategy for ASS1-negative tumors. In nearly 70% of tumors, ASS1 loss has been observed, resulting in more efforts to exploit the metabolic vulnerability for development of arginine deprivation therapy. Arginine deprivation therapy is considered to be more effective in ASS1-negative tumors than tumors with low level of ASS1 expression. Arginine deprivation can induce signal alteration. For example, arginine deprivation impairs mTOR and p70S6K activation with consequent inactivation of PI3K/Akt pathway (Wang et al., 2020). PEGylated arginine deiminase (ADI-PEG20) is an arginine-metabolizing enzyme to mediate arginine degradation, which is currently being tested in many clinical trials. ADI-PEG20 has been verified to exhibit anti-tumor effect in a wide range of tumors, including primary acute myeloid leukemia, metastatic melanoma, hepatocellular carcinoma, thoracic cancer (Izzo et al., 2004; Ascierto et al., 2005; Miraki-Moud et al., 2015; Beddowes et al., 2017). ADI-PEG 20 treatment increases T cell infiltration in the low PD-L1 tumor microenvironment to enhance the anti-tumor effect of PD-1 inhibition (Chang et al., 2021). ADI-PEG20 also significantly improves progression-free survival in patients with ASS1-loss mesothelioma (Szlosarek et al., 2017). In pancreatic tumor, ADI-PEG20 can also augment the anti-tumor effect of radiation *via* the activation of ER stress signaling pathway (Singh et al., 2019). ADI-PEG 20 has also been found to be specifically effective in MYC-driven tumors (Chalishazar et al., 2019). ADI-PEG20 can disrupt pyrimidine pools in ASS1-lacked high-grade gliomas to increase tumor sensitivity to the antifolate and pemetrexed (Hall et al., 2019). Although promising results from the preclinical and clinical trials, there are tumors exhibiting resistance to ADI-PEG20. It has been well-established that the re-expression of ASS1 is a main reason for tumor resistance to ADI-PEG20. In mesothelioma cells exhibiting ADI-PEG20 resistance, it is believed that this resistance is induced by regain of ASS1 expression by demethylation of the ASS1 promoter. Considering the efficacy of ADT as a single agent therapy is limited due to frequently observed resistance, combined arginine deprivation and other therapeutic targets may be an answer to improve the therapeutic effect. For instance, HDAC inhibition has been found to induce degradation of a key DNA repair enzyme C-terminal-binding protein interacting protein (CtIP),

TABLE 1 Summary of ADI-PEG20 utilization in tumor management.

Tumor types	Regimen	Clinical phase	References
Melanoma	ADI-PEG20	1/2	Ott et al. (2013)
Acute myeloid leukemia	ADI-PEG20	2	Tsai et al. (2017)
Mesothelioma	ADI-PEG20	2	Szlosarek et al. (2017)
Hepatocellular carcinoma	ADI-PEG20	2	Yang et al. (2010)
Hepatocellular carcinoma	ADI-PEG20	2	Glazer et al. (2010)
Hepatocellular carcinoma	ADI-PEG20	3	Abou-Alfa et al. (2018)
Acute myeloid leukemia	ADI-PEG20 + cytarabine	1	
Metastatic melanoma	ADI-PEG20 + cisplatin	1	Yao et al. (2021)
Recurrent high-grade glioma	ADI-PEG20 + pemetrexed + cisplatin	1	Hall et al. (2019)
Metastatic Uveal Melanoma	ADI-PEG20 + pemetrexed + cisplatin	1	Chan et al. (2022)
Non-squamous non-small cell lung cancer	ADI-PEG20 + pemetrexed + cisplatin	1	Szlosarek et al. (2021)
Pancreatic adenocarcinoma	ADI-PEG20 + nab-paclitaxel + gemcitabin	1/1B	Lowery et al. (2017)

leading to DNA damage and apoptosis. Arginine deprivation and HDAC inhibition can synergistically mediate DNA damage and degradation of CtIP, resulting in apoptosis (Kim et al., 2020). In addition, a metabolic synthetic lethal strategy has been developed to combine ADI-PEG20 with chloroquine to induce cell death in ASS1-deficient sarcomas (Bean et al., 2016). More importantly, combination treatment of ADI-PEG 20 with chemotherapeutic drugs has been extensively studied. Several clinical trials indicated that this combination treatment has acceptable safety profiles and anti-tumour activity against ASS1-deficient solid tumors (Hall et al., 2019; Szlosarek et al., 2021; Yao et al., 2021; Chan et al., 2022). Summary of ADI-PEG20 utilization in tumor management has been illustrated in Table 1.

The safety and anti-tumor activities of a newly developed arginine depleting drug pegylated recombinant human arginase (PEG-BCT-100) has been verified in patients with advanced arginine auxotrophic tumors (Cheng et al., 2021). PEG-BCT-100 has been tested in chemo naïve post-sorafenib hepatocellular carcinoma, showing well-tolerated with moderate disease control rate (Chan et al., 2021). More clinical studies should be designed to further verify the safety and anti-tumor efficacy, and the potential effects of combination therapy with other chemotherapeutic and targeted agents should also be further explored.

Implicit of ASS1 in cancer treatment

Prognostic and predictive value of ASS1 in cancer

In non-small-cell lung cancer, lack of expression of ASS1 in tumor cells is associated with high angiogenesis. Patients with ASS1 expression in tumor cells exhibit a favorable prognosis, which may be related to high density of iNOS-expressing tumor-infiltrating lymphocytes in tumor cells with ASS1 expression (Giatromanolaki et al., 2021). ASS1 is lower in renal cell carcinomas compared with paired normal tissues, and a lower ASS1 expression is correlated with a worse prognosis in patients with renal cell carcinoma (Wang et al., 2019).

Development of ASS1 activator for cancer treatment

Considering the tumor suppressor role of ASS1 in tumor cells, exploring potent ASS1 agonist to activate ASS1 expression and activity in tumor cells with low ASS1 expression is a promising therapeutic strategy. It has been demonstrated that spinosyn A and its derivative LM-2I exhibit anti-tumor function, which is regulated by activation of ASS1. Mechanistically, spinosyn A binds to ASS1 at the 97th cysteine site in tumor cells, thus increasing ASS1 enzymatic activity and anti-tumor effect (Zou et al., 2021). Currently, the investigation on the development of ASS1 activators is still limited, it is still required to explore deeper to identify the safety and efficacy of ASS1 activators.

Conclusion

ASS1 is an enzyme that catalyzes the conversion of nitrogen from ammonia and aspartate from glutamine to form argininosuccinate. The down-regulation of ASS1 expression are very common in various tumors, and associated with clinicopathological factors and prognosis. For precision therapeutic purposes, it is important to understand the mechanisms driving malignant diseases to identify the most promising therapy for individual patients. Currently, the down-regulation of ASS1 expression is extensively studied in mesothelioma, non-small-cell lung cancer, myxofibrosarcomas and others. Arginine deprivation leads to immunosuppression, and the removal of L-arginine can improve the elimination of arginine-auxotrophic tumors. However, the removal of L-arginine

has no inhibition on the development of non-auxotrophic tumors, given that these tumors synthesize L-arginine from citrulline by expressing ASS1. Therefore, it is necessary to identify the auxotrophic and non-auxotrophic tumors. To date, numerous studies of phase 1–3 clinic trials are being conducted to evaluate the efficacy of ADI-PEG20 therapy in diverse tumor types for its anti-tumor activity, it is still required to explore deeper to identify the cancer types that can be effectively treated with ADI-PEG20 therapy. In addition, combination of ASS1 activators with anti-tumor drugs like chemotherapy and TKIs could augment the anti-tumor effect of traditional regimens. Given that ASS1 is commonly downregulated in multiple tumor types and participates in the regulation of tumor development depending on diverse mechanisms, it may be a robust potential therapeutic target for cancer management.

Author Contributions

NS writing and elaborating the figures. XZ writing and reviewing the final version.

Funding

This work was supported by the Project of Liaoning Province Natural Science Foundation (2021-BS-117).

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., Qin, S., Ryoo, B. Y., Lu, S. N., Yen, C. J., Feng, Y. H., et al. (2018). Phase III randomized study of second line ADI-PEG 20 plus best supportive care versus placebo plus best supportive care in patients with advanced hepatocellular carcinoma. *Ann. Oncol.* 29 (6), 1402–1408. doi:10.1093/annonc/mdy101

Allen, M. D., Luong, P., Hudson, C., Leyton, J., Delage, B., Ghazaly, E., et al. (2014). Prognostic and therapeutic impact of argininosuccinate synthetase 1 control in bladder cancer as monitored longitudinally by PET imaging. *Cancer Res.* 74 (3), 896–907. doi:10.1158/0008-5472.CAN-13-1702

Ascierto, P. A., Scala, S., Castello, G., Daponte, A., Simeone, E., and Ottaiano, A. (2005). Pegylated arginine deiminase treatment of patients with metastatic melanoma: Results from phase I and II studies. *J. Clin. Oncol.* 23 (30), 7660–7668. doi:10.1200/JCO.2005.02.0933

Bateman, L. A., Ku, W. M., Heslin, M. J., Contreras, C. M., Skibola, C. F., Nomura, D. K., et al. (2017). Argininosuccinate synthase 1 is a metabolic regulator of colorectal cancer pathogenicity. *ACS Chem. Biol.* 12 (4), 905–911. doi:10.1021/acschembio.6b01158

Bean, G. R., Kremer, J. C., Prudner, B. C., Schenone, A. D., Yao, J. C., Schultze, M. B., et al. (2016). A metabolic synthetic lethal strategy with arginine deprivation and chloroquine leads to cell death in ASS1-deficient sarcomas. *Cell Death Dis.* 7 (10), e2406. doi:10.1038/cddis.2016.232

Beddowes, E., Spicer, J., Chan, P. Y., Khadeir, R., Corbacho, J. G., Repana, D., et al. (2017). Phase 1 dose-escalation study of pegylated arginine deiminase, cisplatin, and pemetrexed in patients with argininosuccinate synthetase 1-deficient thoracic cancers. *J. Clin. Oncol.* 35 (16), 1778–1785. doi:10.1200/JCO.2016.71.3230

Chalishazar, M. D., Wait, S. J., Huang, F., Ireland, A. S., Mukhopadhyay, A., Lee, Y., et al. (2019). MYC-driven small-cell lung cancer is metabolically distinct and vulnerable to arginine depletion. *Clin. Cancer Res.* 25 (16), 5107–5121. doi:10.1158/1078-0432.CCR-18-4140

Chan, P. Y., Phillips, M. M., Ellis, S., Johnston, A., Feng, X., Arora, A., et al. (2022). A phase 1 study of ADI-PEG20 (pegargiminase) combined with cisplatin and pemetrexed in ASS1-negative metastatic uveal melanoma. *Pigment. Cell Melanoma Res.* 35, 461–470. doi:10.1111/pcmr.13042

Chan, S. L., Cheng, P. N. M., Liu, A. M., Chan, L. L., Li, L., Chu, C. M., et al. (2021). A phase II clinical study on the efficacy and predictive biomarker of pegylated recombinant arginase on hepatocellular carcinoma. *Invest. New Drugs* 39 (5), 1375–1382. doi:10.1007/s10637-021-01111-8

Chang, K. Y., Chiang, N. J., Wu, S. Y., Yen, C. J., Chen, S. H., Yeh, Y. M., et al. (2021). Phase 1b study of pegylated arginine deiminase (ADI-PEG 20) plus Pembrolizumab in advanced solid cancers. *Oncoimmunology* 10 (1), 1943253. doi:10.1080/2162402X.2021.1943253

Chen, C. L., Hsu, S. C., Ann, D. K., Yen, Y., and Kung, H. J. (2021). Arginine signaling and cancer metabolism. *Cancers* 13 (14), 3541. doi:10.3390/cancers13143541

Chen, X., Xu, M., Xu, X., Zeng, K., Liu, X., Pan, B., et al. (2020). METTL14-mediated N6-methyladenosine modification of SOX4 mRNA inhibits tumor metastasis in colorectal cancer. *Mol. Cancer* 19 (1), 106. doi:10.1186/s12943-020-01220-7

Cheng, C. T., Qi, Y., Wang, Y. C., Chi, K. K., Chung, Y., Ouyang, C., et al. (2018). Arginine starvation kills tumor cells through aspartate exhaustion and mitochondrial dysfunction. *Commun. Biol.* 1, 178. doi:10.1038/s42003-018-0178-4

Cheng, P. N. M., Liu, A. M., Bessudo, A., and Mussai, F. (2021). Safety, PK/PD and preliminary anti-tumor activities of pegylated recombinant human arginase 1 (BCT-100) in patients with advanced arginine auxotrophic tumors. *Invest. New Drugs* 39 (6), 1633–1640. doi:10.1007/s10637-021-01149-8

Del Caño-Ochoa, F., Moreno-Morcillo, M., and Ramón-Maiques, S. (2019). CAD, A multienzymatic protein at the head of de Novo pyrimidine biosynthesis. *Subcell. Biochem.* 93, 505538. doi:10.1007/978-3-030-28151-9_17

Giatromanolaki, A., Harris, A. L., and Koukourakis, M. I. (2021). The prognostic and therapeutic implications of distinct patterns of argininosuccinate synthase 1 (ASS1) and arginase-2 (ARG2) expression by cancer cells and tumor stroma in non-small-cell lung cancer. *Cancer Metab.* 9 (1), 28. doi:10.1186/s40170-021-00264-7

Glazer, E. S., Piccirillo, M., Albino, V., Di Giacomo, R., Palaia, R., Mastro, A. A., et al. (2010). Phase II study of pegylated arginine deiminase for nonresectable and metastatic hepatocellular carcinoma. *J. Clin. Oncol.* 28 (13), 2220–2226. doi:10.1200/JCO.2009.26.7765

Hall, P. E., Lewis, R., Syed, N., Shaffer, R., Evanson, J., Ellis, S., et al. (2019). A phase I study of pegylated arginine deiminase (pegargiminase), cisplatin, and pemetrexed in argininosuccinate synthetase 1-deficient recurrent high-grade glioma. *Clin. Cancer Res.* 25 (9), 2708–2716. doi:10.1158/1078-0432.CCR-18-3729

Huang, H. Y., Wu, W. R., Wang, Y. H., Wang, J. W., Fang, F. M., Tsai, J. W., et al. (2013). ASS1 as a novel tumor suppressor gene in myxofibrosarcomas: Aberrant loss via epigenetic DNA methylation confers aggressive phenotypes, negative prognostic impact, and therapeutic relevance. *Clin. Cancer Res.* 19 (11), 2861–2872. doi:10.1158/1078-0432.CCR-12-2641

Izzo, F., Marra, P., Beneduce, G., Castello, G., Vallone, P., De Rosa, V., et al. (2004). Pegylated arginine deiminase treatment of patients with unresectable

hepatocellular carcinoma: Results from phase I/II studies. *J. Clin. Oncol.* 22 (10), 1815–1822. doi:10.1200/JCO.2004.11.120

Jia, H., Yang, Y., Li, M., Chu, Y., Song, H., Zhang, J., et al. (2021). Snail enhances arginine synthesis by inhibiting ubiquitination-mediated degradation of ASS1. *EMBO Rep.* 22 (8), e51780. doi:10.15252/embr.202051780

Keshet, R., Lee, J. S., Adler, L., Iraqi, M., Ariav, Y., Lim, L. Q. J., et al. (2020). Targeting purine synthesis in ASS1-expressing tumors enhances the response to immune checkpoint inhibitors. *Nat. Cancer* 1 (9), 894–908. doi:10.1038/s43018-020-0106-7

Khare, S., Kim, L. C., Lobel, G., Doulias, P. T., Ischiropoulos, H., Nissim, I., et al. (2021). ASS1 and ASL suppress growth in clear cell renal cell carcinoma *via* altered nitrogen metabolism. *Cancer Metab.* 9 (1), 40. doi:10.1186/s40170-021-00271-8

Kim, S., Lee, M., Song, Y., Choi, I., Park, I. S., Kim, J., et al. (2021). Argininosuccinate synthase 1 suppresses tumor progression through activation of PERK/eIF2α/ATF4/CHOP axis in hepatocellular carcinoma. *J. Exp. Clin. Cancer Res.* 40 (1), 127. doi:10.1186/s13046-021-01912-y

Kim, S. S., Xu, S., Cui, J., Poddar, S., Le, T. M., Hayrapetyan, H., et al. (2020). Histone deacetylase inhibition is synthetically lethal with arginine deprivation in pancreatic cancers with low argininosuccinate synthetase 1 expression. *Theranostics* 10 (2), 829–840. doi:10.7150/thno.40195

Kremer, J. C., Prudner, B. C., Lange, S. E. S., Bean, G. R., Schultze, M. B., Brashears, C. B., et al. (2017). Arginine deprivation inhibits the warburg effect and upregulates glutamine anaplerosis and serine biosynthesis in ASS1-deficient cancers. *Cell Rep.* 18 (4), 991–1004. doi:10.1016/j.celrep.2016.12.077

Kulis, M., and Esteller, M. (2010). DNA methylation and cancer. *Adv. Genet.* 70, 27–56. doi:10.1016/B978-0-12-380866-0.60002-2

Li, G., Li, D., Wang, T., and He, S. (2021). Pyrimidine biosynthetic enzyme CAD: Its function, regulation, and diagnostic potential. *Int. J. Mol. Sci.* 22 (19), 10253. doi:10.3390/ijms221910253

Locke, M., Ghazaly, E., Freitas, M. O., Mitsinga, M., Lattanzio, L., Lo Nigro, C., et al. (2016). Inhibition of the polyamine synthesis pathway is synthetically lethal with loss of argininosuccinate synthase 1. *Cell Rep.* 16 (6), 1604–1613. doi:10.1016/j.celrep.2016.06.097

Long, Y., Tsai, W. B., Wang, D., Hawke, D. H., Savaraj, N., Feun, L. G., et al. (2017). Argininosuccinate synthetase 1 (ASS1) is a common metabolic marker of chemosensitivity for targeted arginine- and glutamine-starvation therapy. *Cancer Lett.* 388, 54–63. doi:10.1016/j.canlet.2016.11.028

Long, Y., Tsai, W. B., Wangpaichitr, M., Tsukamoto, T., Savaraj, N., Feun, L. G., et al. (2013). Arginine deiminase resistance in melanoma cells is associated with metabolic reprogramming, glucose dependence, and glutamine addiction. *Mol. Cancer Ther.* 12 (11), 2581–2590. doi:10.1158/1535-7163.MCT-13-0302

Lowery, M. A., Yu, K. H., Kelsen, D. P., Harding, J. J., Bomalaski, J. S., Glassman, D. C., et al. (2017). A phase 1/1B trial of ADI-PEG 20 plus nab-paclitaxel and gemcitabine in patients with advanced pancreatic adenocarcinoma. *Cancer* 123 (23), 4556–4565. doi:10.1002/cncr.30897

Miao, Y. Q., Chen, W., Zhou, J., Shen, Q., Sun, Y., Li, T., et al. (2022). N(6)-adenosine-methyltransferase-14 promotes glioma tumorigenesis by repressing argininosuccinate synthase 1 expression in an m6A-dependent manner. *Bioengineered* 13 (1), 1858–1871. doi:10.1080/21655979.2021.2018386

Miraki-Moud, F., Ghazaly, E., Ariza-McNaughton, L., Hodby, K. A., Clear, A., Anjos-Afonso, F., et al. (2015). Arginine deprivation using pegylated arginine deiminase has activity against primary acute myeloid leukemia cells *in vivo*. *Blood* 125 (26), 4060–4068. doi:10.1182/blood-2014-10-608133

Mollick, T., and Laín, S. (2020). Modulating pyrimidine ribonucleotide levels for the treatment of cancer. *Cancer Metab.* 8, 12. doi:10.1186/s40170-020-00218-5

Mörén, L., Perryman, R., Crook, T., Langer, J. K., Oneill, K., Syed, N., et al. (2018). Metabolomic profiling identifies distinct phenotypes for ASS1 positive and negative GBM. *BMC Cancer* 18 (1), 167. doi:10.1186/s12885-018-4040-3

Nicholson, L. J., Smith, P. R., Hiller, L., Kimberley, C., Sehouli, J., Koensgen, D., et al. (2009). Epigenetic silencing of argininosuccinate synthetase confers resistance to platinum-induced cell death but collateral sensitivity to arginine auxotrophy in ovarian cancer. *Int. J. Cancer* 125 (6), 1454–1463. doi:10.1002/ijc.24546

Ott, P. A., Carvajal, R. D., Pandit-Taskar, N., Jungbluth, A. A., Hoffman, E. W., Wu, B. W., et al. (2013). Phase I/II study of pegylated arginine deiminase (ADI-PEG 20) in patients with advanced melanoma. *Invest. New Drugs* 31 (2), 425–434. doi:10.1007/s10637-012-9862-2

Pavlova, N. N., and Thompson, C. B. (2016). The emerging hallmarks of cancer metabolism. *Cell Metab.* 23 (1), 27–47. doi:10.1016/j.cmet.2015.12.006

Rabinovich, S., Adler, L., Yizhak, K., Sarver, A., Silberman, A., Agron, S., et al. (2015). Diversion of aspartate in ASS1-deficient tumours fosters de novo pyrimidine synthesis. *Nature* 527 (7578), 379–383. doi:10.1038/nature15529

Siddiqui, A., and Ceppi, P. (2020). A non-proliferative role of pyrimidine metabolism in cancer. *Mol. Metab.* 35, 100962. doi:10.1016/j.molmet.2020.02.005

Silberman, A., Goldman, O., Boukobza Assayag, O., Jacob, A., Rabinovich, S., Adler, L., et al. (2019). Acid-induced downregulation of ASS1 contributes to the maintenance of intracellular pH in cancer. *Cancer Res.* 79 (3), 518–533. doi:10.1158/0008-5472.CAN-18-1062

Singh, P. K., Deorukhkar, A. A., Venkatesulu, B. P., Li, X., Tailor, R., Bomalaski, J. S., et al. (2019). Exploiting arginine auxotrophy with pegylated arginine deiminase (ADI-PEG20) to sensitize pancreatic cancer to radiotherapy *via* metabolic dysregulation. *Mol. Cancer Ther.* 18 (12), 2381–2393. doi:10.1158/1535-7163.MCT-18-0708

Somasundaram, V., Basudhar, D., Bharadwaj, G., No, J. H., Ridnour, L. A., Cheng, R. Y. S., et al. (2019). Molecular mechanisms of nitric oxide in cancer progression, signal transduction, and metabolism. *Antioxid. Redox Signal.* 30 (8), 1124–1143. doi:10.1089/ars.2018.7527

Syed, N., Langer, J., Janczar, K., Singh, P., Lo Nigro, C., Lattanzio, L., et al. (2013). Epigenetic status of argininosuccinate synthetase and argininosuccinate lyase modulates autophagy and cell death in glioblastoma. *Cell Death Dis.* 4 (1), e458. doi:10.1038/cddis.2012.197

Szlosarek, P. W., Steele, J. P., Nolan, L., Gilligan, D., Taylor, P., Spicer, J., et al. (2017). Arginine deprivation with pegylated arginine deiminase in patients with argininosuccinate synthetase 1-deficient malignant pleural mesothelioma: A randomized clinical trial. *JAMA Oncol.* 3 (1), 58–66. doi:10.1001/jamaoncol.2016.3049

Szlosarek, P. W., Wimalasingham, A. G., Phillips, M. M., Hall, P. E., Chan, P. Y., Conibear, J., et al. (2021). Phase 1, pharmacogenomic, dose-expansion study of pegargiminase plus pemetrexed and cisplatin in patients with ASS1-deficient non-squamous non-small cell lung cancer. *Cancer Med.* 10 (19), 6642–6652. doi:10.1002/cam4.4196

Tabe, Y., Lorenzi, P. L., and Konopleva, M. (2019). Amino acid metabolism in hematologic malignancies and the era of targeted therapy. *Blood* 134 (13), 1014–1023. doi:10.1182/blood.2019001034

Tong, B. C., and Barbul, A. (2004). Cellular and physiological effects of arginine. *Mini Rev. Med. Chem.* 4 (8), 823–832. doi:10.2174/1389557043403305

Tsai, C. Y., Chi, H. C., Chi, L. M., Yang, H. Y., Tsai, M. M., Lee, K. F., et al. (2018). Argininosuccinate synthetase 1 contributes to gastric cancer invasion and progression by modulating autophagy. *FASEB J.* 32 (5), 2601–2614. doi:10.1096/fj.201700094R

Tsai, H. J., Jiang, S. S., Hung, W. C., Borthakur, G., Lin, S. F., Pemmaraju, N., et al. (2017). A phase II study of arginine deiminase (ADI-PEG20) in relapsed/refractory or poor-risk acute myeloid leukemia patients. *Sci. Rep.* 7 (1), 11253. doi:10.1038/s41598-017-10542-4

Wang, H., Li, Q. F., Chow, H. Y., Choi, S. C., and Leung, Y. C. (2020). Arginine deprivation inhibits pancreatic cancer cell migration, invasion and EMT *via* the down regulation of Snail, Slug, Twist, and MMP1/9. *J. Physiol. Biochem.* 76 (1), 73–83. doi:10.1007/s13105-019-00716-1

Wang, K., Sun, Y., Guo, C., Liu, T., Fei, X., and Chang, C. (2019). Androgen receptor regulates ASS1P3/miR-34a-5p/ASS1 signaling to promote renal cell carcinoma cell growth. *Cell Death Dis.* 10 (5), 339. doi:10.1038/s41419-019-1330-x

Yang, T. S., Lu, S. N., Chao, Y., Sheen, I. S., Lin, C. C., Wang, T. E., et al. (2010). A randomised phase II study of pegylated arginine deiminase (ADI-PEG 20) in Asian advanced hepatocellular carcinoma patients. *Br. J. Cancer* 103 (7), 954–960. doi:10.1038/sj.bjc.6605856

Yao, S., Janku, F., Subbiah, V., Stewart, J., Patel, S. P., Kaseb, A., et al. (2021). Phase 1 trial of ADI-PEG20 plus cisplatin in patients with pretreated metastatic melanoma or other advanced solid malignancies. *Br. J. Cancer* 124 (9), 1533–1539. doi:10.1038/s41416-020-01230-8

Yeon, A., You, S., Kim, M., Gupta, A., Park, M. H., Weisenberger, D. J., et al. (2018). Rewiring of cisplatin-resistant bladder cancer cells through epigenetic regulation of genes involved in amino acid metabolism. *Theranostics* 8 (16), 4520–4534. doi:10.7150/thno.25130

Zeng, J., Li, Y., Wang, Y., Xie, G., Feng, Q., Yang, Y., et al. (2020). lncRNA 00312 attenuates cell proliferation and invasion and promotes apoptosis in renal cell carcinoma *via* miR-34a-5p/ASS1 Axis. *Oxid. Med. Cell Longev.* 2020, 5737289. doi:10.1155/2020/5737289

Zhang, Y., Chung, S. F., Tam, S. Y., Leung, Y. C., and Guan, X. (2021). Arginine deprivation as a strategy for cancer therapy: An insight into drug design and drug combination. *Cancer Lett.* 502, 58–70. doi:10.1016/j.canlet.2020.12.041

Zou, Z., Hu, X., Luo, T., Ming, Z., Chen, X., Xia, L., et al. (2021). Naturally-occurring spinosyn A and its derivatives function as argininosuccinate synthase activator and tumor inhibitor. *Nat. Commun.* 12 (1), 2263. doi:10.1038/s41467-021-22235-8

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 22 July 2022
DOI 10.3389/fphar.2022.935536

OPEN ACCESS

EDITED BY
Na Li,
University of California, San Diego,
United States

REVIEWED BY
Xiawei Cheng,
East China University of Science and Technology, China
Vera Miranda-Gonçalves,
Portuguese Oncology Institute,
Portugal

*CORRESPONDENCE
Yanru Qin,
yanruqin@163.com

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

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 04 May 2022
ACCEPTED 29 June 2022
PUBLISHED 22 July 2022

CITATION
Chen C, Wang Z and Qin Y (2022), Connections between metabolism and epigenetics: mechanisms and novel anti-cancer strategy.
Front. Pharmacol. 13:935536.
doi: 10.3389/fphar.2022.935536

Connections between metabolism and epigenetics: mechanisms and novel anti-cancer strategy

Chen Chen†, Zehua Wang† and Yanru Qin*

Department of Oncology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Cancer cells undergo metabolic adaptations to sustain their growth and proliferation under several stress conditions thereby displaying metabolic plasticity. Epigenetic modification is known to occur at the DNA, histone, and RNA level, which can alter chromatin state. For almost a century, our focus in cancer biology is dominated by oncogenic mutations. Until recently, the connection between metabolism and epigenetics in a reciprocal manner was spotlighted. Explicitly, several metabolites serve as substrates and co-factors of epigenetic enzymes to carry out post-translational modifications of DNA and histone. Genetic mutations in metabolic enzymes facilitate the production of oncometabolites that ultimately impact epigenetics. Numerous evidences also indicate epigenome is sensitive to cancer metabolism. Conversely, epigenetic dysfunction is certified to alter metabolic enzymes leading to tumorigenesis. Further, the bidirectional relationship between epigenetics and metabolism can impact directly and indirectly on immune microenvironment, which might create a new avenue for drug discovery. Here we summarize the effects of metabolism reprogramming on epigenetic modification, and vice versa; and the latest advances in targeting metabolism-epigenetic crosstalk. We also discuss the principles linking cancer metabolism, epigenetics and immunity, and seek optimal immunotherapy-based combinations.

KEYWORDS

cancer metabolism, epigenetics, immunity, novel anti-cancer strategy, oncology

1 Introduction

Cancer metabolism is based on the principle that cancer cells undergo metabolic adaptations to sustain their uncontrolled proliferation. Such adaptations render malignant cells to exhibit altered metabolism compared to the normal cells. In 1920s, Warburg firstly proposed (Kaye, 1998; Chinnaiyan et al., 2012) that cancer cells display enhanced glycolysis and increased secretion of lactate even with abundant oxygen supply. This phenomenon is termed as "Warburg effect" or aerobic glycolysis. Moreover, an emerging class of metabolic alterations enables tumor cells to take up available ample nutrients and utilize them to produce ATP, generate biosynthetic precursors for cell anabolism, and tolerate stresses related to malignancy,

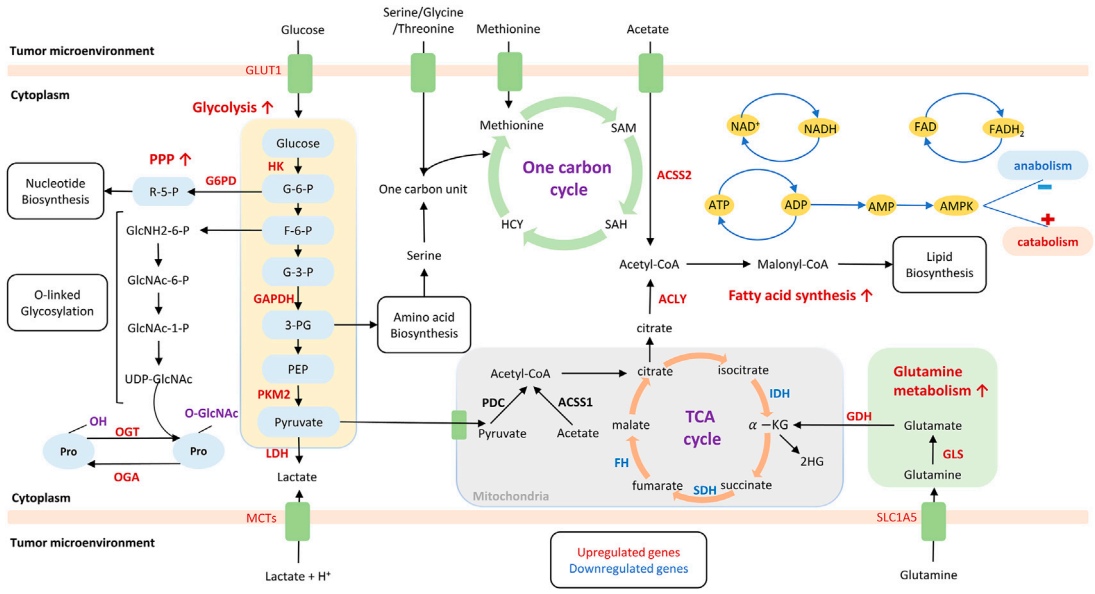


FIGURE 1
Metabolism reprogramming in cancer cells. Metabolism reprogramming is characterized by a class of altered pathway, including enhanced glycolysis with increased lactate production, and enhanced pentose phosphate pathway, fatty acid synthesis, and glutamine metabolism. These metabolic pathways support energy supply and macromolecule biosynthesis, such as nucleotides, amino acids, and lipids. Metabolites that are produced by altered metabolism have the potential to control signaling or epigenetic pathways by regulating reactive oxygen species, acetylation, and methylation. Upregulated genes or proteins are labels red, whereas downregulated genes or proteins are labeled blue. GLUT, glucose transporter; MCT, monocarboxylate transporter; SLC1A5, solute carrier family 1 member 5; TCA, Tricarboxylic acid cycle; G6PD, glucose-6-phosphate dehydrogenase; HK, hexokinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PKM, pyruvate kinase M 2; LDH, lactate dehydrogenase; ACSS2, Acyl-CoA short-chain synthetase-2; ACSS1: Acyl-CoA short-chain synthetase-1; ACLY: ATP citrate lyase; GLS, glutaminase; GDH, glutamate dehydrogenase; PDC: pyruvate dehydrogenase complex; FH, fumarate hydratase; SDH, succinate dehydrogenase; IDH1/2, isocitrate dehydrogenase 1/2; HCY, homocysteine; PPP, pentose phosphate pathway; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; OGT, O-GlcNAc transferase; OGA, O-GlcNAcase.

such as hypoxia and nutrient starvation (Owen et al., 2002; Koppenol et al., 2011; Lunt and Vander Heiden, 2011; Metallo et al., 2011; Mullen et al., 2011; Wise et al., 2011; Cantor and Sabatini, 2012; Ahn and Metallo, 2015). In this context, cancer metabolism provides a selective advantage during tumorigenesis. Metabolic reprogramming (Figure 1) is now recognized as a hallmark of cancer (Hanahan and Weinberg, 2011; Pavlova and Thompson, 2016), which could be intrinsically regulated by genotype and epigenotype, or extrinsically affected by tumor microenvironment (TME).

Epigenetics was firstly established by Conrad Waddington in 1942 (Cairns et al., 2011), which refers to the study of modification in gene expression or cellular phenotype that occurs without changes in DNA nucleotide sequences (Possemato et al., 2011). The basic unit of chromatin organization is nucleosome, which is composed of DNA and histone octamer. Chromatin state is a dynamic event that controls gene transcription. Epigenetic modification of gene expression occurs at the DNA, histone, and RNA level. The most well-characterized examples are DNA methylation, histone methylation, acetylation, phosphorylation, ubiquitination, and microRNA-dependent gene silencing (Margueron and Reinberg, 2010). It is widely recognized that epigenetic dysfunction is a common feature of many cancers (Ribich et al., 2017). Numerous excellent reviews have summarized the biology fundamentals of chromatin-modified proteins (CMPs) (Tessarz and Kouzarides, 2014; Piunti and Shilatifard, 2016; Soshnev et al., 2016) and the therapeutic potentials to target CMPs in tumor (Pfister and Ashworth, 2017).

For almost a century, our focus in cancer is dominated by oncogenic mutations. Until recently, the connection between metabolism and epigenetics was emphasized in cancer biology. Metabolism reprogramming is known to affect epigenetic landscapes through different mechanisms. Conversely, epigenetic regulation contributes to altered metabolic activities. Hence, cancer metabolism and epigenetics are highly interwoven in a reciprocal manner. This great breakthrough has gained wide interest in targeting both altered metabolism and modified epigenetics. However, whether these two hallmarks synergistically attack tumor remains unknown. Noteworthy, such a complex relationship has the potential to affect immune system, such as trained immunity, T cell activation, macrophage activation. A novel strategy is to target epigenetics-metabolism axis in combination with immunotherapy, potentially boosting more potent antitumor responses.

TABLE 1 Fundamental interface of metabolism and epigenetics.

Metabolism pathway	Metabolic enzyme	Metabolites	Epigenetic enzyme	Epigenetic regulation
One-carbon cycle	MAT	SAM/SAH	KMT, PRMT	DNA and histone methylation
TCA cycle	FADS	FAD/FADH2	LSD	Histone demethylation
TCA cycle	IDH, GLUD	α-KG	TET and JmjC demethylase	DNA and histone demethylation
TCA cycle	ACSS1, ACSS2, ACLY	Acetyl-CoA/CoA	HAT	Histone acetylation
Glycolysis/TCA cycle	NMNAT	NAD+/NADH	SIRT, PARP	Histone deacetylation
TCA cycle	NA	AMP/ATP	AMPK	Phospharylation
Hexosamine	NA	GlcNac	OGT	GlcNacylation

MAT, methionine adenosyltransferase; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; KMT, Lysine methyltransferase; PRMT, protein arginine methyltransferase; TCA, Tricarboxylic acid; ACSS, acetyl-CoA synthetase short-chain family member; ACLY, ATP citrate lyase; HAT, histone acetyltransferase; NMNAT, nicotinamide mononucleotide adenylytransferase; PARP, poly-ADP ribose polymerase; FADS, flavin adenine dinucleotides; LSD, lysine specific demethylase; IDH, isocitrate dehydrogenase; GLUD, glutamate dehydrogenase; TET, ten-eleven translocation methylcytosine dioxygenase; JmjC, Jumonji N/C-terminal domains; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; GlcNac, O-linked N-acetylglucosamine; OGT, O-linked N-acetylglucosamine transferase; NA, Not Applicable

In this review article, we firstly summarize the metabolic alterations that drive epigenetic changes in cancer, and vice versa. We next describe the therapeutic opportunities by targeting metabolism-epigenetic crosstalk. Further, we discuss the principles linking metabolism, epigenetics to immunity and introduce the rationale for novel immunotherapy-based combinations. Our aim is to introduce the fundamentals of connection between metabolism and epigenetics in cancer biology and discuss potential pharmacological strategies that can exploit the metabolism and epigenetics in malignancy.

2 Metabolism shapes the epigenetic state of cancer cells

Tumors are likely to harbor epigenetic changes driven by their cellular metabolism. There are several different mechanisms explaining the influx from metabolism to chromatin.

2.1 Metabolites are either substrates or co-factors for epigenetic enzymes

Epigenetic enzymes employ several metabolic intermediates as substrates or co-factors to carry out post-translational modifications of DNA and histone (Katada et al., 2012), which in turn influence metabolic gene expression. Examples of such metabolites include: SAM, α-KG, and FAD that participate in DNA and histone methylation; acetate, acetyl-CoA and NAD^+ that mediate histone acetylation (Thakur and Chen, 2019). These key metabolites are produced in multiple pathways mediated by metabolic enzymes: SAM from one-carbon metabolism, α-KG and FAD^+ from the TCA cycle, acetyl-CoA from glycolysis and glutamine metabolism, and NAD^+ from the conjunction of glycolysis and oxidative phosphorylation (Wang and Lei, 2018). The fundamental interface between metabolism and epigenetics has been summarized in Table 1.

2.2 SAM/SAH ratio affects DNA and histone methylation

2.2.1 SAM/SAH

DNA and histone methylation are respectively mediated by DNA methyltransferase (DNMT) enzymes and histone methyltransferase (HMT) enzymes (Varier and Timmers, 2011), both of which utilize S-Adenosyl-methionine (SAM) as a major methyl donor. Methylation is to transfer a methyl group from SAM to the receptor, and the remaining residue is S-adenosyl-homocysteine (SAH) that is inhibitory to methyltransferase. SAM is derived from one-carbon metabolism that plays integral roles in DNA synthesis and methylation reaction. The most studied metabolites, like glucose and glutamine, feed into the one-carbon cycle and increase the availability of SAM. Both global DNA hypomethylation and site-specific CpG hypermethylation are frequent epigenetic abnormities observed in cancer (Sandoval and Esteller, 2012), while histone methylation may activate or repress gene transcription (Vakoc et al., 2005; Berger, 2007; Bernstein et al., 2007). Therefore, SAM/SAH ratio directly affect the methylation status of chromatin.

2.3 TCA cycle metabolites regulate DNA and histone demethylation

2.3.1 TCA cycle metabolites

Reversal of DNA and histone methylation is catalyzed by DNA and histone demethylase. Histone demethylation is regulated by two classes of enzymes: lysine-specific demethylase family (LSD1 and LSD2) (Fang et al., 2010) and

JmjC-containing family, both of which are dependent on ferrous adenine dinucleotide (FAD). Also, JmjC family is ferrous ion-dependent oxygenase requiring α-KG for the enzymatic activation (Shi et al., 2005; Klose et al., 2006). Likewise, DNA demethylation is modulated by TET-family proteins (TET1, TET2, and TET3), which are also FAD- and α-KG-dependent dioxygenase (Bhutani et al., 2011; He et al., 2011; Ito et al., 2011). Both FAD and α-KG are intermediary metabolites produced in TCA cycle. Other TCA metabolites, such as succinate and fumarate, are identified as antagonists for JmjC-containing family demethylase (Xiao et al., 2012). Therefore, TCA cycle metabolites regulate epigenetic marks on DNA and histone.

2.4 Acetyl-CoA, NAD^+ and acetate influence histone acetylation

2.4.1 Acetyl-CoA

Histone acetylation is another important epigenetic modification that depends on histone acetyltransferase (HAT) and histone deacetylase (HDAC) (Shahbazian and Grunstein, 2007). Acetyl-CoA is a pivotal metabolite for energy production and anabolic process (Wellen and Thompson, 2012; Pietrocola et al., 2015). HAT transfers the acetyl moiety of acetyl-CoA to lysine residues of histone, while HDAC is responsible for removing the acetyl group to reverse histone acetylation. It is well-known histone acetylation can increase nucleosome mobility and activate transcription elongation (Racey and Byvoet, 1971; Cai et al., 2011). Previous study figured out, in yeast and mammalian cells, the glycolysis dynamically governs the acetyl-CoA quantity and correspondingly regulates HAT-dependent histone acetylation (Friis et al., 2009; Cai et al., 2011; Lee et al., 2014).

2.4.2 NAD^+

Histone deacetylation is catalyzed by two kinds of deacetylases: zinc-dependent and NAD^+-dependent proteins. Deacetylation results in the tight wrapping of DNA by histone and hence promotes gene repression and silence (Imai et al., 2000; Finkel et al., 2009). Similarly, some metabolites function as antagonists that inhibit the activities of HDAC. For example, butyrate can robustly antagonize HDACs I, II and IV (Candido et al., 1978). Also, NAD^+ is regarded as a catalytic co-factor for HDAC III to mediate histone deacetylation (Thakur and Chen, 2019). Further, evidence illustrated higher histone deacetylation levels are associated with poorer prognosis (Kurdistani, 2011).

2.4.3 Acetate

Acetate has been implicated in driving histone acetylation and deacetylation. Recently, the role of acetate in the interaction between metabolism and epigenetics has been emphasized during tumorigenesis. Under hypoxia, cancer cells decrease the reliance on glucose and glutamate and inversely increase the demand of acetate as a substitute carbon source for lipid synthesis (Kamphorst et al., 2014). Consequently, acetate must be converted to acetyl-CoA either by ACSS1 in mitochondria or by ACSS2 in the cytoplasm or nucleus (Figure 1). There is already evidence that both acetate and acetyl-CoA facilitate tumor growth by histone acetylation in yeast (Cai et al., 2011). ACSS2, as the only known enzyme utilizing free acetate in nucleus (Moffett et al., 2020), could shape the epigenetic landscape via selective histone acetylation. More specifically, ACSS2 is translocated from cytoplasm to the nucleus supplying a local of acetyl-CoA (Chen et al., 2017), which contributes to all kinds of acetylation reactions in cell nuclei. One study indicated (Gao et al., 2016), under hypoxia condition, ACSS2 catalyzes the conversion of acetate to acetyl-CoA in the hepatoma carcinoma cells, facilitating the hyper-acetylation of histone K3K9, H3K27, and H3K56 and thereby upregulating the expression of lipogenic enzymes. This explains how acetate links metabolite levels to epigenetic regulation and gene transcription. Otherwise, ACSS2 acts to recycle acetate generated from HDAC-mediated deacetylation reactions under metabolic stresses, replenishing the cytoplasmic and nuclear storage and thus supporting chromatin remodeling events (Moffett et al., 2020).

2.5 ATP/AMP ratio controls histone phosphorylation

2.5.1 ATP/AMP

Some kinase could be translocated to nucleus and straightly phosphorylate histone (Baek, 2011). For example, AMP-activated protein kinase (AMPK) acts as sensory signal of ATP/AMP ratio (Hardie, 2011). Conversion of ATP to AMP aids in anabolic process via AMPK-mediated pathway, whereas catabolism relies on the opposite switch from AMP to ATP. Owing to metabolic stress and low ATP/AMP ratio, AMPK is activated to phosphorylate histone H2B on serine 36 that triggers gene expression in favor of tumor survival (Bungard et al., 2010).

2.6 Hexosamine biosynthetic pathway mediates protein glycosylation

2.6.1 O-GlcNAc

Protein glycosylation is carried by opposite actions of O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively responsible for the addition and removal of O-GlcNAc from proteins. One of the most common features that cancer cells demonstrate is OGT overexpression leading to protein hyper-glycosylation (Pinho and Reis, 2015). Typically, O-GlcNAc is produced in Hexosamine biosynthetic pathway (HBP). In this pathway, glucose is firstly converted into glucose-6-P and then fructose-6-P. A series of metabolites, such as acetyl-CoA, UTP, glutamine, subsequently participate in the production

TABLE 2 Metabolites are either substrates or co-factors for epigenetic enzymes in cancer biology.

Epigenetic enzymes	Examples	Substrates or Co-factors	Mechanisms
DNA methylation and demethylation			
DNA methyltransferase	DNMTs	SAM/SAH (methionine cycle)	Methyl donors for methyltransferases
DNA demethylase	TETs	α-KG, 2HG, succinate, fumarate, vitamin C, $FAD/FADH_2$	Co-factors for α-KG-utilizing dioxygenases; Inhibition of α-KG-utilizing dioxygenases
Histone acetylation and deacetylation			
Histone acetyltransferase	HATs	Acetyl-CoA (TCA cycle/acetate)	Acetyl donors for acetyltransferases
Histone deacetylases	HDAC, SIRT	NAD^+, nicotinamide, β-Hydroxybutyrate, succinyl-CoA, butyrate	Activation or inhibition of histone deacetylase; Histone succinylation
Histone methylation and demethylation			
Histone methyltransferase	Lysine: PKMTs, Arginine: PRMTs	SAM/SAH (methionine cycle)	Methyl donors for methyltransferases
Histone demethylases	KDMs: LSD, JmjC	α-KG, 2HG, succinate, fumarate, vitamin C, $FADH_2$	Co-factors for α-KG-utilizing dioxygenases; Positive regulators of LSD; Inhibition of α-KG-utilizing dioxygenases
Histone phosphorylation			
Histone kinase	AMPK	ATP/AMP	Phosphate donors for protein kinase
Protein glycosylation			
Protein glycosylase	OGT, OGA	O-GlcNAc	O-GlcNAc donors for protein glycosylation

of UDP-GlcNAc, the activated substrate for O-GlcNAcylation. Therefore, HBP integrated various metabolism pathways. Upregulation of HBP is associated with abnormal O-GlcNAcylation and more invasive behavior (Caldwell et al., 2010; Wellen et al., 2010; Itkonen et al., 2013; Onodera et al., 2014; Lucena et al., 2016). Recently, studies confirm that enhanced glycolysis aids in protein glycosylation (Wong et al., 2017). Moreover, OGT is associated with TETs to control O-GlcNAcylation of histone H2B for activation of gene transcription (Chen et al., 2013; Ito et al., 2014), while OGT is coordinated with EZH2 to modulate H3K27me3 for silence of tumor suppressor genes (Chu et al., 2014).

Taken together, either methylation or acetylation controls the activation and repression of gene transcription. This event is balanced by various epigenetic enzymes. The cellular metabolites, such as SAM/SAH, acetyl-CoA/CoA, NAD^+/NADH, ATP/AMP ratio, commonly act as substrate or co-factors for these epigenetic-based enzymes (Table 2, Figure 2). Their fluctuating concentrations could regulate the epigenetic profile and affect gene transcription.

2.7 Genetic mutations of metabolic enzyme that modify epigenome

Mutations in metabolic enzymes subject the cells to tumorigenesis. Such changes facilitate the accumulation of metabolites that ultimately lead to epigenetic dysfunction (DeBerardinis and Chandel, 2016) and immunosuppression (Table 3).

One example is to generate oncometabolite. Oncometabolite refers to metabolites whose great quantity increases markedly in tumors compared with normal cells (Nowicki and Gottlieb, 2015). This new term is used to describe metabolites for which 1) there is a well-characterized mechanism connecting mutations in metabolic enzymes to accumulation of a certain

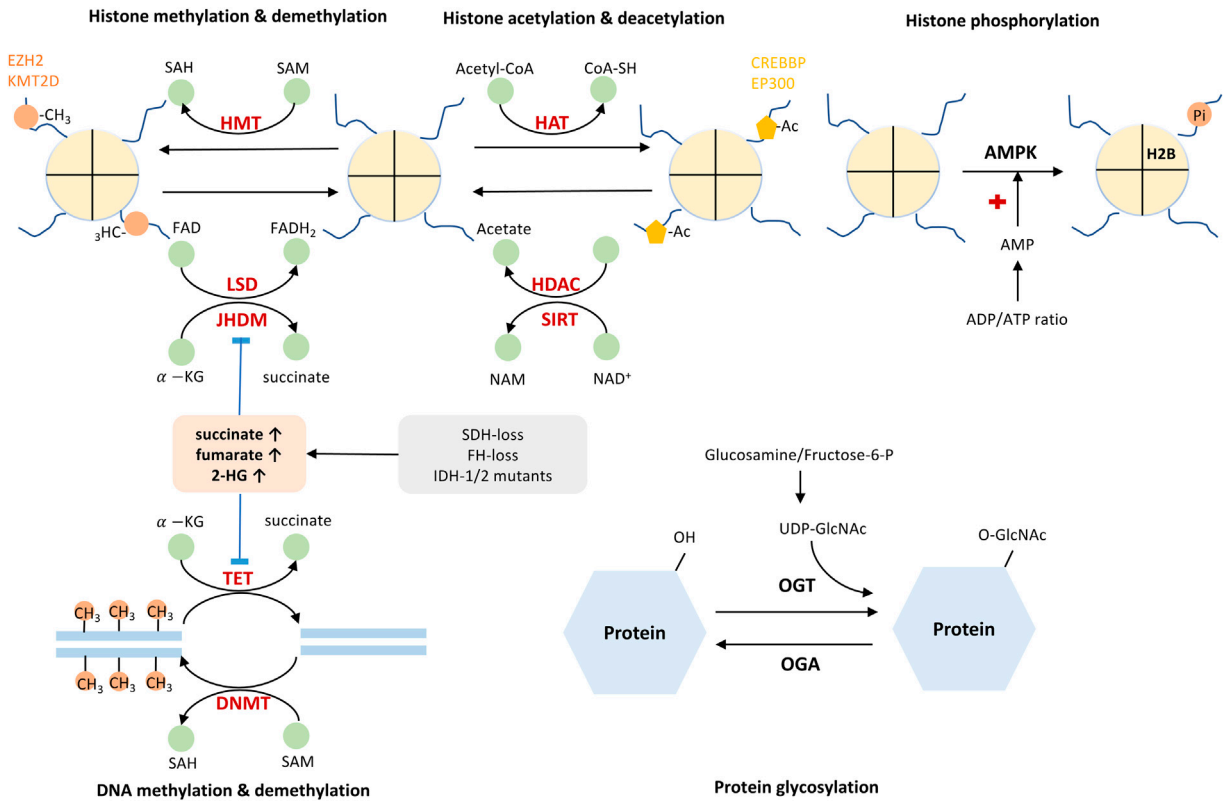


FIGURE 2
Cellular metabolites serve as co-factors or substrates for epigenetic enzymes. Addition or removal of epigenetic marks is catalyzed by epigenetic enzymes, of which process relies on several critical metabolites. SAH/SAM, NAD^+/NADH, Acetyl-CoA/Co-A, ATP/ADP ratio act as important molecules or signals governing epigenetic modifications. In addition, Metabolites such as succinate, fumarate, 2-HG, and lactate could inhibit the activity of epigenetic enzymes. HMT, histone methyltransferase; LSD, lysine-specific histone demethylase; JHDM, Jumonji domain-containing histone demethylase; HAT, histone acetyltransferase; HDAC, histone deacetylase; SIRT, sirtuins; DNMT, DNA methyltransferase; TET, ten-eleven translocation methylcytosine dioxygenase; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; α– KG, α-ketoglutarate; NAM, nicotinamide; NAD^+, nicotinamide adenine dinucleotide (oxidized); FAD, flavin adenine dinucleotide (oxidized); $FADH_2$, flavin adenine dinucleotide (reduced); FH, fumarate hydratase; SDH, succinate dehydrogenase; IDH1/2, isocitrate dehydrogenase 1/2; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; KMT2D, histone-lysine N-methyltransferase 2D. AMPK, AMP-activated protein kinase; Pi, phosphate group; OGT, O-GlcNAc transferase; OGA, O-GlcNAcase.

metabolite; 2) there is convincing evidence for some metabolites as a predisposition to tumorigenesis. Oncometabolites are frequently associated with aberrant DNA damage and enable the tumor microenvironment (TME) more invasive. Currently, D-2-hydroxyglutarate (D2HG), L-2-hydroxyglutarate (L2HG), succinate, fumarate, and lactate are recognized oncometabolites.

2.7.1 D2HG and L2HG

The first emphasized oncometabolite is D2HG, a reduced form of the TCA cycle intermediate α-ketoglutarate, which is scarce in normal tissues but rises to a higher concentration in tumors (Xu et al., 2011). This oncometabolite is caused by $NADP^+$-dependent isocitrate dehydrogenase (IDH1 or IDH2) mutation. High levels of D2HG inhibit the activity of TET-family DNA and JmjC family histone demethylase. Overall, cancer cells harboring IDH1/IDH2 mutations display hypermethylation of DNA and histone (Figueroa et al., 2010; Losman et al., 2013). Mutant-IDH1/IDH2 and their relationship to D2HG have been reviewed extensively elsewhere (Losman and Kaelin, 2013). These mutations frequently occur in gliomas, blood cancer, glioblastoma multiforme, and cholangiocarcinoma (Yan et al., 2009; Vatrinet et al., 2017). Another reduced form of α-ketoglutarate is L2HG that is accumulated due to loss-of-function mutations of L-2-hydroxyglutarate dehydrogenase (L2HGDH) (Aghili et al., 2009; Rogers et al., 2010). The increased levels of L2HG have been observed in renal cell carcinoma and brain tumors (Shim et al., 2014).

2.7.2 Succinate and fumarate

This principle also applies to another two oncometabolites: succinate and fumarate (Yang et al., 2013). Mutational inactivation of succinate dehydrase (SDH) and fumarate hydratase (FH)

TABLE 3 The effect of oncometabolites on epigenetic dysfunction and immunosuppression.

Oncometabolite	Metabolic enzymes	Epigenetic dysfunction	Immunosuppressive effect	Malignancies	References
D-2-hydroxyglutarate	IDH1/2	DNA and histone hypermethylation	NA	Glioblastoma multiforme, ALL, Chondrosarcoma, Cholangiocarcinoma	Dang et al. (2009); Amary et al. (2011); Borger et al. (2014); Shim et al. (2014); Waterfall et al. (2014); Colvin et al. (2016)
L-2-hydroxyglutarate	L2HGDH	DNA and histone hypermethylation	NA	Brain tumors, Renal cell carcinoma	Aghili et al. (2009); Rogers et al. (2010)
Succinate	SDH	DNA and histone hypermethylation	TAM marker gene expression ↑ IL-6 secretion ↑	Pheochromocytomas, Paragangliomas	Hao et al. (2009); Bardella et al. (2011); Zhang et al. (2011); Yang et al. (2013); Williamson et al. (2015); Jiang and Yan, (2017); Mu et al. (2017)
Fumarate	FH	DNA and histone hypermethylation	Neutrophils, T-cell, B-cell response ↓ Inhibiting DC maturation CD150, CD40, CD86 expression ↓ CTLA-4, PD-L1 expression ↑ IL-6, IL-1β, TNF-α secretion ↓	Pheochromocytomas, Paragangliomas	Kinch et al. (2011); Fieuw et al. (2012); Sullivan et al. (2013); Zheng et al. (2013b); Castro-Vega et al. (2014); Shanmugasundaram et al. (2014); Yang et al. (2014); Jin et al. (2015); Zheng et al. (2015)
Lactate	MCT/LDH	Histone acetylation	PD-1, PD-L1, CTLA-4 expression ↑ Inhibiting the differentiation of monocytes to DCs Inhibiting the differentiation of progenitor cells to $CD4^+$ and $CD8^+$ T-cell	Lung carcinoma, Melanoma, Prostate cancer	(Colegio et al., 2014; El-Kenawi et al., 2019)

IDH1/2, isocitrate dehydrogenase; L2HGDH, L-2-hydroxyglutarate dehydrogenase; SDH, succinate dehydrogenase; FH, fumarate hydratase; MCT, monocarboxylate transporter; LDH, lactate dehydrogenase; TAM, tumor-associated macrophages; ALL, acute lymphoblastic leukemia; NA, not applicable.

respectively contributes to the stacking up of succinate and fumarate (Baysal et al., 2000; Tomlinson et al., 2002; Gottlieb and Tomlinson, 2005), both of which interfere with α KG-dependent dioxygenases, namely DNA and histone demethylase (Nowicki and Gottlieb, 2015). Consequently, deficiency of SDH and FH activity results in DNA and histone hypermethylation, supporting the notion that oncometabolites are potent modifiers of the epigenome. Other studies provided additional layers of metabolic control of epigenome. FH is observed to be O-GlycNAcylated and consequently bring changes in histone methylation (Wang et al., 2017). Another research proposed that the enrichment of fumarate facilitates epithelial-to-mesenchymal-transition (EMT) through inhibiting TET methylase (Sciacovelli et al., 2016). Therefore, oncometabolites perform their biological functions outside of conventional pathways and play quantitative roles leading to aberrant epigenome. Additionally, emerging evidence supports that both succinate and fumarate contribute to immunosuppressive polarization and T cell exhaustion, thereby making the tumor microenvironment more suitable for cell migration. Explicitly, succinate can upregulate tumor-associated macrophages (TAM) marker gene expression, such as Arg1, Fizz1, Mhl1, and Mgl2. The expression of succinate receptor 1 is also associated with immune inhibitory proteins, such as PD-L1, PD-1, and CTLA-4. Moreover, fumarate could downregulate neutrophils, T-cell, and B-cell responses, inhibit dendritic cell (DC) maturation, and motivate CTLA-4 and PD-L1 expression.

2.7.3 Lactate

To ensure adequate ATP supply, the malignant transformation is associated with an upregulated glycolysis (de Groof et al., 2009). Cancer cells upregulate glycolytic enzymes and metabolic transporters, which is connected with lactate overproduction. A new discovery considered lactate might have an effect on lysine residues of histone, acting in a similar way to acetylation and gene activation (Hou et al., 2019; Zhang et al., 2019). This phenomenon is based on the conversion of lactate to acetyl residues and thereby stimulates tumor angiogenesis. The accumulation of lactate also exerts an immunosuppressive effect on TME through inhibiting the differentiation and maturation of DC and T cell (Gottfried et al., 2006).

2.7.4 PHGDH, PRODH, and NNMT

Cancer-specific mutations of metabolic enzymes with implications in epigenetic regulation have been reported. Phosphoglycerate dehydrogenase (PHGDH) is overexpressed in breast cancer and melanoma (Locasale et al., 2011;

Possemato et al., 2011), directing the metabolism toward the serine biosynthesis pathway. Serine provides methyl donors to one-carbon metabolism, thereby affecting cellular epigenetics (Locasale, 2013). Conversely, PHGDH silence can downregulate serine synthesis leading to tumor growth suppression (Locasale et al., 2011; Possemato et al., 2011). Another example is proline dehydrogenase (PRODH) that catalyzes proline to produce pyrroline-5-carbonxylate (P5C), which is sequentially converted into glutamate and α-KG to affect epigenome (Phang et al., 2013). Studies showed amplification of PRODH in immunodeficient mice displayed tumor-suppressive characters (Liu et al., 2010). Nicotinamide N-methyltransferase (NNMT) also modulates epigenetic events in cancer cells. NNMT catalyzes the transfer of methyl group from SAM to nicotinamide. Overexpression of NNMT hampers SAM-dependent methylation of DNA and histone, along with the procurement of more invasive phenotype (Ulanovskaya et al., 2013).

As summarized, mutations in genes encoding metabolic enzymes have been recognized in caner, but they are rare. These lesions in genes related to metabolism constitute a new class of cancer-associated mutations that is able to subvert normal epigenetic regulation. It is tempting to speculate that these mutations provide the hope of identifying novel targets.

3 Epigenetic events contribute to altered metabolism in cancer

3.1 DNA methylation

A number of metabolic enzymes are altered attributing to DNA methylation. Examples of such enzymes involve Fructose-1,6-bisphosphastase (FBP-1), fructose-1,6-bisphosphatase (FBP-2), glucose transporter 1 (GLUT-1), Hexokinase (HK2), and pyruvate kinase isozyme 2 (PKM-2).

As reported, promoter hypermethylation leads to the silence of FBP-1 and FBP-2 in gastric, colon, liver, and breast cancers (Kamphorst et al., 2014; Gao et al., 2016). Both FBP-1 and FBP-2 are rate-limiting enzymes for gluconeogenesis that antagonize glycolysis. Theoretically, the silence of FBP-1 or FBP-2 contributes to glycolytic phenotype, supporting macromolecular biosynthesis and energy production. DNA methylation also mediates the gene overexpression of GLUT-1 that transports glucose from tumor microenvironment to cytoplasm (Lopez-Serra et al., 2014). Oppositely, promoter hypomethylation results in the upregulation of HK2 in glioblastoma and hepatic carcinoma (Chen et al., 2011; Wolf et al., 2011) and the overexpression of PKM2 in multiple cancer types (Desai et al., 2014).

In brief, increased HK2 and PKM-2 levels promote enhanced glycolysis, while the silence of FBP-1 and FBP-2 limit gluconeogenesis. DNA methylation contributes to a higher glycolytic influx, which is beneficial to the proliferation of tumor cells.

3.2 Histone modifications

Sirtuins (SIRTs), an enzyme catalyzing histone deacetylation, has been shown to function in cancer metabolism. Examples of epigenetic enzymes are SIRT6, SIRT7, and SIRT2.

3.2.1 SIRT6

NAD^+-dependent SIRT6 optimizes energy homeostasis by regulating histone acetylation (Xiao et al., 2010). SIRT6 could directly repress glycolysis in the HIF1 α-dependent way, thereby it acts as a tumor suppressor by inhibiting the Warburg effect (Zhong et al., 2010; Sebastián et al., 2012). Instead, SIRT6 knockdown shifts the cell metabolism towards a "glycolytic phenotype" inducing malignancy aggressiveness. Specific deletions in SIRT6 have been observed in colon, pancreatic, and hepatocellular cells (Zhang and Qin, 2014). Also, a growing body of evidence demonstrates that SIRT6 upregulates hepatic gluconeogenic gene expression and increases glycerol release from adipose tissue. These findings underline the potential to target SIRT6 for modulating cancer metabolism (Roichman et al., 2021).

3.2.2 SIRT7

SIRT7 could directly interacts with MYC that mediates the transcription of almost all the genes involved in glycolysis and glutaminolysis (Barber et al., 2012; Shin et al., 2013). SIRT7 selectively catalyzes H3K18 deacetylation that is a repressive mark (Wong et al., 2017). Hence, SIRT7 plays an opposite role in MYC-mediated metabolic reprogramming.

3.2.3 SIRT2

Compared to SIRT6/7, SIRT2 promotes cancer metabolism through stabilizing MYC (Liu et al., 2013). SIRT2 specifically deacetylases H4K16, resulting in decreased expression of ubiquitin-protein ligase NEDD4. NEDD4 serves as a negative regulator of MYC through ubiquitination and degradation (Wong et al., 2017). Consequently, SIRT2 facilitates MYC-dependent transcription and oncogenesis.

4 Novel cancer therapy targeting metabolism-epigenetic crosstalk

4.1 Novel targets for cancer metabolism

Targeting metabolic enzymes might be novel strategy for cancer therapy. LDH-A, a metabolic enzyme responsible for the conversion of pyruvate to lactate, was recognized as the

first metabolic target of the oncogene MYC (Shim et al., 1997). Appealing evidence manifested genetic or pharmacologic ablation of LDH-A is able to dwindle MYC-driven tumors in the xenograft models (Fantin et al., 2006; Le et al., 2010). Inhibition of LDH-A could delay the progression of myeloid leukemia (Wang et al., 2014) and diminish NSCLC without systemic toxicity in genetically engineered mouse models (Xie et al., 2014). Hence, LDH-A is a promising target in MYC-mutant tumors. Another attractive target is the glycolytic protein Hexokinase (HK2). Many tumors express high levels of HK2. Specific inhibition of HK2 delays tumor progression in mouse models of NSCLC and breast cancer (Patra et al., 2013). Targeting HK2 might be efficacious in highly glycolytic tumors. Besides, PHGDH, an enzyme that functions in the *de novo* serine synthesis, is found to overexpress in human melanoma and breast cancers (Locasale et al., 2011; Possemato et al., 2011). Targeting PHGDH in the one-carbon metabolism has been shown to delay tumor progression, though more studies are needed to confirm it. Additionally, the concept of oncometabolite opened a new window for targeted therapy. Small molecules targeting IDH1/IDH2 demonstrate positive outcomes in ongoing clinical trials (Yen et al., 2017). Taken together, targeting metabolic enzyme holds great promise in the treatment of malignancy (Olivares et al., 2015).

Targeting metabolism pathways, such as glycolysis, glutamine metabolism, mitochondrial metabolism, and autophagy, provides new opportunities for drug discovery scheme. In the certain context, metabolites produced from these metabolic pathways are able to affect epigenome. For example, metformin, an anti-diabetic drug, has been spotlighted on mitochondrial-mediated metabolic activity emerging as a key target for cancer therapy (Weinberg and Chandel, 2015). Because diabetic patients treated with metformin not only control their blood glucose level but also improve survival rate if cancer was diagnosed already (Evans et al., 2005). Biguanide phenformin also displayed anti-tumor effect by inhibiting mitochondrial complex I (Birsoy et al., 2014). Another example is BPTES [bis-2-(5-phenylacetamido-1, 2, 4-thiadiazol-2-yl) ethyl sulfide], one inhibitor of glutaminase activity, is being explored for anti-cancer characteristics (Xiang et al., 2015). Autography offers amino acids that fuel TCA cycle. Autography inhibition is confirmed to decrease tumor progression without significant toxicity in the mouse models of NSCLC and pancreatic cancers (Son et al., 2013; Karsli-Uzunbas et al., 2014). An alternative approach is to target acetate metabolism. As discussed above, mitochondria conventionally provide acetyl-CoA to the normal cells, whereas cancer cells also utilize acetate to support cell survival under hypoxia or nutrient deprivation (Schug et al., 2015). ACCS2, a cytosolic enzyme that converts acetate to acetyl-CoA, is dispensable for acetate metabolism and holds great promise for cancer therapy. In models of hepatocellular carcinoma, genetic loss of ACSS2 is likely to reduce tumor burden (Comerford et al., 2014). Human glioblastoma is sensitive to inhibitors of ACSS2 as well (Mashimo et al., 2014).

4.2 Reversal of epigenetic dysfunction by targeting metabolism

Over the past decades, a few studies represent how advances of metabolic effects on epigenetics can be translated into potential therapies. One strategy is to reverse epigenetic dysfunction by targeting cancer metabolism (Table 4).

Glycolysis inhibitors could reverse global histone hyperacetylation. 2-Deoxyglucose (2-DG), a glucose analog, is a rate-limiting enzyme for glycolysis. The use of 2-DG inhibits acetyl-CoA levels, which rationally promotes histone deacetylation in multiple cancer cell lines. Hence, glycolysis inhibition represents a candidate target for regulating histone acetylation. Glutaminolysis produces α–KG and acetyl-CoA. Glutaminase (GLS) is an extensively investigated target. Relevant inhibitors include CB-839, compound 968, and BPTEs. For example, compound-968 suppresses histone H3K4me3 in breast cancer and Zaprinast decreases H3K9Me3 in IDH-mutant cancer cells. The utility of GLS inhibitors could restore epigenetic dysfunction, particularly in IDH 1/2-mutant tumors. In addition, IDH 1/2 inhibitors specifically reduce the production of 2-HG that is an oncometabolite in IDH 1/2-mutant cells. For instance, AG-221 and AGI-6780 treatment result in demethylation status of DNA and histone in IDH 2-mutant tumors; AGI-5198 prompts demethylation of H3K9me3 and H3K27me3 in chondrosarcoma cells; GSK-321 causes DNA hypomethylation in AML cells. NNMT inhibitors lead to reduced SAM levels, which in turn downregulate histone methylation. The summarized concepts are illustrated in Table 4.

4.3 Reversal of metabolism rewiring by targeting epigenetics

Instead, using epigenetic drugs could modulate metabolism rewiring as well (Table 5).

There are two kinds of DNMT inhibitors therapeutically targeting DNA methylation, respectively named 5-azacytidine and 5-aza-2′-deoxycytidine. Both of them have been approved by FDA to treat myelodysplastic syndrome (MDS). IDH 1/2-mutant tumors carrying DNA hypermethylation show a high sensitivity to DNMT inhibitor. In IDH 1-mutant glioma models, both of 5-azacytidine and 5-aza-2′-deoxycytidine induced tumor regression. When inducing the differentiation of IDH-mutant

TABLE 4 Reversal of epigenetic dysfunction by targeting metabolism.

Target pathway	Metabolic enzyme	Pharmacological agents	Mechanism	Indications	References
Glycolysis	Hexokinases	2-DG (phase-I/II)	2-DG suppresses hexokinase that is a rate-limiting enzyme for glycolysis; 2-DG reduces acetyl-CoA level, which inhibits the acetylation of histones in various cancer cell lines	lung cancer, breast cancer, pancreatic cancer, prostate cancer, lymphoma	Chen and Guéron, (1992); Liu et al. (2015)
Glutaminolysis	Glutaminase (GLS)	CB-839 (phase-I); Compound-968; Zaprinast	GLS inhibitors reduce acetyl-CoA and 2-HG level; Compound-968 decreases histone H3K4me3 in breast cancer and Zaprinast reduces H3K9me3 in IDH1-mutant cancer cells	AML, ALL, MM, NHL, pancreatic carcinoma	Robinson et al. (2007); Wang et al. (2010a); Simpson et al. (2012a); Simpson et al. (2012b); Elhammali et al. (2014)
Serine/glycine metabolism	PHGDH	shRNA to PHGDH	Inhibiting the process of *de novo* serine synthesis	NA	Locasale et al. (2011); Possemato et al. (2011)
One-carbon cycle	SAH hydrolase	DZNep; Adenosine Dialdehyde	Both agents could increase the SAH/SAM ratio and decrease DNA and histone methylation	NA	Jiang et al. (2008); Miranda et al. (2009); Momparler et al. (2012); Schäfer and Balleyguier, (2013); Momparler and Côté, (2015)
IDH1 inhibitor	IDH1-mutant	AG-120, IDH305, AG-881, BAY1436032, FT-2102, AGI-5198, GSK-321	IDH1 inhibitors suppress the production of 2-HG that is a kind of oncometabolite in IDH1-mutant cells; AGI-5198 prompts demethylation of H3K9me3 and H3K27me3 in IDH1-mutant chondrosarcoma cells; GSK-321 induces DNA hypomethylation in IDH1-mutant AML cells	AML, solid tumors, gliomas, hematologic malignancies	Rohle et al. (2013); Zheng et al. (2013a); Davis et al. (2014); Deng et al. (2015); Kim et al. (2015); Li et al. (2015); Okoye-Okafor et al. (2015)
IDH2 inhibitor	IDH2-mutant	AG-221, AG-881, AGI-6780	IDH2 inhibitors suppress the production of 2-HG that is a kind of oncometabolite in IDH2-mutant cells; AG-221 and AGI-6780 prompt demethylation of DNA and histone in IDH2-mutant cancer cells	AML, solid tumors, gliomas, hematologic malignancies	Wang et al. (2013); Kernytsky et al. (2015)
NNMT inhibitor	N-Methylnicotinamide	Nicotinamide N-methyltransferase (NNMT)	NNMT inhibitors reduce SAM level and histone methylation in NNMT-overexpressed cells	NA	Kraus et al. (2014)

2-DG, 2-Deoxyglucose; GLS, glutaminase; AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; MM, multiple myeloma; NHL, Non-Hodgkin Lymphoma; NA, not applicable.

glioma cells, 5-aza-2′-deoxycytidine displayed a more potent efficacy than IDH inhibitors. Therefore, targeting epigenetics is a complementary approach to modulate the effect of oncometabolites in tumor. HDAC inhibitors could induce histone acetylation and reverse gene silence caused by HDACs. Growing evidence suggests HDAC inhibitors significantly suppressed glycolysis in various cancer types, such as lung cancer, breast cancer, and multiple myeloma. These findings manifest that inhibition of HDAC might reverse glycolytic phenotype. The modulation of SIRT activator and inhibitor holds promise as their regulatory roles in metabolism reprogramming. MiRNA-based therapeutics, such as miRNA-143, also inhibit glycolysis by targeting hexokinase-II 3′-UTR. More examples are summarized in Table 5.

4.4 Combination therapy of metabolism and epigenetics

Advancements in the area of cancer drug discovery have spotlighted on the inhibitors of metabolic pathways and cancer epigenetics. However, the efficacy of epigenetic inhibitors alone is not satisfactory, and this approach is usually prone to drug resistance (Zhang et al., 2020). Also, cancer cell could be drug-resistant to suppression of a particular metabolic pathway by upregulating compensatory pathways or expressing alternative isoforms. Further, inhibitions of metabolic enzymes might produce systemic toxicity owing to their physiological role in normal cells (Pearce et al., 2013; Ito and Suda, 2014; Erez and DeBerardinis, 2015). To achieve the

TABLE 5 Reversal of metabolism reprogramming by targeting epigenetics.

Inhibitors	Target enzyme	Pharmacological agents	Mechanism	Indication	References
DNMT inhibitor	DNA methyltransferases	Azacitidine (approved) Decitabine (approved) Guadecitabine (phase-III)	Non-selective inactivating DNMT1, DNMT3A, and DNMT3B; Reversing the hypermethylation status in IDH1-mutant glioma cells	MDS, AML	Borodovsky et al. (2013); Turcan et al. (2013)
KDM inhibitor	LSD1 (Lysine demethylase)	ORY-1001 (phase-I) GSK2879552 (phase-I)	Inhibiting histone demethylation	AML, SCLC, MDS	NCT02913443 NCT02177812 NCT02034123
HDAC inhibitor	Histone deacetylases	Romidepsin (approved) Vorinostat (approved) Panobinstat (approved) Belinostat (approved)	Prompting histone acetylation; Reducing glucose uptake, glycolytic flux, and lactate metabolism	T-cell Lymphoma, MM	Wardell et al. (2009); Alcarraz-Vizán et al. (2010); Amoêdo et al. (2011); Rodrigues et al. (2015)
SIRT activator and inhibitor	SIRT6 (Histone deacetylases)	Linoleic acid Myristic acid Oleic acid	Activating or inhibiting histone deacetylation; Free fatty acid activates SIRT6 that inhibits glycolysis	Unknown	Feldman et al. (2013)
miRNA modulator	miRNAs	miRNA mimics miRNA sponges antisense oligonucleotides	miRNA reversed silenced miRNA function; miRNA-143 could inhibit glycolysis by targeting hexokinase-II 3′-UTR; Anti-miRNA-21 could restore PTEN expression	Unknown	Meng et al. (2007); Gregersen et al. (2012)

DNMT, DNA, methyltransferase; KDM, lysine demethylase; HDAC, histone deacetylase; SIRT, sirtuin; miRNA, microRNA; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; SCLC, small cell lung cancer; MM, multiple myeloma; 3′-UTR, 3′-untranslated region.

purpose of less toxicity and potent efficiency, a rational strategy is to develop multiple drug combinations.

As an epigenetic regulator, enhancer of zeste homology (EZH2) inhibits gene transcription by trimethylation of histone H3K27 in cancer cells. Mounting evidence has suggested that EZH2 participated in the alteration of metabolic profiles in cancer through diverse pathways, covering glucose, lipid, amino acid metabolism. Meanwhile, metabolic activities also affect the stability and methyltransferase activity of EZH2, as some metabolites offer the donors for EZH2 post-translational modifications (Zhang et al., 2020). As a promising target, EZH2 inhibitors have been investigated in preclinical trials, but the effectiveness of EZH2 inhibitors alone is not satisfactory (De Raedt et al., 2011; Baude et al., 2014; Huang X. et al., 2018). Recently, researchers have found EZH2 inhibitor is able to weaken drug resistance caused by metabolic activities in tumor. Solid tumor is subject to hypoxia and glutamine deficiency because of the underdeveloped vascular system. Hypoxia induces a metabolic switch from oxidative to glycolytic metabolism, promoting the dedifferentiation of tumor cells and inducing resistance to radio- and chemotherapy. However, EZH2 inhibitors could directly block H3K27 methylation and consequently activate the transcription of pro-differentiation genes. Also, metabolic pathway is likely to downregulate EZH2 activity and thereby acts synergistically with EZH2 inhibitors (Zhang et al., 2020). More specifically, AMPK is activated in response to energy stress (glucose deficiency) and phosphorylates EZH2 (Cha et al., 2005). AKT-mediated phosphorylation of EZH2 suppresses trimethylation of lysine 27 in histone H3, facilitating the transcription of target genes to suppress tumor growth (Cha et al., 2005; Priebe et al., 2011; Gao et al., 2014; Kim and Yeom, 2018). Therefore, a combination of EZH2 inhibitors with metabolic regulators is a novel strategy to rescue the poor effectiveness of EZH2 inhibitor alone (Zhang et al., 2020). Briefly, epigenetic and metabolic alterations mediated by EZH2 are highly interlaced, demonstrating a synergistic effect in treating malignancy.

A model whereby linked metabolic-epigenetic programs reflects a new idea to target such an integrated axis. A study (McDonald et al., 2017) on the evolution of pancreatic ductal adenocarcinoma (PDAC) introduced an epigenetic mechanism that links glucose metabolism to distant metastasis. Remarkably, oxidative branch of the Pentose Phosphate Pathway (ox-PPP)

TABLE 6 Ongoing clinical trials of combined anti-epigenetic drugs and anti-metabolism drugs.

Identifier	Start year	Combination therapy		Conditions	Phase	Enrollment
		Anti-epigenetics drug	Anti-metabolism drug			
NCT02719574	2016	Azacitidine	FT-2102	AML/MDS	I/II	336
NCT02677922	2016	Azacitidine	AG-120	AML	I/II	131
NCT03173248	2017	Azacitidine	AG-120	AML	III	148
NCT03471260	2018	Azacitidine	AG-120	Hematologic malignancies	I/II	30
NCT03683433	2018	Azacitidine	AG-221	AML	II	50
NCT03684811	2018	Azacitidine	FT-2102	Solid tumors and gliomas	I/II	200
NCT04774393	2021	Decitabine	AG-120/AG-221	AML	I/II	84

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; DNMT, inhibitors: Azacitidine; Decitabine. IDH, inhibitors: AG-120 (Ivosidenib); AG-221 (Enasidenib); FT-2102.

was a driving force for epigenetic programming (histone H3K9 and DNA methylation) that enhanced tumorigenic fitness during the distant metastasis. Hence, targeting ox-PPP to reverse malignant epigenetic programs could be effective in metastatic PDAC. Another best-studied example is the use of AMPK activator metformin, which decreased EZHIP protein concentrations, elevated H3K27me3, inhibited TCA cycle, and suppressed tumor growth. Consequently, targeting integrated epigenetic-metabolic pathway shows hopeful therapeutic efficacy in mice models transplanted with PFA ependymomas (Panwalkar et al., 2021).

Oncogenic signal pathways also play important roles in novel combination therapy. A distinct work on melanoma demonstrated that reduced α-KG levels result in histone hypermethylation and develop the resistance to BRAF inhibitors. The combination of histone methyltransferase and BRAF inhibitors was sufficient to overcome resistance (Pan et al., 2016). Also, liver kinase B1 (LKB1)-deficiency tumors carrying KRAS activation would accompany with SAM production, leading to more potent methyltransferase activity and increased DNA methylation levels (Kottakis et al., 2016). Combined inhibition of DNA methyltransferase and serine metabolism could attack LKB-loss tumors with KRAS-positive more aggressively.

Taken together, our understanding in targeting both altered metabolism and epigenetics remains at a very early stage. Whether these two hallmarks exert synergistic functions in tumor is less explored, though there are a few well-elaborated agents in ongoing clinical trials (Table 6).

5 Epigenetic, metabolic, and immune crosstalk

5.1 Principles linking cancer metabolism, epigenetics, and immunity

In the traditional viewpoint, immunological memory is a unique feature of the adaptive immune system (Netea et al., 2020a). However, "Trained immunity" is a relatively new term that refers to myeloid cells from the innate immune system also display memory capacity after pathogen exposure (Dominguez-Andres and Netea, 2019; Netea et al., 2020b; O'Neill and Netea, 2020). After the first stimuli, innate immune cells, such as macrophage and monocyte, are epigenetically programmed (Fanucchi et al., 2021). These epigenetic modifications unfold chromatin and expose promoter and enhancer regions controlling immune-associated genes, enabling them accessible to transcription factors (Klemm et al., 2019) and permitting cells to maintain a "trained" state after rechallenge (Saeed et al., 2014). Specifically, H3K4me3 frequently occurs on gene promoters; H3K4me1 and H3K27Ac accumulates on enhancers (Quintin et al., 2012; Novakovic et al., 2016). As such, upon the secondary stimulus, immune genes are more robustly transcribed (Fanucchi et al., 2021).

In addition, some metabolites act as substrates or co-factors for epigenetic enzymes, which alter chromatin state to cause transcriptional changes that are causal to trained immunity (Fanucchi et al., 2021). For example, acetyl-CoA mediates histone acetylation following immune stimuli (Wellen et al., 2009; Christ and Latz, 2019), while SAM level regulates DNA and histone methylation to control trained immunity (Mentch et al., 2015; Ji et al., 2019). On the contrary, NAD^+ assist histone deacetylation to block trained immunity (Yeung et al., 2004; Zhong et al., 2010; Lo Sasso et al., 2014; Jia et al., 2018). α-KG-derived metabolites reduce histone demethylation by competing with α-KG-dependent KDM5 histone demethylase (Sowter et al., 2003; Cheng et al., 2014). Explicitly, human monocytes exposed to β-glucan will have higher concentrations of α-KG-derived metabolites and lower activity of KDM5 demethylases, which is associated with less H3K4me3 demethylation and higher gene expression (Fanucchi et al., 2021). Overall, the induction, maintenance, and regulation of "trained immunity" is based on the complex interplay between epigenetics and metabolism.

TABLE 7 Ongoing clinical trials of combined anti-epigenetic drugs and immune checkpoint inhibitors.

Identifier	Start year	Combination therapy		Conditions	Phase	Enrollment
		DNMT inhibitors	Checkpoint inhibitor			
NCT02608437	2015	Guadecitabine	Ipilimumab	Metastatic melanoma	I	19
NCT02530463	2015	Azacitidine	Ipilimumab/Nivolumab	MDS/Leukemia	II	160
NCT02957968	2016	Decitabine	Pembrolizumab	Breast cancer	II	32
NCT02890329	2016	Decitabine	Ipilimumab	MDS/AML	I	48
NCT02664181	2017	Decitabine	Nivolumab	NSCLC	II	13
NCT03094637	2017	Azacitidine	Pembrolizumab	High-risk MDS	II	37
NCT03264404	2017	Azacitidine	Pembrolizumab	Pancreas cancer	II	31
NCT03019003	2017	Azacitidine	Durvalumab	Head and neck cancer	I/II	13
NCT03308396	2017	Guadecitabine	Durvalumab	Kidney cancer	Ib/II	57
NCT04510610	2019	Decitabine	Camrelizumab	Hodgkin lymphoma	II/III	100
NCT04353479	2020	Decitabine	Camrelizumab	AML	II	29
Identifier	**Start Year**	**Combination Therapy**		**Conditions**	**Phase**	**Enrollment**
		HDAC Inhibitors	**Checkpoint Inhibitor**			
NCT02616965	2015	Romidepsin	Brentuximab vedotin	T-cell lymphoma	I	27
NCT03024437	2017	Entinostat	Atezolizumab	Renal cancer	I/II	72
NCT03848754	2019	Pracinostat	Gemtuzumab ozogamicin	AML	I	14
NCT03903458	2019	Tinostamustine	Nivolumab	Advanced melanoma	IB	21
NCT03820596	2019	Chidamide	Sintilimab	NK/T-cell lymphoma	I/II	50
NCT04651127	2020	Chidamide	Toripalimab	Cervical cancer	I/II	40
NCT04562311	2020	Chidamide	Tislelizumab	Bladder cancer	II	43
Identifier	**Start Year**	**Combination Therapy**		**Conditions**	**Phase**	**Enrollment**
		KMT6A Inhibitor	**Checkpoint Inhibitor**			
NCT03525795	2018	CPI-1205	Ipilimumab	Advanced solid tumor	I	24
NCT03854474	2019	Tazemetostat	Pembrolizumab	Bladder cancer	I/II	30
Identifier	**Start Year**	**Combination Therapy**		**Conditions**	**Phase**	**Enrollment**
		KDM1A inhibitor	**Checkpoint Inhibitor**			
NCT02712905	2016	INCB059872	Nivolumab	Hematologic tumor	I/II	116
NCT02959437	2017	INCB059872	Pembrolizumab	Hematologic tumor	I/II	70

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; NSCLC, non-small cell lung cancer.

Apart from trained immunity, the crosstalk of metabolism and epigenetics has been reported in T cell (Bailis et al., 2019) and macrophage activation (Liu et al., 2017). A recent study has shown that both mitochondrial citrate export and malate-aspartate shuttle favor histone acetylation and influence the expression of specific genes involved in T cell activation (Bailis et al., 2019). Also, a research figured out α-KG produced from glutamine metabolism orchestrates M2 macrophage activation by Jmjd3-dependent epigenetic remodeling (Liu et al., 2017). Specifically, H3K27me3 is a repressive epigenetic marker that downregulates the expression of M2 macrophage marker genes (Ishii et al., 2009). It is notable Jmjd3 is a crucial enzyme for demethylation of H3K27 (Satoh et al., 2010). α-KG derived from glutamine metabolism could facilitate epigenetic changes in a Jmjd3-dependent demethylation of H3K27 on the promoters of M2-specific marker genes (Bailis et al., 2019). This result indicates α-KG and Jmjd3 synergistically

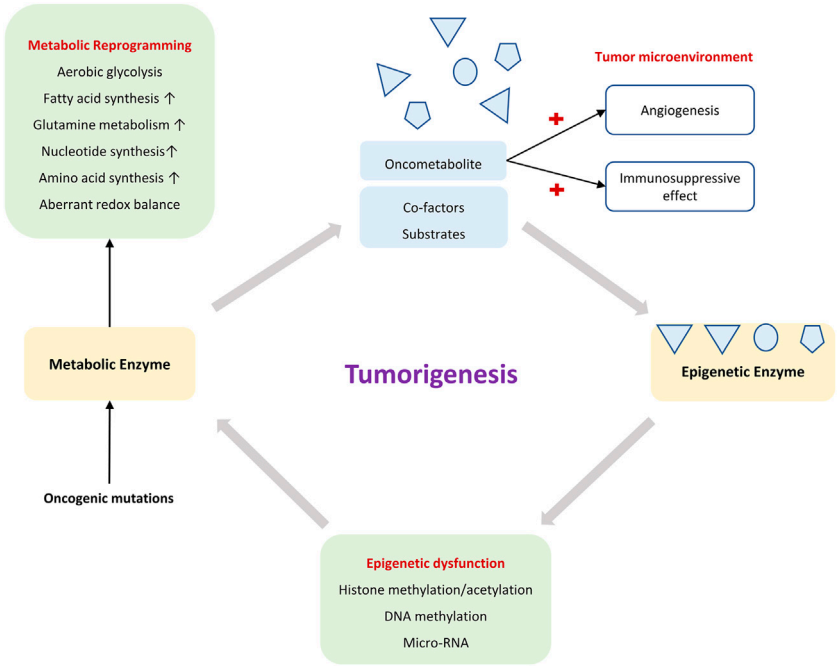


FIGURE 3
The crosstalk between metabolism and epigenetics in tumorigenesis.

promotes macrophage activation. Consequently, an attractive strategy is to modulate glutamine metabolism to harness macrophage-mediated immune responses.

5.2 Rational for novel immunotherapy-based combinations

Cancer immunotherapy is rapidly developing in various research settings, including CAR-T cell therapy, immune checkpoint inhibitors, and adoptive transfer of tumor infiltrating lymphocytes (Rosenberg et al., 1988; Zhao et al., 2005; Robbins et al., 2011; Rosenberg et al., 2011; Rosenberg, 2012; Topalian et al., 2012; Maude et al., 2014). An innovative strategy is the combination of immunotherapy with either epigenetic inhibitors or metabolic inhibitors, or a triple combination of them.

Epigenetics and immunology are both fast-developing fields in cancer biology. Recent evidence provides unique opportunities to combine epigenetics-based drugs with immunotherapy (Zhang et al., 2020). Epigenetic-based drugs include four pan-HDAC inhibitors and two DNMT inhibitors approved by FDA before 2020 (Knutson et al., 2012; Yu et al., 2017). These agents are able to change the immunosuppressive tumor microenvironment and increased tumor-infiltrating lymphocytes (Yanagida et al., 2001; Wang L. et al., 2010; Li et al., 2013; Anwar et al., 2018), leading to enhanced tumor-associated antigen presentation, activation of DC cells, suppression of T cell exhaustion. Similar changes in TME are also observed in tumor tissues treated with other agents, such as inhibitors of KMT6A (EZH2), KDM1A (LSD1), PRMT5, and BET proteins (Hemmings and Restuccia, 2012; Kikuchi et al., 2015; Garcia and Shaw, 2017; Herzig and Shaw, 2018; Hoxhaj and Manning, 2020). Consequently, given that epigenetic drugs boosting antitumor immune response, immune checkpoint blockade therapies (ICBTs) and epigenetic-based inhibitors exert synergistic functions to sensitize less-immunogenic tumors and prevent both primary and acquired resistance (Zhang et al., 2020). There are numerous ongoing clinical trials summarized in Table 7.

Metabolism can be modulated *in vivo* to govern anti-tumor T cell longevity and functionality, which determines the efficacy of immunotherapy (Chang and Pearce, 2016; O'Neill et al., 2016). The modulation of T cell metabolism is a promising strategy to enhance or suppress immune response (O'Sullivan and Pearce, 2015), as the characteristics of T cells are critical to determine clinical outcomes (Klebanoff et al., 2012). Several advances have been made in preclinical models. For example, when treating vascularized melanoma, limiting the ability of T cells engaged in glycolysis through suppression of hexokinase by 2-DG could ultimately leads to enhanced anti-tumor efficacy (Sukumar et al., 2013). Additionally, metabolic reprogramming occurs in other immune cells within tumor microenvironment, such as

macrophages and dendritic cells (DCs). One research (Yan et al., 2021) put forward strategies to enhance cancer immunotherapy by manipulating metabolism reprogramming. For example, CB-839 is a glutaminase inhibitor that has been explored in numerous clinical trials with or without the combinations of immunotherapy (Cerezo and Rocchi, 2020). Acetyl-CoA acetyltransferase 1 (ACAT1) inhibitors could enhance the activity of $CD8^+$ T cells and reduce the inflammatory response. Hence, ACAT1 might be a potential target to optimize immunotherapy (Yang et al., 2016; Huang L. H. et al., 2018; Bi et al., 2019). Indoleamine 2,3-dioxygenase (IDO) is responsible for the conversion of tryptophan to kynurenine in tumors. Blocking IDO can decrease Treg cells and preserve the functionality of T cells. Combination of IDO inhibitors (epacadostat) and immune checkpoint inhibitor (pembrolizumab) has been shown safe enough in clinical trials, though its efficacy needs further investigation (Prendergast et al., 2017; Komiya and Huang, 2018; Long et al., 2019). In summary, glutamine, acetyl-CoA acetyltransferase 1 (ATAC1), indoleamine 2,3-dioxygenase (IDO), lactate, and Toll-like receptors (TLRs) are likely to be considered as novel "metabolic checkpoints", targeting of which could assist immune cells to achieve better anti-tumor effect.

Noteworthily, epigenetic, metabolism, and immune crosslink in germinal-cancer-derived B-cell lymphomas (GCB) uncover a rational triple combination therapy (Serganova et al., 2021). GCB lymphoma is significantly heterogenous based on genetic, epigenetic, and clinical characteristics. Epigenetic dysfunction, such as gain-of-function mutations of EZH2 and loss-of-function mutations of CREBP and EP300, disrupts the normal biological link between lymphoma cells and immune TME, and motivates immune evasion in GCB lymphoma. Also, lymphoma metabolism adaptions might aggravate immunosuppression, leading to poorly infiltrated effector T-cell. Considering the impacts of cancer metabolism on epigenetic modifier and immune microenvironment, triple combination therapy is a logic and feasible strategy for future treatment.

6 Perspectives

As reviewed, epigenetics and metabolism are highly interconnected in a reciprocal manner (Figure 3). Such a relationship is accentuated by the reversibility of both processes (Henikoff and Matzke, 1997). A major goal in exploring metabolism-dependent epigenetic modifications is the hope of identifying novel targets for cancer therapy. However, some aspects pertaining to metabolic-epigenetic axis in cancers remain poorly understood.

Firstly, tumor heterogeneity is a major challenge that limits our understanding (Hensley et al., 2016). Inconsistent metabolic phenotypes were observed in various tumor tissues. Hence, tumor heterogeneity allows cancer cells to escape the deleterious attacks of inhibitors (Thakur and Chen, 2019). Secondly, the downstream factors mediating the tumorigenic activity of oncometabolites remains largely unknown. Thirdly, enzymatic parameters, such as K_m, V_{max}, and allosteric and inhibitory binding constants, constitute the basic element of the biochemistry (Reid et al., 2017). It is difficult to define physiological conditions in which the concentration dynamics of substrates and co-factors causally underlie an alteration of chromatin status. Discrepancies exist between artificial culture *in vitro* and physiological environment *in vivo* (Davidson et al., 2016). Another complexity is the precise input of metabolism into chromatin modifications, as both activation and suppression of histone marks need metabolites. For instance, how to predict the changes of SAM level establish the overall chromatin state and epigenetic phenotype. Additionally, though a bunch of metabolic enzymes function in nucleus have been identified, their individual contribution to epigenetic alterations was less defined. Robust experimental methods are needed to obtain accurate measurements of metabolites in specific cellular domain. Despite much interest in targeting both metabolism and epigenetics, poorly understood layers that whether these two hallmarks confer dependencies in tumors synergistically still exist.

In-depth connection between oncogenic signaling, metabolism, epigenetics, and immunity in cancer would facilitates effective designing of novel targeted drugs, which is the premise of precision medicine. It is anticipated that multiple combination therapies hold opportunities to improve care of cancer patients. Nevertheless, several outstanding challenges will be the major goal of future study.

Author contributions

YQ designed the study and reviewed the manuscript. CC and ZW participated in the study design and wrote the original draft of the manuscript. CC was mainly responsible for the design of tables and figures. All authors agreed to the submission of the final manuscript.

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

Aghili, M., Zahedi, F., and Rafiee, E. (2009). Hydroxyglutaric aciduria and malignant brain tumor: A case report and literature review. *J. Neurooncol.* 91 (2), 233–236. doi:10.1007/s11060-008-9706-2

Ahn, C. S., and Metallo, C. M. (2015). Mitochondria as biosynthetic factories for cancer proliferation. *Cancer Metab.* 3 (1), 1. doi:10.1186/s40170-015-0128-2

Alcarraz-Vizán, G., Boren, J., Lee, W. N., and Cascante, M. (2010). Histone deacetylase inhibition results in a common metabolic profile associated with HT29 differentiation. *Metabolomics* 6 (2), 229–237. doi:10.1007/s11306-009-0192-0

Amary, M. F., Bacsi, K., Maggiani, F., Damato, S., Halai, D., Berisha, F., et al. (2011). IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. *J. Pathol.* 224 (3), 334–343. doi:10.1002/path.2913

Amoêdo, N. D., Rodrigues, M. F., Pezzuto, P., Galina, A., da Costa, R. M., de Almeida, F. C., et al. (2011). Energy metabolism in H460 lung cancer cells: Effects of histone deacetylase inhibitors. *PLoS One* 6 (7), e22264. doi:10.1371/journal.pone.0022264

Anwar, T., Arellano-Garcia, C., Ropa, J., Chen, Y. C., Kim, H. S., Yoon, E., et al. (2018). p38-mediated phosphorylation at T367 induces EZH2 cytoplasmic localization to promote breast cancer metastasis. *Nat. Commun.* 9 (1), 2801. doi:10.1038/s41467-018-05078-8

Baek, S. H. (2011). When signaling kinases meet histones and histone modifiers in the nucleus. *Mol. Cell.* 42 (3), 274–284. doi:10.1016/j.molcel.2011.03.022

Bailis, W., Shyer, J. A., Zhao, J., Canaveras, J. C. G., Al Khazal, F. J., Qu, R., et al. (2019). Distinct modes of mitochondrial metabolism uncouple T cell differentiation and function. *Nature* 571 (7765), 403–407. doi:10.1038/s41586-019-1311-3

Barber, M. F., Michishita-Kioi, E., Xi, Y., Tasselli, L., Kioi, M., Moqtaderi, Z., et al. (2012). SIRT7 links H3K18 deacetylation to maintenance of oncogenic transformation. *Nature* 487 (7405), 114–118. doi:10.1038/nature11043

Bardella, C., Pollard, P. J., and Tomlinson, I. (2011). SDH mutations in cancer. *Biochim. Biophys. Acta* 1807 (11), 1432–1443. doi:10.1016/j.bbabio.2011.07.003

Baude, A., Lindroth, A. M., and Plass, C. (2014). PRC2 loss amplifies Ras signaling in cancer. *Nat. Genet.* 46 (11), 1154–1155. doi:10.1038/ng.3124

Baysal, B. E., Ferrell, R. E., Willett-Brozick, J. E., Lawrence, E. C., Myssiorek, D., Bosch, A., et al. (2000). Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 287 (5454), 848–851. doi:10.1126/science.287.5454.848

Berger, S. L. (2007). The complex language of chromatin regulation during transcription. *Nature* 447 (7143), 407–412. doi:10.1038/nature05915

Bernstein, B. E., Meissner, A., and Lander, E. S. (2007). The mammalian epigenome. *Cell.* 128 (4), 669–681. doi:10.1016/j.cell.2007.01.033

Bhutani, N., Burns, D. M., and Blau, H. M. (2011). DNA demethylation dynamics. *Cell.* 146 (6), 866–872. doi:10.1016/j.cell.2011.08.042

Bi, M., Qiao, X., Zhang, H., Wu, H., Gao, Z., Zhou, H., et al. (2019). Effect of inhibiting ACAT-1 expression on the growth and metastasis of Lewis lung carcinoma. *Oncol. Lett.* 18 (2), 1548–1556. doi:10.3892/ol.2019.10427

Birsoy, K., Possemato, R., Lorbeer, F. K., Bayraktar, E. C., Thiru, P., Yucel, B., et al. (2014). Metabolic determinants of cancer cell sensitivity to glucose limitation and biguanides. *Nature* 508 (7494), 108–112. doi:10.1038/nature13110

Borger, D. R., Goyal, L., Yau, T., Poon, R. T., Ancukiewicz, M., Deshpande, V., et al. (2014). Circulating oncometabolite 2-hydroxyglutarate is a potential surrogate biomarker in patients with isocitrate dehydrogenase-mutant intrahepatic cholangiocarcinoma. *Clin. Cancer Res.* 20 (7), 1884–1890. doi:10.1158/1078-0432.Ccr-13-2649

Borodovsky, A., Salmasi, V., Turcan, S., Fabius, A. W., Baia, G. S., Eberhart, C. G., et al. (2013). 5-azacytidine reduces methylation, promotes differentiation and induces tumor regression in a patient-derived IDH1 mutant glioma xenograft. *Oncotarget* 4 (10), 1737–1747. doi:10.18632/oncotarget.1408

Bungard, D., Fuerth, B. J., Zeng, P. Y., Faubert, B., Maas, N. L., Viollet, B., et al. (2010). Signaling kinase AMPK activates stress-promoted transcription via histone H2B phosphorylation. *Science* 329 (5996), 1201–1205. doi:10.1126/science.1191241

Cai, L., Sutter, B. M., Li, B., and Tu, B. P. (2011). Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. *Mol. Cell.* 42 (4), 426–437. doi:10.1016/j.molcel.2011.05.004

Cairns, R. A., Harris, I. S., and Mak, T. W. (2011). Regulation of cancer cell metabolism. *Nat. Rev. Cancer* 11 (2), 85–95. doi:10.1038/nrc2981

Caldwell, S. A., Jackson, S. R., Shahriari, K. S., Lynch, T. P., Sethi, G., Walker, S., et al. (2010). Nutrient sensor O-GlcNAc transferase regulates breast cancer tumorigenesis through targeting of the oncogenic transcription factor FoxM1. *Oncogene* 29 (19), 2831–2842. doi:10.1038/onc.2010.41

Candido, E. P., Reeves, R., and Davie, J. R. (1978). Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell.* 14 (1), 105–113. doi:10.1016/0092-8674(78)90305-7

Cantor, J. R., and Sabatini, D. M. (2012). Cancer cell metabolism: One hallmark, many faces. *Cancer Discov.* 2 (10), 881–898. doi:10.1158/2159-8290.Cd-12-0345

Castro-Vega, L. J., Buffet, A., De Cubas, A. A., Cascón, A., Menara, M., Khalifa, E., et al. (2014). Germline mutations in FH confer predisposition to malignant pheochromocytomas and paragangliomas. *Hum. Mol. Genet.* 23 (9), 2440–2446. doi:10.1093/hmg/ddt639

Cerezo, M., and Rocchi, S. (2020). Cancer cell metabolic reprogramming: A keystone for the response to immunotherapy. *Cell. Death Dis.* 11 (11), 964. doi:10.1038/s41419-020-03175-5

Cha, T. L., Zhou, B. P., Xia, W., Wu, Y., Yang, C. C., Chen, C. T., et al. (2005). Akt-mediated phosphorylation of EZH2 suppresses methylation of lysine 27 in histone H3. *Science* 310 (5746), 306–310. doi:10.1126/science.1118947

Chang, C. H., and Pearce, E. L. (2016). Emerging concepts of T cell metabolism as a target of immunotherapy. *Nat. Immunol.* 17 (4), 364–368. doi:10.1038/ni.3415

Chen, M., Zhang, J., Li, N., Qian, Z., Zhu, M., Li, Q., et al. (2011). Promoter hypermethylation mediated downregulation of FBP1 in human hepatocellular carcinoma and colon cancer. *PLoS One* 6 (10), e25564. doi:10.1371/journal.pone.0025564

Chen, Q., Chen, Y., Bian, C., Fujiki, R., and Yu, X. (2013). TET2 promotes histone O-GlcNAcylation during gene transcription. *Nature* 493 (7433), 561–564. doi:10.1038/nature11742

Chen, R., Xu, M., Nagati, J., and Garcia, J. A. (2017). Coordinate regulation of stress signaling and epigenetic events by Acss2 and HIF-2 in cancer cells. *PLoS One* 12 (12), e0190241. doi:10.1371/journal.pone.0190241

Chen, W., and Guéron, M. (1992). The inhibition of bovine heart hexokinase by 2-deoxy-D-glucose-6-phosphate: Characterization by 31P NMR and metabolic implications. *Biochimie* 74 (9-10), 867–873. doi:10.1016/0300-9084(92)90070-u

Cheng, S. C., Quintin, J., Cramer, R. A., Shepardson, K. M., Saeed, S., Kumar, V., et al. (2014). mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* 345 (6204), 1250684. doi:10.1126/science.1250684

Chinnaiyan, P., Kensicki, E., Bloom, G., Prabhu, A., Sarcar, B., Kahali, S., et al. (2012). The metabolomic signature of malignant glioma reflects accelerated anabolic metabolism. *Cancer Res.* 72 (22), 5878–5888. doi:10.1158/0008-5472.Can-12-1572-t

Christ, A., and Latz, E. (2019). The Western lifestyle has lasting effects on metaflammation. *Nat. Rev. Immunol.* 19 (5), 267–268. doi:10.1038/s41577-019-0156-1

Chu, C. S., Lo, P. W., Yeh, Y. H., Hsu, P. H., Peng, S. H., Teng, Y. C., et al. (2014). O-GlcNAcylation regulates EZH2 protein stability and function. *Proc. Natl. Acad. Sci. U. S. A.* 111 (4), 1355–1360. doi:10.1073/pnas.1323226111

Colegio, O. R., Chu, N. Q., Szabo, A. L., Chu, T., Rhebergen, A. M., Jairam, V., et al. (2014). Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* 513 (7519), 559–563. doi:10.1038/nature13490

Colvin, H., Nishida, N., Konno, M., Haraguchi, N., Takahashi, H., Nishimura, J., et al. (2016). Oncometabolite D-2-hydroxyglurate directly induces epithelial-mesenchymal transition and is associated with distant metastasis in colorectal cancer. *Sci. Rep.* 6, 36289. doi:10.1038/srep36289

Comerford, S. A., Huang, Z., Du, X., Wang, Y., Cai, L., Witkiewicz, A. K., et al. (2014). Acetate dependence of tumors. *Cell.* 159 (7), 1591–1602. doi:10.1016/j.cell.2014.11.020

Dang, L., White, D. W., Gross, S., Bennett, B. D., Bittinger, M. A., Driggers, E. M., et al. (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 462 (7274), 739–744. doi:10.1038/nature08617

Davidson, S. M., Papagiannakopoulos, T., Olenchock, B. A., Heyman, J. E., Keibler, M. A., Luengo, A., et al. (2016). Environment impacts the metabolic dependencies of ras-driven non-small cell lung cancer. *Cell. Metab.* 23 (3), 517–528. doi:10.1016/j.cmet.2016.01.007

Davis, M. I., Gross, S., Shen, M., Straley, K. S., Pragani, R., Lea, W. A., et al. (2014). Biochemical, cellular, and biophysical characterization of a potent inhibitor of mutant isocitrate dehydrogenase IDH1. *J. Biol. Chem.* 289 (20), 13717–13725. doi:10.1074/jbc.M113.511030

de Groof, A. J., te Lindert, M. M., van Dommelen, M. M., Wu, M., Willemse, M., Smift, A. L., et al. (2009). Increased OXPHOS activity precedes rise in glycolytic rate in H-RasV12/E1A transformed fibroblasts that develop a Warburg phenotype. *Mol. Cancer* 8, 54. doi:10.1186/1476-4598-8-54

De Raedt, T., Walton, Z., Yecies, J. L., Li, D., Chen, Y., Malone, C. F., et al. (2011). Exploiting cancer cell vulnerabilities to develop a combination therapy for ras-driven tumors. *Cancer Cell.* 20 (3), 400–413. doi:10.1016/j.ccr.2011.08.014

DeBerardinis, R. J., and Chandel, N. S. (2016). Fundamentals of cancer metabolism. *Sci. Adv.* 2 (5), e1600200. doi:10.1126/sciadv.1600200

Deng, G., Shen, J., Yin, M., McManus, J., Mathieu, M., Gee, P., et al. (2015). Selective inhibition of mutant isocitrate dehydrogenase 1 (IDH1) via disruption of a metal binding network by an allosteric small molecule. *J. Biol. Chem.* 290 (2), 762–774. doi:10.1074/jbc.M114.608497

Desai, S., Ding, M., Wang, B., Lu, Z., Zhao, Q., Shaw, K., et al. (2014). Tissue-specific isoform switch and DNA hypomethylation of the pyruvate kinase PKM gene in human cancers. *Oncotarget* 5 (18), 8202–8210. doi:10.18632/oncotarget.1159

Dominguez-Andres, J., and Netea, M. G. (2019). Long-term reprogramming of the innate immune system. *J. Leukoc. Biol.* 105 (2), 329–338. doi:10.1002/jlb.Mr0318-104r

El-Kenawi, A., Gatenbee, C., Robertson-Tessi, M., Bravo, R., Dhillon, J., Balagurunathan, Y., et al. (2019). Acidity promotes tumour progression by altering macrophage phenotype in prostate cancer. *Br. J. Cancer* 121 (7), 556–566. doi:10.1038/s41416-019-0542-2

Elhammali, A., Ippolito, J. E., Collins, L., Crowley, J., Marasa, J., Piwnica-Worms, D., et al. (2014). A high-throughput fluorimetric assay for 2-hydroxyglutarate identifies Zaprinast as a glutaminase inhibitor. *Cancer Discov.* 4 (7), 828–839. doi:10.1158/2159-8290.Cd-13-0572

Erez, A., and DeBerardinis, R. J. (2015). Metabolic dysregulation in monogenic disorders and cancer - Finding method in madness. *Nat. Rev. Cancer* 15 (7), 440–448. doi:10.1038/nrc3949

Evans, J. M., Donnelly, L. A., Emslie-Smith, A. M., Alessi, D. R., and Morris, A. D. (2005). Metformin and reduced risk of cancer in diabetic patients. *Bmj* 330 (7503), 1304–1305. doi:10.1136/bmj.38415.708634.F7

Fang, R., Barbera, A. J., Xu, Y., Rutenberg, M., Leonor, T., Bi, Q., et al. (2010). Human LSD2/KDM1b/AOF1 regulates gene transcription by modulating intragenic H3K4me2 methylation. *Mol. Cell.* 39 (2), 222–233. doi:10.1016/j.molcel.2010.07.008

Fantin, V. R., St-Pierre, J., and Leder, P. (2006). Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell.* 9 (6), 425–434. doi:10.1016/j.ccr.2006.04.023

Fanucchi, S., Domínguez-Andrés, J., Joosten, L. A. B., Netea, M. G., and Mhlanga, M. M. (2021). The intersection of epigenetics and metabolism in trained immunity. *Immunity* 54 (1), 32–43. doi:10.1016/j.immuni.2020.10.011

Feldman, J. L., Baeza, J., and Denu, J. M. (2013). Activation of the protein deacetylase SIRT6 by long-chain fatty acids and widespread deacylation by mammalian sirtuins. *J. Biol. Chem.* 288 (43), 31350–31356. doi:10.1074/jbc.C113.511261

Fieuw, A., Kumps, C., Schramm, A., Pattyn, F., Menten, B., Antonacci, F., et al. (2012). Identification of a novel recurrent 1q42.2-1qter deletion in high risk MYCN single copy 11q deleted neuroblastomas. *Int. J. Cancer* 130 (11), 2599–2606. doi:10.1002/ijc.26317

Figueroa, M. E., Abdel-Wahab, O., Lu, C., Ward, P. S., Patel, J., Shih, A., et al. (2010). Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell.* 18 (6), 553–567. doi:10.1016/j.ccr.2010.11.015

Finkel, T., Deng, C. X., and Mostoslavsky, R. (2009). Recent progress in the biology and physiology of sirtuins. *Nature* 460 (7255), 587–591. doi:10.1038/nature08197

Friis, R. M., Wu, B. P., Reinke, S. N., Hockman, D. J., Sykes, B. D., Schultz, M. C., et al. (2009). A glycolytic burst drives glucose induction of global histone acetylation by picNuA4 and SAGA. *Nucleic Acids Res.* 37 (12), 3969–3980. doi:10.1093/nar/gkp270

Gao, M., Liang, J., Lu, Y., Guo, H., German, P., Bai, S., et al. (2014). Site-specific activation of AKT protects cells from death induced by glucose deprivation. *Oncogene* 33 (6), 745–755. doi:10.1038/onc.2013.2

Gao, X., Lin, S. H., Ren, F., Li, J. T., Chen, J. J., Yao, C. B., et al. (2016). Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. *Nat. Commun.* 7, 11960. doi:10.1038/ncomms11960

Garcia, D., and Shaw, R. J. (2017). Ampk: Mechanisms of cellular energy sensing and restoration of metabolic balance. *Mol. Cell.* 66 (6), 789–800. doi:10.1016/j.molcel.2017.05.032

Gottfried, E., Kunz-Schughart, L. A., Ebner, S., Mueller-Klieser, W., Hoves, S., Andreesen, R., et al. (2006). Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. *Blood* 107 (5), 2013–2021. doi:10.1182/blood-2005-05-1795

Gottlieb, E., and Tomlinson, I. P. (2005). Mitochondrial tumour suppressors: A genetic and biochemical update. *Nat. Rev. Cancer* 5 (11), 857–866. doi:10.1038/nrc1737

Gregersen, L. H., Jacobsen, A., Frankel, L. B., Wen, J., Krogh, A., Lund, A. H., et al. (2012). MicroRNA-143 down-regulates Hexokinase 2 in colon cancer cells. *BMC Cancer* 12, 232. doi:10.1186/1471-2407-12-232

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell.* 144 (5), 646–674. doi:10.1016/j.cell.2011.02.013

Hao, H. X., Khalimonchuk, O., Schraders, M., Dephoure, N., Bayley, J. P., Kunst, H., et al. (2009). SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science* 325 (5944), 1139–1142. doi:10.1126/science.1175689

Hardie, D. G. (2011). Adenosine monophosphate-activated protein kinase: A central regulator of metabolism with roles in diabetes, cancer, and viral infection. *Cold Spring Harb. Symp. Quant. Biol.* 76, 155–164. doi:10.1101/sqb.2011.76.010819

He, Y. F., Li, B. Z., Li, Z., Liu, P., Wang, Y., Tang, Q., et al. (2011). Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science* 333 (6047), 1303–1307. doi:10.1126/science.1210944

Hemmings, B. A., and Restuccia, D. F. (2012). PI3K-PKB/Akt pathway. *Cold Spring Harb. Perspect. Biol.* 4 (9), a011189. doi:10.1101/cshperspect.a011189

Henikoff, S., and Matzke, M. A. (1997). Exploring and explaining epigenetic effects. *Trends Genet.* 13 (8), 293–295. doi:10.1016/s0168-9525(97)01219-5

Hensley, C. T., Faubert, B., Yuan, Q., Lev-Cohain, N., Jin, E., Kim, J., et al. (2016). Metabolic heterogeneity in human lung tumors. *Cell.* 164 (4), 681–694. doi:10.1016/j.cell.2015.12.034

Herzig, S., and Shaw, R. J. (2018). Ampk: Guardian of metabolism and mitochondrial homeostasis. *Nat. Rev. Mol. Cell. Biol.* 19 (2), 121–135. doi:10.1038/nrm.2017.95

Hou, X. M., Yuan, S. Q., Zhao, D., Liu, X. J., and Wu, X. A. (2019). LDH-A promotes malignant behavior via activation of epithelial-to-mesenchymal transition in lung adenocarcinoma. *Biosci. Rep.* 39 (1), BSR20181476. doi:10.1042/bsr20181476

Hoxhaj, G., and Manning, B. D. (2020). The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. *Nat. Rev. Cancer* 20 (2), 74–88. doi:10.1038/s41568-019-0216-7

Huang, L. H., Melton, E. M., Li, H., Sohn, P., Jung, D., Tsai, C. Y., et al. (2018a). Myeloid-specific Acat1 ablation attenuates inflammatory responses in macrophages, improves insulin sensitivity, and suppresses diet-induced obesity. *Am. J. Physiol. Endocrinol. Metab.* 315 (3), E340-E356. doi:10.1152/ajpendo.00174.2017

Huang, X., Yan, J., Zhang, M., Wang, Y., Chen, Y., Fu, X., et al. (2018b). Targeting epigenetic crosstalk as a therapeutic strategy for EZH2-aberrant solid tumors. *Cell.* 175 (1), 186–199. e119. doi:10.1016/j.cell.2018.08.058

Imai, S., Armstrong, C. M., Kaeberlein, M., and Guarente, L. (2000). Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403 (6771), 795–800. doi:10.1038/35001622

Ishii, M., Wen, H., Corsa, C. A., Liu, T., Coelho, A. L., Allen, R. M., et al. (2009). Epigenetic regulation of the alternatively activated macrophage phenotype. *Blood* 114 (15), 3244–3254. doi:10.1182/blood-2009-04-217620

Itkonen, H. M., Minner, S., Guldvik, I. J., Sandmann, M. J., Tsourlakis, M. C., Berge, V., et al. (2013). O-GlcNAc transferase integrates metabolic pathways to regulate the stability of c-MYC in human prostate cancer cells. *Cancer Res.* 73 (16), 5277–5287. doi:10.1158/0008-5472.Can-13-0549

Ito, K., and Suda, T. (2014). Metabolic requirements for the maintenance of self-renewing stem cells. *Nat. Rev. Mol. Cell. Biol.* 15 (4), 243–256. doi:10.1038/nrm3772

Ito, R., Katsura, S., Shimada, H., Tsuchiya, H., Hada, M., Okumura, T., et al. (2014). TET3-OGT interaction increases the stability and the presence of OGT in chromatin. *Genes. cells.* 19 (1), 52–65. doi:10.1111/gtc.12107

Ito, S., Shen, L., Dai, Q., Wu, S. C., Collins, L. B., Swenberg, J. A., et al. (2011). Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science* 333 (6047), 1300–1303. doi:10.1126/science.1210597

Ji, J., Xu, Y., Zheng, M., Luo, C., Lei, H., Qu, H., et al. (2019). Methionine attenuates lipopolysaccharide-induced inflammatory responses via DNA methylation in macrophages. *ACS Omega* 4 (1), 2331–2336. doi:10.1021/acsomega.8b03571

Jia, Y., Li, Z., Cai, W., Xiao, D., Han, S., Han, F., et al. (2018). SIRT1 regulates inflammation response of macrophages in sepsis mediated by long noncoding RNA. *Biochim. Biophys. Acta. Mol. Basis Dis.* 1864 (3), 784–792. doi:10.1016/j.bbadis.2017.12.029

Jiang, S., and Yan, W. (2017). Succinate in the cancer-immune cycle. *Cancer Lett.* 390, 45–47. doi:10.1016/j.canlet.2017.01.019

Jiang, X., Tan, J., Li, J., Kivimäe, S., Yang, X., Zhuang, L., et al. (2008). DACT3 is an epigenetic regulator of Wnt/beta-catenin signaling in colorectal cancer and is a therapeutic target of histone modifications. *Cancer Cell.* 13 (6), 529–541. doi:10.1016/j.ccr.2008.04.019

Jin, L., Li, D., Alesi, G. N., Fan, J., Kang, H. B., Lu, Z., et al. (2015). Glutamate dehydrogenase 1 signals through antioxidant glutathione peroxidase 1 to regulate redox homeostasis and tumor growth. *Cancer Cell.* 27 (2), 257–270. doi:10.1016/j.ccell.2014.12.006

Kamphorst, J. J., Chung, M. K., Fan, J., and Rabinowitz, J. D. (2014). Quantitative analysis of acetyl-CoA production in hypoxic cancer cells reveals substantial contribution from acetate. *Cancer Metab.* 2, 23. doi:10.1186/2049-3002-2-23

Karsli-Uzunbas, G., Guo, J. Y., Price, S., Teng, X., Laddha, S. V., Khor, S., et al. (2014). Autophagy is required for glucose homeostasis and lung tumor maintenance. *Cancer Discov.* 4 (8), 914–927. doi:10.1158/2159-8290.Cd-14-0363

Katada, S., Imhof, A., and Sassone-Corsi, P. (2012). Connecting threads: Epigenetics and metabolism. *Cell.* 148 (1-2), 24–28. doi:10.1016/j.cell.2012.01.001

Kaye, S. B. (1998). New antimetabolites in cancer chemotherapy and their clinical impact. *Br. J. Cancer* 78 (Suppl. 3Suppl 3), 1–7. doi:10.1038/bjc.1998.747

Kernytsky, A., Wang, F., Hansen, E., Schalm, S., Straley, K., Gliser, C., et al. (2015). IDH2 mutation-induced histone and DNA hypermethylation is progressively reversed by small-molecule inhibition. *Blood* 125 (2), 296–303. doi:10.1182/blood-2013-10-533604

Kikuchi, J., Koyama, D., Wada, T., Izumi, T., Hofgaard, P. O., Bogen, B., et al. (2015). Phosphorylation-mediated EZH2 inactivation promotes drug resistance in multiple myeloma. *J. Clin. Investig.* 125 (12), 4375–4390. doi:10.1172/jci80325

Kim, H. J., Choi, B. Y., and Keum, Y. S. (2015). Identification of a new selective chemical inhibitor of mutant isocitrate dehydrogenase-1. *J. Cancer Prev.* 20 (1), 78–83. doi:10.15430/jcp.2015.20.1.78

Kim, J. A., and Yeom, Y. I. (2018). Metabolic signaling to epigenetic alterations in cancer. *Biomol. Ther.* 26 (1), 69–80. doi:10.4062/biomolther.2017.185

Kinch, L., Grishin, N. V., and Brugarolas, J. (2011). Succination of Keap1 and activation of Nrf2-dependent antioxidant pathways in FH-deficient papillary renal cell carcinoma type 2. *Cancer Cell.* 20 (4), 418–420. doi:10.1016/j.ccr.2011.10.005

Klebanoff, C. A., Gattinoni, L., and Restifo, N. P. (2012). Sorting through subsets: Which T-cell populations mediate highly effective adoptive immunotherapy? *J. Immunother.* 35 (9), 651–660. doi:10.1097/CJI.0b013e31827806e6

Klemm, S. L., Shipony, Z., and Greenleaf, W. J. (2019). Chromatin accessibility and the regulatory epigenome. *Nat. Rev. Genet.* 20 (4), 207–220. doi:10.1038/s41576-018-0089-8

Klose, R. J., Kallin, E. M., and Zhang, Y. (2006). JmjC-domain-containing proteins and histone demethylation. *Nat. Rev. Genet.* 7 (9), 715–727. doi:10.1038/nrg1945

Knutson, S. K., Wigle, T. J., Warholic, N. M., Sneeringer, C. J., Allain, C. J., Klaus, C. R., et al. (2012). A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nat. Chem. Biol.* 8 (11), 890–896. doi:10.1038/nchembio.1084

Komiya, T., and Huang, C. H. (2018). Updates in the clinical development of epacadostat and other indoleamine 2, 3-dioxygenase 1 inhibitors (Ido1) for human cancers. *Front. Oncol.* 8, 423. doi:10.3389/fonc.2018.00423

Koppenol, W. H., Bounds, P. L., and Dang, C. V. (2011). Otto Warburg's contributions to current concepts of cancer metabolism. *Nat. Rev. Cancer* 11 (5), 325–337. doi:10.1038/nrc3038

Kottakis, F., Nicolay, B. N., Roumane, A., Karnik, R., Gu, H., Nagle, J. M., et al. (2016). LKB1 loss links serine metabolism to DNA methylation and tumorigenesis. *Nature* 539 (7629), 390–395. doi:10.1038/nature20132

Kraus, D., Yang, Q., Kong, D., Banks, A. S., Zhang, L., Rodgers, J. T., et al. (2014). Nicotinamide N-methyltransferase knockdown protects against diet-induced obesity. *Nature* 508 (7495), 258–262. doi:10.1038/nature13198

Kurdistani, S. K. (2011). Histone modifications in cancer biology and prognosis. *Prog. Drug Res.* 67, 91–106. doi:10.1007/978-3-7643-8989-5_5

Le, A., Cooper, C. R., Gouw, A. M., Dinavahi, R., Maitra, A., Deck, L. M., et al. (2010). Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proc. Natl. Acad. Sci. U. S. A.* 107 (5), 2037–2042. doi:10.1073/pnas.0914433107

Lee, J. V., Carrer, A., Shah, S., Snyder, N. W., Wei, S., Venneti, S., et al. (2014). Akt-dependent metabolic reprogramming regulates tumor cell histone acetylation. *Cell. Metab.* 20 (2), 306–319. doi:10.1016/j.cmet.2014.06.004

Li, K., Liu, C., Zhou, B., Bi, L., Huang, H., Lin, T., et al. (2013). Role of EZH2 in the growth of prostate cancer stem cells isolated from LNCaP cells. *Int. J. Mol. Sci.* 14 (6), 11981–11993. doi:10.3390/ijms140611981

Li, L., Paz, A. C., Wilky, B. A., Johnson, B., Galoian, K., Rosenberg, A., et al. (2015). Treatment with a small molecule mutant IDH1 inhibitor suppresses tumorigenic activity and decreases production of the oncometabolite 2-hydroxyglutarate in human chondrosarcoma cells. *PLoS One* 10 (9), e0133813. doi:10.1371/journal.pone.0133813

Liu, P. S., Wang, H., Li, X., Chao, T., Teav, T., Christen, S., et al. (2017). α-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. *Nat. Immunol.* 18 (9), 985–994. doi:10.1038/ni.3796

Liu, P. Y., Xu, N., Malyukova, A., Scarlett, C. J., Sun, Y. T., Zhang, X. D., et al. (2013). The histone deacetylase SIRT2 stabilizes Myc oncoproteins. *Cell. Death Differ.* 20 (3), 503–514. doi:10.1038/cdd.2012.147

Liu, W., Zabirnyk, O., Wang, H., Shiao, Y. H., Nickerson, M. L., Khalil, S., et al. (2010). miR-23b targets proline oxidase, a novel tumor suppressor protein in renal cancer. *Oncogene* 29 (35), 4914–4924. doi:10.1038/onc.2010.237

Liu, X. S., Little, J. B., and Yuan, Z. M. (2015). Glycolytic metabolism influences global chromatin structure. *Oncotarget* 6 (6), 4214–4225. doi:10.18632/oncotarget.2929

Lo Sasso, G., Menzies, K. J., Mottis, A., Piersigilli, A., Perino, A., Yamamoto, H., et al. (2014). SIRT2 deficiency modulates macrophage polarization and susceptibility to experimental colitis. *PLoS One* 9 (7), e103573. doi:10.1371/journal.pone.0103573

Locasale, J. W., Grassian, A. R., Melman, T., Lyssiotis, C. A., Mattaini, K. R., Bass, A. J., et al. (2011). Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. *Nat. Genet.* 43 (9), 869–874. doi:10.1038/ng.890

Locasale, J. W. (2013). Serine, glycine and one-carbon units: Cancer metabolism in full circle. *Nat. Rev. Cancer* 13 (8), 572–583. doi:10.1038/nrc3557

Long, G. V., Dummer, R., Hamid, O., Gajewski, T. F., Caglevic, C., Dalle, S., et al. (2019). Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): A phase 3, randomised, double-blind study. *Lancet. Oncol.* 20 (8), 1083–1097. doi:10.1016/s1470-2045(19)30274-8

Lopez-Serra, P., Marcilla, M., Villanueva, A., Ramos-Fernandez, A., Palau, A., Leal, L., et al. (2014). A DERL3-associated defect in the degradation of SLC2A1 mediates the Warburg effect. *Nat. Commun.* 5, 3608. doi:10.1038/ncomms4608

Losman, J. A., and Kaelin, W. G., Jr. (2013). What a difference a hydroxyl makes: Mutant IDH, (R)-2-hydroxyglutarate, and cancer. *Genes. Dev.* 27 (8), 836–852. doi:10.1101/gad.217406.113

Losman, J. A., Looper, R. E., Koivunen, P., Lee, S., Schneider, R. K., McMahon, C., et al. (2013). (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. *Science* 339 (6127), 1621–1625. doi:10.1126/science.1231677

Lucena, M. C., Carvalho-Cruz, P., Donadio, J. L., Oliveira, I. A., de Queiroz, R. M., Marinho-Carvalho, M. M., et al. (2016). Epithelial mesenchymal transition induces aberrant glycosylation through hexosamine biosynthetic pathway activation. *J. Biol. Chem.* 291 (25), 12917–12929. doi:10.1074/jbc.M116.729236

Lunt, S. Y., and Vander Heiden, M. G. (2011). Aerobic glycolysis: Meeting the metabolic requirements of cell proliferation. *Annu. Rev. Cell. Dev. Biol.* 27, 441–464. doi:10.1146/annurev-cellbio-092910-154237

Margueron, R., and Reinberg, D. (2010). Chromatin structure and the inheritance of epigenetic information. *Nat. Rev. Genet.* 11 (4), 285–296. doi:10.1038/nrg2752

Mashimo, T., Pichumani, K., Vemireddy, V., Hatanpaa, K. J., Singh, D. K., Sirasanagandla, S., et al. (2014). Acetate is a bioenergetic substrate for human glioblastoma and brain metastases. *Cell.* 159 (7), 1603–1614. doi:10.1016/j.cell.2014.11.025

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. Engl. J. Med.* 371 (16), 1507–1517. doi:10.1056/NEJMoa1407222

McDonald, O. G., Li, X., Saunders, T., Tryggvadottir, R., Mentch, S. J., Warmoes, M. O., et al. (2017). Epigenomic reprogramming during pancreatic cancer progression links anabolic glucose metabolism to distant metastasis. *Nat. Genet.* 49 (3), 367–376. doi:10.1038/ng.3753

Meng, F., Henson, R., Wehbe-Janek, H., Ghoshal, K., Jacob, S. T., Patel, T., et al. (2007). MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 133 (2), 647–658. doi:10.1053/j.gastro.2007.05.022

Mentch, S. J., Mehrmohamadi, M., Huang, L., Liu, X., Gupta, D., Mattocks, D., et al. (2015). Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism. *Cell. Metab.* 22 (5), 861–873. doi:10.1016/j.cmet.2015.08.024

Metallo, C. M., Gameiro, P. A., Bell, E. L., Mattaini, K. R., Yang, J., Hiller, K., et al. (2011). Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* 481 (7381), 380–384. doi:10.1038/nature10602

Miranda, T. B., Cortez, C. C., Yoo, C. B., Liang, G., Abe, M., Kelly, T. K., et al. (2009). DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. *Mol. Cancer Ther.* 8 (6), 1579–1588. doi:10.1158/1535-7163.Mct-09-0013

Moffett, J. R., Puthillathu, N., Vengilote, R., Jaworski, D. M., and Namboodiri, A. M. (2020). Acetate revisited: A key biomolecule at the nexus of metabolism, epigenetics, and oncogenesis - Part 2: Acetate and ACSS2 in health and disease. *Front. Physiol.* 11, 580171. doi:10.3389/fphys.2020.580171

Momparler, R. L., and Côté, S. (2015). Targeting of cancer stem cells by inhibitors of DNA and histone methylation. *Expert Opin. Investig. Drugs* 24 (8), 1031–1043. doi:10.1517/13543784.2015.1051220

Momparler, R. L., Idaghdour, Y., Marquez, V. E., and Momparler, L. F. (2012). Synergistic antileukemic action of a combination of inhibitors of DNA methylation and histone methylation. *Leuk. Res.* 36 (8), 1049–1054. doi:10.1016/j.leukres.2012.03.001

Mu, X., Zhao, T., Xu, C., Shi, W., Geng, B., Shen, J., et al. (2017). Oncometabolite succinate promotes angiogenesis by upregulating VEGF expression through GPR91-mediated STAT3 and ERK activation. *Oncotarget* 8 (8), 13174–13185. doi:10.18632/oncotarget.14485

Mullen, A. R., Wheaton, W. W., Jin, E. S., Chen, P. H., Sullivan, L. B., Cheng, T., et al. (2011). Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature* 481 (7381), 385–388. doi:10.1038/nature10642

Netea, M. G., Domínguez-Andrés, J., Barreiro, L. B., Chavakis, T., Divangahi, M., Fuchs, E., et al. (2020a). Defining trained immunity and its role in health and disease. *Nat. Rev. Immunol.* 20 (6), 375–388. doi:10.1038/s41577-020-0285-6

Netea, M. G., Giamarellos-Bourboulis, E. J., Domínguez-Andrés, J., Curtis, N., van Crevel, R., van de Veerdonk, F. L., et al. (2020b). Trained immunity: A tool for reducing susceptibility to and the severity of SARS-CoV-2 infection. *Cell.* 181 (5), 969–977. doi:10.1016/j.cell.2020.04.042

Novakovic, B., Habibi, E., Wang, S. Y., Arts, R. J. W., Davar, R., Megchelenbrink, W., et al. (2016). β-Glucan reverses the epigenetic state of LPS-induced immunological tolerance. *Cell.* 167 (5), 1354–1368. e1314. doi:10.1016/j.cell.2016.09.034

Nowicki, S., and Gottlieb, E. (2015). Oncometabolites: Tailoring our genes. *Febs J.* 282 (15), 2796–2805. doi:10.1111/febs.13295

O'Neill, L. A. J., and Netea, M. G. (2020). BCG-Induced trained immunity: Can it offer protection against COVID-19? *Nat. Rev. Immunol.* 20 (6), 335–337. doi:10.1038/s41577-020-0337-y

O'Neill, L. A., Kishton, R. J., and Rathmell, J. (2016). A guide to immunometabolism for immunologists. *Nat. Rev. Immunol.* 16 (9), 553–565. doi:10.1038/nri.2016.70

O'Sullivan, D., and Pearce, E. L. (2015). Targeting T cell metabolism for therapy. *Trends Immunol.* 36 (2), 71–80. doi:10.1016/j.it.2014.12.004

Okoye-Okafor, U. C., Bartholdy, B., Cartier, J., Gao, E. N., Pietrak, B., Rendina, A. R., et al. (2015). New IDH1 mutant inhibitors for treatment of acute myeloid leukemia. *Nat. Chem. Biol.* 11 (11), 878–886. doi:10.1038/nchembio.1930

Olivares, O., Däbritz, J. H. M., King, A., Gottlieb, E., and Halsey, C. (2015). Research into cancer metabolomics: Towards a clinical metamorphosis. *Semin. Cell. Dev. Biol.* 43, 52–64. doi:10.1016/j.semcdb.2015.09.008

Onodera, Y., Nam, J. M., and Bissell, M. J. (2014). Increased sugar uptake promotes oncogenesis via EPAC/RAP1 and O-GlcNAc pathways. *J. Clin. Investig.* 124 (1), 367–384. doi:10.1172/jci63146

Owen, O. E., Kalhan, S. C., and Hanson, R. W. (2002). The key role of anaplerosis and cataplerosis for citric acid cycle function. *J. Biol. Chem.* 277 (34), 30409–30412. doi:10.1074/jbc.R200006200

Pan, M., Reid, M. A., Lowman, X. H., Kulkarni, R. P., Tran, T. Q., Liu, X., et al. (2016). Regional glutamine deficiency in tumours promotes dedifferentiation through inhibition of histone demethylation. *Nat. Cell. Biol.* 18 (10), 1090–1101. doi:10.1038/ncb3410

Panwalkar, P., Tamrazi, B., Dang, D., Chung, C., Sweha, S., Natarajan, S. K., et al. (2021). Targeting integrated epigenetic and metabolic pathways in lethal childhood PFA ependymomas. *Sci. Transl. Med.* 13 (614), eabc0497. doi:10.1126/scitranslmed.abc0497

Patra, K. C., Wang, Q., Bhaskar, P. T., Miller, L., Wang, Z., Wheaton, W., et al. (2013). Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. *Cancer Cell.* 24 (2), 213–228. doi:10.1016/j.ccr.2013.06.014

Pavlova, N. N., and Thompson, C. B. (2016). The emerging hallmarks of cancer metabolism. *Cell. Metab.* 23 (1), 27–47. doi:10.1016/j.cmet.2015.12.006

Pearce, E. L., Poffenberger, M. C., Chang, C. H., and Jones, R. G. (2013). Fueling immunity: Insights into metabolism and lymphocyte function. *Science* 342 (6155), 1242454. doi:10.1126/science.1242454

Pfister, S. X., and Ashworth, A. (2017). Marked for death: Targeting epigenetic changes in cancer. *Nat. Rev. Drug Discov.* 16 (4), 241–263. doi:10.1038/nrd.2016.256

Phang, J. M., Liu, W., and Hancock, C. (2013). Bridging epigenetics and metabolism: Role of non-essential amino acids. *Epigenetics* 8 (3), 231–236. doi:10.4161/epi.24042

Pietrocola, F., Galluzzi, L., Bravo-San Pedro, J. M., Madeo, F., and Kroemer, G. (2015). Acetyl coenzyme A: A central metabolite and second messenger. *Cell. Metab.* 21 (6), 805–821. doi:10.1016/j.cmet.2015.05.014

Pinho, S. S., and Reis, C. A. (2015). Glycosylation in cancer: Mechanisms and clinical implications. *Nat. Rev. Cancer* 15 (9), 540–555. doi:10.1038/nrc3982

Piunti, A., and Shilatifard, A. (2016). Epigenetic balance of gene expression by Polycomb and COMPASS families. *Science* 352 (6290), aad9780. doi:10.1126/science.aad9780

Possemato, R., Marks, K. M., Shaul, Y. D., Pacold, M. E., Kim, D., Birsoy, K., et al. (2011). Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 476 (7360), 346–350. doi:10.1038/nature10350

Prendergast, G. C., Malachowski, W. P., DuHadaway, J. B., and Muller, A. J. (2017). Discovery of Ido1 inhibitors: From bench to bedside. *Cancer Res.* 77 (24), 6795–6811. doi:10.1158/0008-5472.Can-17-2285

Priebe, A., Tan, L., Wahl, H., Kueck, A., He, G., Kwok, R., et al. (2011). Glucose deprivation activates AMPK and induces cell death through modulation of Akt in ovarian cancer cells. *Gynecol. Oncol.* 122 (2), 389–395. doi:10.1016/j.ygyno.2011.04.024

Quintin, J., Saeed, S., Martens, J. H. A., Giamarellos-Bourboulis, E. J., Ifrim, D. C., Logie, C., et al. (2012). Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. *Cell. Host Microbe* 12 (2), 223–232. doi:10.1016/j.chom.2012.06.006

Racey, L. A., and Byvoet, P. (1971). Histone acetyltransferase in chromatin. Evidence for *in vitro* enzymatic transfer of acetate from acetyl-coenzyme A to histones. *Exp. Cell. Res.* 64 (2), 366–370. doi:10.1016/0014-4827(71)90089-9

Reid, M. A., Dai, Z., and Locasale, J. W. (2017). The impact of cellular metabolism on chromatin dynamics and epigenetics. *Nat. Cell. Biol.* 19 (11), 1298–1306. doi:10.1038/ncb3629

Ribich, S., Harvey, D., and Copeland, R. A. (2017). Drug discovery and chemical biology of cancer epigenetics. *Cell. Chem. Biol.* 24 (9), 1120–1147. doi:10.1016/j.chembiol.2017.08.020

Robbins, P. F., Morgan, R. A., Feldman, S. A., Yang, J. C., Sherry, R. M., Dudley, M. E., et al. (2011). Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J. Clin. Oncol.* 29 (7), 917–924. doi:10.1200/jco.2010.32.2537

Robinson, M. M., McBryant, S. J., Tsukamoto, T., Rojas, C., Ferraris, D. V., Hamilton, S. K., et al. (2007). Novel mechanism of inhibition of rat kidney-type glutaminase by bis-2-(5-phenylacetamido-1, 2, 4-thiadiazol-2-yl)ethyl sulfide (BPTES). *Biochem. J.* 406 (3), 407–414. doi:10.1042/bj20070039

Rodrigues, M. F., Carvalho, É., Pezzuto, P., Rumjanek, F. D., and Amoêdo, N. D. (2015). Reciprocal modulation of histone deacetylase inhibitors sodium butyrate and trichostatin A on the energy metabolism of breast cancer cells. *J. Cell. Biochem.* 116 (5), 797–808. doi:10.1002/jcb.25036

Rogers, R. E., Deberardinis, R. J., Klesse, L. J., Boriack, R. L., Margraf, L. R., Rakheja, D., et al. (2010). Wilms tumor in a child with L-2-hydroxyglutaric aciduria. *Pediatr. Dev. Pathol.* 13 (5), 408–411. doi:10.2350/09-12-0768-cr.1

Rohle, D., Popovici-Muller, J., Palaskas, N., Turcan, S., Grommes, C., Campos, C., et al. (2013). An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science* 340 (6132), 626–630. doi:10.1126/science.1236062

Roichman, A., Elhanati, S., Aon, M. A., Abramovich, I., Di Francesco, A., Shahar, Y., et al. (2021). Restoration of energy homeostasis by SIRT6 extends healthy lifespan. *Nat. Commun.* 12 (1), 3208. doi:10.1038/s41467-021-23545-7

Rosenberg, S. A., Packard, B. S., Aebersold, P. M., Solomon, D., Topalian, S. L., Toy, S. T., et al. (1988). Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. *N. Engl. J. Med.* 319 (25), 1676–1680. doi:10.1056/nejm198812223192527

Rosenberg, S. A. (2012). Raising the bar: The curative potential of human cancer immunotherapy. *Sci. Transl. Med.* 4 (127), 127ps8. doi:10.1126/scitranslmed.3003634

Rosenberg, S. A., Yang, J. C., Sherry, R. M., Kammula, U. S., Hughes, M. S., Phan, G. Q., et al. (2011). Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin. Cancer Res.* 17 (13), 4550–4557. doi:10.1158/1078-0432.Ccr-11-0116

Saeed, S., Quintin, J., Kerstens, H. H., Rao, N. A., Aghajanirefah, A., Matarese, F., et al. (2014). Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* 345 (6204), 1251086. doi:10.1126/science.1251086

Sandoval, J., and Esteller, M. (2012). Cancer epigenomics: Beyond genomics. *Curr. Opin. Genet. Dev.* 22 (1), 50–55. doi:10.1016/j.gde.2012.02.008

Satoh, T., Takeuchi, O., Vandenbon, A., Yasuda, K., Tanaka, Y., Kumagai, Y., et al. (2010). The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat. Immunol.* 11 (10), 936–944. doi:10.1038/ni.1920

Schäfer, F., Balleyguier, C., et al. (2013). Letter to editors regarding "breast elasticity: Principles, technique, results: An update and overview of commercially available software", C. Balleyguier et al. http://dx.doi.org/10.1016/j.ejrad.2012.03.001. *Eur. J. Radiol.* 82 (2), 385–386. doi:10.1016/j.ejrad.2012.03.00110.1016/j.ejrad.2012.11.016

Schug, Z. T., Peck, B., Jones, D. T., Zhang, Q., Grosskurth, S., Alam, I. S., et al. (2015). Acetyl-CoA synthetase 2 promotes acetate utilization and maintains cancer cell growth under metabolic stress. *Cancer Cell.* 27 (1), 57–71. doi:10.1016/j.ccell.2014.12.002

Sciacovelli, M., Gonçalves, E., Johnson, T. I., Zecchini, V. R., da Costa, A. S., Gaude, E., et al. (2016). Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition. *Nature* 537 (7621), 544–547. doi:10.1038/nature19353

Sebastián, C., Zwaans, B. M., Silberman, D. M., Gymrek, M., Goren, A., Zhong, L., et al. (2012). The histone deacetylase SIRT6 is a tumor suppressor that controls cancer metabolism. *Cell.* 151 (6), 1185–1199. doi:10.1016/j.cell.2012.10.047

Serganova, I., Chakraborty, S., Yamshon, S., Isshiki, Y., Bucktrout, R., Melnick, A., et al. (2021). Epigenetic, metabolic, and immune crosstalk in germinal-center-derived B-cell lymphomas: Unveiling new vulnerabilities for rational combination therapies. *Front. Cell. Dev. Biol.* 9, 805195. doi:10.3389/fcell.2021.805195

Shahbazian, M. D., and Grunstein, M. (2007). Functions of site-specific histone acetylation and deacetylation. *Annu. Rev. Biochem.* 76, 75–100. doi:10.1146/annurev.biochem.76.052705.162114

Shanmugasundaram, K., Nayak, B., Shim, E. H., Livi, C. B., Block, K., Sudarshan, S., et al. (2014). The oncometabolite fumarate promotes pseudohypoxia through noncanonical activation of NF-κB signaling. *J. Biol. Chem.* 289 (35), 24691–24699. doi:10.1074/jbc.M114.568162

Shi, Y. J., Matson, C., Lan, F., Iwase, S., Baba, T., Shi, Y., et al. (2005). Regulation of LSD1 histone demethylase activity by its associated factors. *Mol. Cell.* 19 (6), 857–864. doi:10.1016/j.molcel.2005.08.027

Shim, E. H., Livi, C. B., Rakheja, D., Tan, J., Benson, D., Parekh, V., et al. (2014). L-2-Hydroxyglutarate: An epigenetic modifier and putative oncometabolite in renal cancer. *Cancer Discov.* 4 (11), 1290–1298. doi:10.1158/2159-8290.Cd-13-0696

Shim, H., Dolde, C., Lewis, B. C., Wu, C. S., Dang, G., Jungmann, R. A., et al. (1997). c-Myc transactivation of LDH-A: Implications for tumor metabolism and growth. *Proc. Natl. Acad. Sci. U. S. A.* 94 (13), 6658–6663. doi:10.1073/pnas.94.13.6658

Shin, J., He, M., Liu, Y., Paredes, S., Villanova, L., Brown, K., et al. (2013). SIRT7 represses Myc activity to suppress ER stress and prevent fatty liver disease. *Cell. Rep.* 5 (3), 654–665. doi:10.1016/j.celrep.2013.10.007

Simpson, N. E., Tryndyak, V. P., Beland, F. A., and Pogribny, I. P. (2012a). An *in vitro* investigation of metabolically sensitive biomarkers in breast cancer progression. *Breast Cancer Res. Treat.* 133 (3), 959–968. doi:10.1007/s10549-011-1871-x

Simpson, N. E., Tryndyak, V. P., Pogribna, M., Beland, F. A., and Pogribny, I. P. (2012b). Modifying metabolically sensitive histone marks by inhibiting glutamine metabolism affects gene expression and alters cancer cell phenotype. *Epigenetics* 7 (12), 1413–1420. doi:10.4161/epi.22713

Son, J., Lyssiotis, C. A., Ying, H., Wang, X., Hua, S., Ligorio, M., et al. (2013). Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* 496 (7443), 101–105. doi:10.1038/nature12040

Soshnev, A. A., Josefowicz, S. Z., and Allis, C. D. (2016). Greater than the sum of parts: Complexity of the dynamic epigenome. *Mol. Cell.* 62 (5), 681–694. doi:10.1016/j.molcel.2016.05.004

Sowter, H. M., Raval, R. R., Moore, J. W., Ratcliffe, P. J., and Harris, A. L. (2003). Predominant role of hypoxia-inducible transcription factor (Hif)-1alpha versus Hif-2alpha in regulation of the transcriptional response to hypoxia. *Cancer Res.* 63 (19), 6130–6134.

Sukumar, M., Liu, J., Ji, Y., Subramanian, M., Crompton, J. G., Yu, Z., et al. (2013). Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J. Clin. Investig.* 123 (10), 4479–4488. doi:10.1172/jci69589

Sullivan, L. B., Martinez-Garcia, E., Nguyen, H., Mullen, A. R., Dufour, E., Sudarshan, S., et al. (2013). The proto-oncometabolite fumarate binds glutathione to amplify ROS-dependent signaling. *Mol. Cell.* 51 (2), 236–248. doi:10.1016/j.molcel.2013.05.003

Tessarz, P., and Kouzarides, T. (2014). Histone core modifications regulating nucleosome structure and dynamics. *Nat. Rev. Mol. Cell. Biol.* 15 (11), 703–708. doi:10.1038/nrm3890

Thakur, C., and Chen, F. (2019). Connections between metabolism and epigenetics in cancers. *Semin. Cancer Biol.* 57, 52–58. doi:10.1016/j.semcancer.2019.06.006

Tomlinson, I. P., Alam, N. A., Rowan, A. J., Barclay, E., Jaeger, E. E., Kelsell, D., et al. (2002). Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat. Genet.* 30 (4), 406–410. doi:10.1038/ng849

Topalian, S. L., Hodi, F. S., Brahmer, J. R., Gettinger, S. N., Smith, D. C., McDermott, D. F., et al. (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* 366 (26), 2443–2454. doi:10.1056/NEJMoa1200690

Turcan, S., Fabius, A. W., Borodovsky, A., Pedraza, A., Brennan, C., Huse, J., et al. (2013). Efficient induction of differentiation and growth inhibition in IDH1 mutant glioma cells by the DNMT Inhibitor Decitabine. *Oncotarget* 4 (10), 1729–1736. doi:10.18632/oncotarget.1412

Ulanovskaya, O. A., Zuhl, A. M., and Cravatt, B. F. (2013). NNMT promotes epigenetic remodeling in cancer by creating a metabolic methylation sink. *Nat. Chem. Biol.* 9 (5), 300–306. doi:10.1038/nchembio.1204

Vakoc, C. R., Mandat, S. A., Olenchock, B. A., and Blobel, G. A. (2005). Histone H3 lysine 9 methylation and HP1gamma are associated with transcription elongation through mammalian chromatin. *Mol. Cell.* 19 (3), 381–391. doi:10.1016/j.molcel.2005.06.011

Varier, R. A., and Timmers, H. T. (2011). Histone lysine methylation and demethylation pathways in cancer. *Biochim. Biophys. Acta* 1815 (1), 75–89. doi:10.1016/j.bbcan.2010.10.002

Vatrinet, R., Leone, G., De Luise, M., Girolimetti, G., Vidone, M., Gasparre, G., et al. (2017). The α-ketoglutarate dehydrogenase complex in cancer metabolic plasticity. *Cancer Metab.* 5, 3. doi:10.1186/s40170-017-0165-0

Wang, F., Travins, J., DeLaBarre, B., Penard-Lacronique, V., Schalm, S., Hansen, E., et al. (2013). Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science* 340 (6132), 622–626. doi:10.1126/science.1234769

Wang, J. B., Erickson, J. W., Fuji, R., Ramachandran, S., Gao, P., Dinavahi, R., et al. (2010a). Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. *Cancer Cell.* 18 (3), 207–219. doi:10.1016/j.ccr.2010.08.009

Wang, L., Jin, Q., Lee, J. E., Su, I. H., and Ge, K. (2010b). Histone H3K27 methyltransferase Ezh2 represses Wnt genes to facilitate adipogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 107 (16), 7317–7322. doi:10.1073/pnas.1000031107

Wang, T., Yu, Q., Li, J., Hu, B., Zhao, Q., Ma, C., et al. (2017). O-GlcNAcylation of fumarase maintains tumour growth under glucose deficiency. *Nat. Cell. Biol.* 19 (7), 833–843. doi:10.1038/ncb3562

Wang, Y. H., Israelsen, W. J., Lee, D., Yu, V. W. C., Jeanson, N. T., Clish, C. B., et al. (2014). Cell-state-specific metabolic dependency in hematopoiesis and leukemogenesis. *Cell.* 158 (6), 1309–1323. doi:10.1016/j.cell.2014.07.048

Wang, Y. P., and Lei, Q. Y. (2018). Metabolic recoding of epigenetics in cancer. *Cancer Commun.* 38 (1), 25. doi:10.1186/s40880-018-0302-3

Wardell, S. E., Ilkayeva, O. R., Wieman, H. L., Frigo, D. E., Rathmell, J. C., Newgard, C. B., et al. (2009). Glucose metabolism as a target of histone deacetylase inhibitors. *Mol. Endocrinol.* 23 (3), 388–401. doi:10.1210/me.2008-0179

Waterfall, J. J., Killian, J. K., and Meltzer, P. S. (2014). The role of mutation of metabolism-related genes in genomic hypermethylation. *Biochem. Biophys. Res. Commun.* 455 (1-2), 16–23. doi:10.1016/j.bbrc.2014.08.003

Weinberg, S. E., and Chandel, N. S. (2015). Targeting mitochondria metabolism for cancer therapy. *Nat. Chem. Biol.* 11 (1), 9–15. doi:10.1038/nchembio.1712

Wellen, K. E., Hatzivassiliou, G., Sachdeva, U. M., Bui, T. V., Cross, J. R., Thompson, C. B., et al. (2009). ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* 324 (5930), 1076–1080. doi:10.1126/science.1164097

Wellen, K. E., Lu, C., Mancuso, A., Lemons, J. M., Ryczko, M., Dennis, J. W., et al. (2010). The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism. *Genes. Dev.* 24 (24), 2784–2799. doi:10.1101/gad.1985910

Wellen, K. E., and Thompson, C. B. (2012). A two-way street: Reciprocal regulation of metabolism and signalling. *Nat. Rev. Mol. Cell. Biol.* 13 (4), 270–276. doi:10.1038/nrm3305

Williamson, S. R., Eble, J. N., Amin, M. B., Gupta, N. S., Smith, S. C., Sholl, L. M., et al. (2015). Succinate dehydrogenase-deficient renal cell carcinoma: Detailed characterization of 11 tumors defining a unique subtype of renal cell carcinoma. *Mod. Pathol.* 28 (1), 80–94. doi:10.1038/modpathol.2014.86

Wise, D. R., Ward, P. S., Shay, J. E., Cross, J. R., Gruber, J. J., Sachdeva, U. M., et al. (2011). Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability. *Proc. Natl. Acad. Sci. U. S. A.* 108 (49), 19611–19616. doi:10.1073/pnas.1117773108

Wolf, A., Agnihotri, S., Munoz, D., and Guha, A. (2011). Developmental profile and regulation of the glycolytic enzyme hexokinase 2 in normal brain and glioblastoma multiforme. *Neurobiol. Dis.* 44 (1), 84–91. doi:10.1016/j.nbd.2011.06.007

Wong, C. C., Qian, Y., and Yu, J. (2017). Interplay between epigenetics and metabolism in oncogenesis: Mechanisms and therapeutic approaches. *Oncogene* 36 (24), 3359–3374. doi:10.1038/onc.2016.485

Xiang, Y., Stine, Z. E., Xia, J., Lu, Y., O'Connor, R. S., Altman, B. J., et al. (2015). Targeted inhibition of tumor-specific glutaminase diminishes cell-autonomous tumorigenesis. *J. Clin. Investig.* 125 (6), 2293–2306. doi:10.1172/jci75836

Xiao, C., Kim, H. S., Lahusen, T., Wang, R. H., Xu, X., Gavrilova, O., et al. (2010). SIRT6 deficiency results in severe hypoglycemia by enhancing both basal and insulin-stimulated glucose uptake in mice. *J. Biol. Chem.* 285 (47), 36776–36784. doi:10.1074/jbc.M110.168039

Xiao, M., Yang, H., Xu, W., Ma, S., Lin, H., Zhu, H., et al. (2012). Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. *Genes. Dev.* 26 (12), 1326–1338. doi:10.1101/gad.191056.112

Xie, H., Hanai, J., Ren, J. G., Kats, L., Burgess, K., Bhargava, P., et al. (2014). Targeting lactate dehydrogenase--a inhibits tumorigenesis and tumor progression in mouse models of lung cancer and impacts tumor-initiating cells. *Cell. Metab.* 19 (5), 795–809. doi:10.1016/j.cmet.2014.03.003

Xu, W., Yang, H., Liu, Y., Yang, Y., Wang, P., Kim, S. H., et al. (2011). Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell.* 19 (1), 17–30. doi:10.1016/j.ccr.2010.12.014

Yan, H., Parsons, D. W., Jin, G., McLendon, R., Rasheed, B. A., Yuan, W., et al. (2009). IDH1 and IDH2 mutations in gliomas. *N. Engl. J. Med.* 360 (8), 765–773. doi:10.1056/NEJMoa0808710

Yan, L., Tan, Y., Chen, G., Fan, J., and Zhang, J. (2021). Harnessing metabolic reprogramming to improve cancer immunotherapy. *Int. J. Mol. Sci.* 22 (19), 10268. doi:10.3390/ijms221910268

Yanagida, O., Kanai, Y., Chairoungdua, A., Kim, D. K., Segawa, H., Nii, T., et al. (2001). Human L-type amino acid transporter 1 (LAT1): Characterization of function and expression in tumor cell lines. *Biochim. Biophys. Acta* 1514 (2), 291–302. doi:10.1016/s0005-2736(01)00384-4

Yang, M., Soga, T., and Pollard, P. J. (2013). Oncometabolites: Linking altered metabolism with cancer. *J. Clin. Investig.* 123 (9), 3652–3658. doi:10.1172/jci67228

Yang, M., Ternette, N., Su, H., Dabiri, R., Kessler, B. M., Adam, J., et al. (2014). The succinated proteome of FH-mutant tumours. *Metabolites* 4 (3), 640–654. doi:10.3390/metabo4030640

Yang, W., Bai, Y., Xiong, Y., Zhang, J., Chen, S., Zheng, X., et al. (2016). Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. *Nature* 531 (7596), 651–655. doi:10.1038/nature17412

Yen, K., Travins, J., Wang, F., David, M. D., Artin, E., Straley, K., et al. (2017). AG-221, a first-in-class therapy targeting acute myeloid leukemia harboring oncogenic IDH2 mutations. *Cancer Discov.* 7 (5), 478–493. doi:10.1158/2159-8290.Cd-16-1034

Yeung, F., Hoberg, J. E., Ramsey, C. S., Keller, M. D., Jones, D. R., Frye, R. A., et al. (2004). Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *Embo J.* 23 (12), 2369–2380. doi:10.1038/sj.emboj.7600244

Yu, T., Wang, Y., Hu, Q., Wu, W., Wu, Y., Wei, W., et al. (2017). The EZH2 inhibitor GSK343 suppresses cancer stem-like phenotypes and reverses mesenchymal transition in glioma cells. *Oncotarget* 8 (58), 98348–98359. doi:10.18632/oncotarget.21311

Zhang, D., Tang, Z., Huang, H., Zhou, G., Cui, C., Weng, Y., et al. (2019). Metabolic regulation of gene expression by histone lactylation. *Nature* 574 (7779), 575–580. doi:10.1038/s41586-019-1678-1

Zhang, T., Gong, Y., Meng, H., Li, C., and Xue, L. (2020). Symphony of epigenetic and metabolic regulation-interaction between the histone methyltransferase EZH2 and metabolism of tumor. *Clin. Epigenetics* 12 (1), 72. doi:10.1186/s13148-020-00862-0

Zhang, Z. G., and Qin, C. Y. (2014). Sirt6 suppresses hepatocellular carcinoma cell growth via inhibiting the extracellular signal-regulated kinase signaling pathway. *Mol. Med. Rep.* 9 (3), 882–888. doi:10.3892/mmr.2013.1879

Zhang, Z., Tan, M., Xie, Z., Dai, L., Chen, Y., Zhao, Y., et al. (2011). Identification of lysine succinylation as a new post-translational modification. *Nat. Chem. Biol.* 7 (1), 58–63. doi:10.1038/nchembio.495

Zhao, Y., Zheng, Z., Robbins, P. F., Khong, H. T., Rosenberg, S. A., Morgan, R. A., et al. (2005). Primary human lymphocytes transduced with NY-ESO-1 antigen-specific TCR genes recognize and kill diverse human tumor cell lines. *J. Immunol.* 174 (7), 4415–4423. doi:10.4049/jimmunol.174.7.4415

Zheng, B., Yao, Y., Liu, Z., Deng, L., Anglin, J. L., Jiang, H., et al. (2013a). Crystallographic investigation and selective inhibition of mutant isocitrate dehydrogenase. *ACS Med. Chem. Lett.* 4 (6), 542–546. doi:10.1021/ml400036z

Zheng, L., Cardaci, S., Jerby, L., MacKenzie, E. D., Sciacovelli, M., Johnson, T. I., et al. (2015). Fumarate induces redox-dependent senescence by modifying glutathione metabolism. *Nat. Commun.* 6, 6001. doi:10.1038/ncomms7001

Zheng, L., MacKenzie, E. D., Karim, S. A., Hedley, A., Blyth, K., Kalna, G., et al. (2013b). Reversed argininosuccinate lyase activity in fumarate hydratase-deficient cancer cells. *Cancer Metab.* 1 (1), 12. doi:10.1186/2049-3002-1-12

Zhong, L., D'Urso, A., Toiber, D., Sebastian, C., Henry, R. E., Vadysirisack, D. D., et al. (2010). The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha. *Cell.* 140 (2), 280–293. doi:10.1016/j.cell.2009.12.041

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 24 August 2022
DOI 10.3389/fphar.2022.932154

OPEN ACCESS

EDITED BY
Lixiang Xue,
Peking University Third Hospital, China

REVIEWED BY
Xiawei Cheng,
East China University of Science and Technology, China
Giovanni Mario Pes,
University of Sassari, Italy
Hung-Chi Yang,
Yuanpei University of Medical Technology, Taiwan

*CORRESPONDENCE
Honglan Zhou,
whlzhou@jlu.edu.cn
Yishu Wang,
wangys@jlu.edu.cn
Zhi-Xiang Xu,
zhixiangxu08@gmail.com

†These authors have contributed equally to this work.

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 29 April 2022
ACCEPTED 01 August 2022
PUBLISHED 24 August 2022

CITATION
Meng Q, Zhang Y, Hao S, Sun H, Liu B, Zhou H, Wang Y and Xu Z-X (2022), Recent findings in the regulation of G6PD and its role in diseases. *Front. Pharmacol.* 13:932154. doi: 10.3389/fphar.2022.932154

Recent findings in the regulation of G6PD and its role in diseases

Qingfei Meng[1], Yanghe Zhang[1], Shiming Hao[1], Huihui Sun[1], Bin Liu[2], Honglan Zhou[2*†], Yishu Wang[1*†] and Zhi-Xiang Xu[1,2,3*†]

[1]Key Laboratory of Pathobiology, Ministry of Education, Jilin University, Changchun, China, [2]Department of Urology, The First Hospital of Jilin University, Changchun, China, [3]School of Life Sciences, Henan University, Kaifeng, China

Glucose-6-phosphate dehydrogenase (G6PD) is the only rate-limiting enzyme in the pentose phosphate pathway (PPP). Rapidly proliferating cells require metabolites from PPP to synthesize ribonucleotides and maintain intracellular redox homeostasis. G6PD expression can be abnormally elevated in a variety of cancers. In addition, G6PD may act as a regulator of viral replication and vascular smooth muscle function. Therefore, G6PD-mediated activation of PPP may promote tumor and non-neoplastic disease progression. Recently, studies have identified post-translational modifications (PTMs) as an important mechanism for regulating G6PD function. Here, we provide a comprehensive review of various PTMs (e.g., phosphorylation, acetylation, glycosylation, ubiquitination, and glutarylation), which are identified in the regulation of G6PD structure, expression and enzymatic activity. In addition, we review signaling pathways that regulate G6PD and evaluate the role of oncogenic signals that lead to the reprogramming of PPP in tumor and non-neoplastic diseases as well as summarize the inhibitors that target G6PD.

KEYWORDS

glucose-6-phosphate dehydrogenase, pentose phosphate pathway, post-translational modifications, metabolic reprogramming, tumorigenesis

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is the only rate-limiting enzyme in the pentose phosphate pathway (PPP). PPP flow is therefore mainly regulated through G6PD expression or enzyme activity. PPP involves the formation of a bypass from glucose-6-phosphate, an intermediate product of glycolysis, which produces fructose-6-phosphate

Abbreviations: cRNA, complementary RNA; G6PD, Glucose-6-phosphate dehydrogenase; HATs, Histone acetyltransferase; HDACs, Histone deacetylases; mRNA, messenger RNA; NADPH, Nicotinamide adenine dinucleotide phosphate; non-oxPPP, Nonoxidative pentose phosphate pathway; OGA, O-GlcNAcase; O-GlcNAc, O-linked β-N-Acetylglucosamine; OGT, O-GlcNAc transferase; Oxppp, Oxidative pentose phosphate pathway; PKA, protein kinase A; PPP, Pentose phosphate pathway; PTM, post-translational modifications; R5P, Ribose-5-phosphate; ROS, Reactive oxygen species; R5P, Ribose-5-phosphate; SIK3, salt-inducible kinase 3; Sirt2, Silent information regulator 2; SMCs, Vascular smooth muscle cells; VHL, von Hippel-Lindau; vRNA, viral RNA; ZIKV, Zika virus.

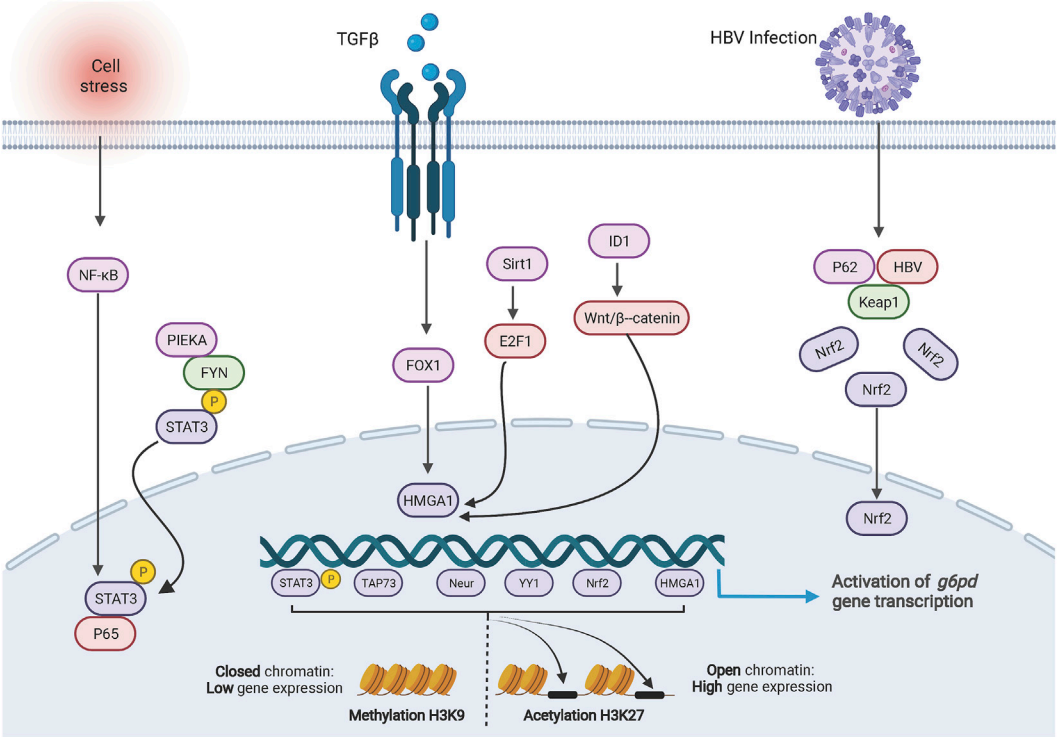


FIGURE 1
Transcriptional regulation of G6PD. The cartoon diagram on display consists of three main parts. On the left, activation of NF-κB in response to cellular stresses or the PIEKA-FYN complex leads to the phosphorylation and activation of STAT3, which results in the translocation of p-STAT3 to the nucleus and binding to the G6PD promoter enhancing transcription. In the middle section, signals regulate the expression of HMGA1 to promote G6PD transcription. On the right side, HBV protein forms a complex with intracellular protein p62 and KEAP1, resulting in translocation of NRF2 into the nucleus to promote G6PD expression. At the bottom, methylation and acetylation of histones are involved in transcriptional regulation of G6PD.

and glyceraldehyde-3-phosphate through two stages of oxidation and group transfer back to glycolysis, also referred to as the hexose monophosphate shunt.

PPP takes place in the cytoplasm and comprises oxidative (oxPPP) and nonoxidative (non-oxPPP) phases. In the oxidative phase, G6PD catalyzes glucose-6-phosphate to generate nicotinamide adenine dinucleotidephosphate (NADPH) and 6-phosphogluconolactone in an $NADP^+$-dependent manner. NADPH is required for the synthesis of both intracellular fatty acids and cholesterol. It also scavenges reactive oxygen species (ROS) and maintains the reduction state of glutathione to combat oxidative stress. As a consequence, cells with a high demand for NADPH, such as tumor cells, exhibit a metabolic vulnerability that could be targeted by the inhibition of G6PD as a therapeutic strategy (Ju et al., 2020). Another important product of the non-oxPPP is ribose-5-phosphate (R5P), which provides important precursors for nucleotide synthesis. Rapidly proliferating cells require products to build cell blocks and maintain intracellular redox homeostasis (Rao et al., 2015). In addition, metabolites in the PPP can function as signaling molecules for the regulation of gene expression (Lin et al., 2015; Gao et al., 2019).

In this review, we focus on current findings in post-translational modifications (PTM) of G6PD and their roles in tumorigenesis and pathogenesis of non-neoplastic diseases.

Transcriptional regulation of G6PD

Transcription factors regulate G6PD expression

G6PD consists of 13 exons and 12 introns, which encode a product of 1,545 bp. The characterization of the promoter region shows 1) a high level (70%) of guanine and cytosine content; 2) a TATA box, which controls the accuracy and frequency of transcription initiation and is located in the -202 bp region upstream of the *G6PD* transcription start site (Gomez-Manzo et al., 2016). The promoter region of *G6PD* contains multiple binding sites for transcription factors. The transcription factors

NeuroD1 (Li Z. et al., 2021), HMGA1 (Zhang R. et al., 2019; Gong et al., 2020), YY1 (Wu et al., 2018), c-MYC (Yin et al., 2017), p65 (Zhang et al., 2020), TAp73 (Du et al., 2013), Nrf2 (Liu et al., 2015; Zhang H.-S. et al., 2019; Lv et al., 2022), and pSTAT3 (Zhang et al., 2020; Sun M. et al., 2021) can directly and individually regulate *G6PD* transcription by binding to the *G6PD* promoter region (Figure 1). Additionally, dual transcription factors from the p65/pSTAT3 complex bind to the pSTAT3 binding site rather than the p65-binding site in the *G6PD* promoter region to stimulate *G6PD* transcription (Zhang et al., 2020).

Transcriptional coactivators/repressors regulate G6PD expression

Transcriptional coactivators or corepressors are also involved in the regulation of *G6PD* transcription. Coactivators and repressors, which are cellular proteins that contain a DNA binding domain without directly binding to the promoter, assemble with transcription factors to form transcriptional complexes that enhance or repress gene transcription, respectively. In pancreatic ductal adenocarcinoma cells, the transcriptional coactivator yes-associated protein 1 interacts with TEA domain transcription factor 1 to regulate *G6PD* expression (Nie et al., 2021). In addition, HATs are involved in the regulation of transcription as coactivators. Acetylation of histones regulated by HATs loosens chromosome structure and facilitates the binding of DNA to transcription factors (Li W. et al., 2021). Histone deacetylase inhibitors (HDACs), NaBu, increases *G6PD* transcription by recruiting transcription factor Sp1 (Makarona et al., 2014). On the other hand, HDACs are transcriptional corepressors capable of transcriptional repression or silencing. For example, liver kinase B1 (LKB1)–AMP-activated protein kinase (AMPK) axis-mediated phosphorylation of histone deacetylase 10 (HDAC10) promotes its translocation to the nucleus to regulate *G6PD* expression (Shan et al., 2019).

Non-coding RNA regulates the expression of G6PD

Small non-coding RNAs are also involved in the regulation of *G6PD* expression. Multiple microRNA binding sites exist in the 3′UTR region of *G6PD*. *MIR-206*, a skeletal muscle-specific microRNA, is a key regulator in skeletal muscle development. *MIR-206* functions pro-myogenically through direct binding of *G6PD* to restore differentiation of rhabdomyosarcoma cells (Coda et al., 2015). In addition, it can inhibit skeletal muscle cell proliferation by targeting *G6PD* (Jiang et al., 2019). MicroRNA has also been reported to inhibit tumor growth by targeting *G6PD*. In renal cell carcinoma, large-scale transcriptome and metabolic analyses showed that miR-146a-5p and miR-155-5p were involved in PPP reprogramming (Boguslawska et al., 2019). Furthermore, LINC00242 competitively bound miR-1-3p to free G6PD from miR-1-3p-mediated repression promoting gastric cancer progression (Deng et al., 2021).

Post-Translational modification regulates G6PD expression in tumorigenesis

PTM of histones is an important epigenetic mechanism regulating the transcriptional activity of G6PD. Both acetylation and methylation modifications of histones have been identified as regulators of G6PD expression. Inhibition of histone deacetylase leads to the recruitment of transcription factor sp1 to the promoter region of G6PD (Makarona et al., 2014), which result in the increase in G6PD expression, suggesting that acetylation may be involved in the transcriptional regulation of G6PD. Recently, increased levels of H3K27Ac have been identified in the G6PD promoter region promoting HDAC10-driven transcription (Shan et al., 2019). Methylation modifications of histone lysine residues were also characterized as regulators of G6PD transcription. H3K9 methylation at G6PD promoter was significantly enriched, leading to the inhibition of G6PD expression (Lu et al., 2022). However, the specific lysine methyltransferases or demethylases that mediate histone methylation in G6PD transcription remains unclear.

In addition to regulating G6PD expression at the transcriptional level, PTMs are also involved in the stability of G6PD through the ubiquitin-proteasome system. Hypoxia activates G6PD expression, which could be reversed by ROS scavengers, suggesting that hypoxia may increase G6PD expression by inducing ROS accumulation. On the other hand, although G6PD expression is significantly reduced under hypoxic conditions and reversed by the proteasome inhibitor MG132, the specific mechanism remains unclear (Chettimada et al., 2015). Recently, von Hippel-Lindau (VHL) E3, an ubiquitin ligase, was found to be involved in the regulation of G6PD stability. VHL directly binds and ubiquitinates G6PD at the K366 and K403, which in turn degrades G6PD (Wang et al., 2019). In addition, SUMOylation and ubiquitination synergistically regulate the stability of G6PD. Silent information regulator 2 (Sirt2) directly binds to G6PD to increase enzyme activity through enhanced SUMOylation and inhibition of ubiquitination (Ni et al., 2021).

Post-Translational modification of G6PD regulates enzyme activity in tumorigenesis

G6PD phosphorylation

Phosphorylation modifications occur mainly on serine, tyrosine, and threonine residues, in which the hydroxyl group

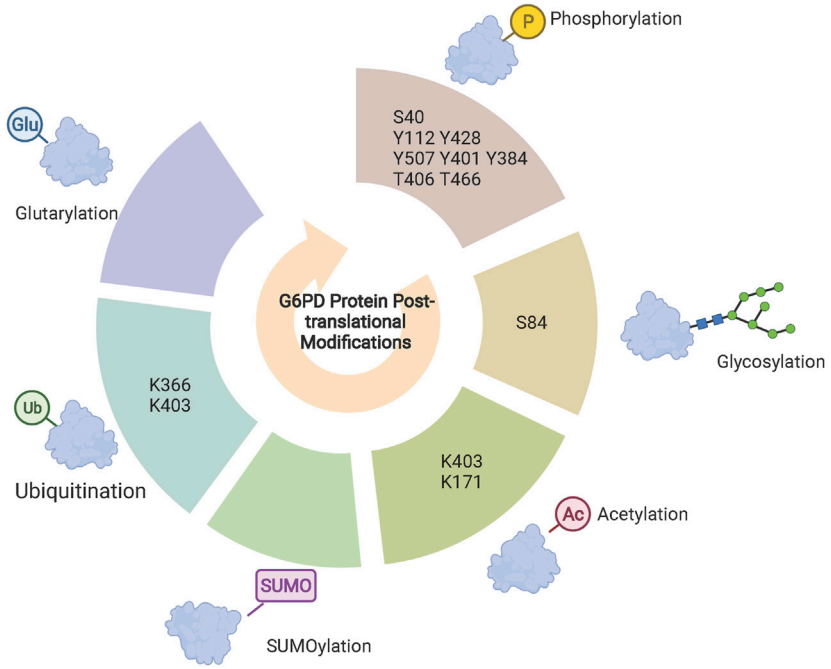


FIGURE 2
G6PD post-translational modifications. Phosphorylation, glycosylation, acetylation and glutarylation modifications regulate G6PD enzyme activity and specific sites identified are shown in the central circle. Ubiquitination and SUMOylation are synergistically involved in the regulation of G6PD protein stability. Acetylation and methylation of histones H3K27 and H3K9 regulate G6PD transcriptional expression, respectively.

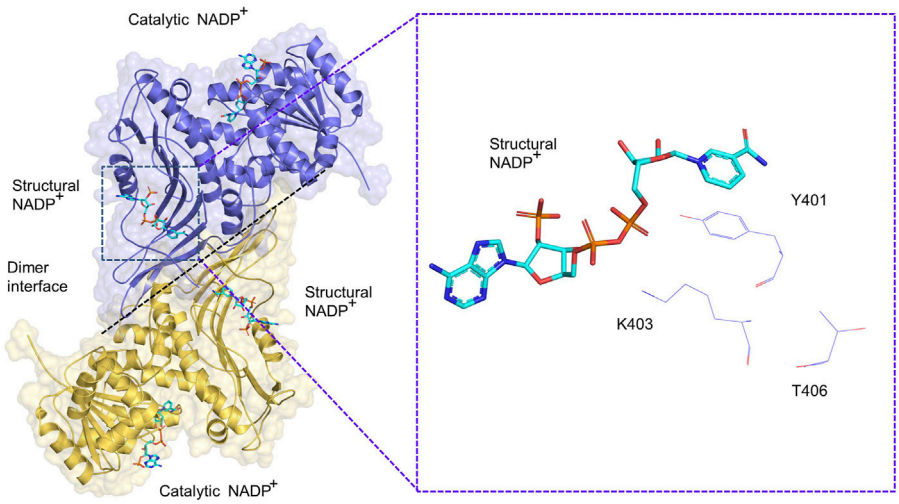


FIGURE 3
Schematic diagram of G6PD (PDB: 2BH9) dimer. A dimer consisting of two G6PD monomers, each of which includes a catalytic $NADP^+$ and structural $NADP^+$, respectively. The G6PD K403, Y401 and T406 sites are located close to the structural $NADP^+$.

can be dehydrated with the phosphate group to form phosphate esters. Gu et determined, using mass spectrometry, that NF-κB-inducing kinase phosphorylation of G6PD at S40 enhances the enzymatic activity and promotes $CD8^+$ effector T cells (Gu et al., 2021). Most reports have focused on the phosphorylation of G6PD tyrosine sites (Pan et al., 2009; Ma et al., 2021). G6PD is a substrate of the non-receptor tyrosine kinase family member Src. Several tyrosine sites of G6PD can be phosphorylated by Src,

including Y112, Y428, and Y507. Among them, Y112 is considered to be the most important phosphorylation site of Src and phosphorylation at this site increases the enzymatic activity of G6PD and enhances PPP flow to promote tumorigenesis (Pan et al., 2009; Ma et al., 2021). Other members of the Src family can also directly bind phosphorylated G6PD. Fyn, a member of the SRC family, phosphorylates Y401 increasing the enzymatic activity of G6PD more than three-fold in erythrocytes (Mattè et al., 2020). In addition, salt-inducible kinase 3 (SIK3), a serine/threonine kinase, binds and phosphorylates G6PD at Y384 enhancing its enzymatic activity (Teesalu et al., 2017). Protein kinase A (PKA) inhibits the expression of SIK3 (Wang et al., 2011), which suggests that PKA and SIK3 may play opposing roles in the regulation of G6PD activity. This is consistent with previous reports that PKA inhibits G6PD enzyme activity (Xu et al., 2005). In addition to tyrosine and serine as potential phosphorylation sites for G6PD, G6PD is phosphorylated by polo-like kinase 1 at T406 and T466 sites increasing its enzymatic activity (Ma et al., 2017).

G6PD O-linked GlcNAc

O-linked β-N-Acetylglucosamine (O-GlcNAc) is a reversible post-translational modification that occurs on serine or threonine residues. This process is regulated by the addition or removal of O-GlcNAc for O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively (Zeng et al., 2016). Recent findings indicate that G6PD is dynamically O-GlcNAcylated at serine 84, which dramatically increases the enzymatic activity of G6PD. Meanwhile, G6PD glycosylation enhances PPP flow to the building blocks of macromolecular biosynthesis promoting the proliferation of tumor cells (Rao et al., 2015). Hypoxic or ERK-induced G6PD O-GlcNAcylation levels are increased in an OGT-dependent manner (Rao et al., 2015; Su et al., 2021). Thus, in addition to directly targeting the enzymatic activity of G6PD, targeting OGT may also be an effective strategy for inhibiting G6PD enzyme activity.

G6PD acetylation

The level of acetylation of certain proteins in cells is determined by the balance between histone deacetylases (HDACs) and histone acetyltransferase (HATs), enzymes that add or remove acetyl groups from lysine residues, respectively (Li W. et al., 2021). KAT9/ELP3, an acetyltransferase, mediates G6PD K403 acetylation to inhibit the enzymatic activity of G6PD (Wang et al., 2014). Conversely, deacetylation of G6PD mediated by deacetylase Sirt2 enhances the enzymatic activity of G6PD and counteracts excessive oxidative stress (Wang et al., 2014; Xu et al., 2016). Furthermore, a report by Zhang et al. indicates that Sirt2 can bind to G6PD and regulate the deacetylation of G6PD K171 promoting the progression of hepatocellular carcinoma (Zhang et al., 2021). In addition to its role as a deacetylase involved in the regulation of G6PD enzyme activity, Sirt2 also maintains the stability of G6PD (Ni et al., 2021). Aspirin, a common clinical analgesic and antipyretic drug, has also been reported to be involved in the regulation of acetylation. It has been shown that aspirin inhibits tumor cell proliferation by inducing G6PD acetylation and correspondingly reducing the enzymatic activity of G6PD to increases oxidative stress (Raza et al., 2011; Ai et al., 2016).

Newly identified post-translational modifications of G6PD

Several novel post-translational modifications located on histone lysine residues have been identified including propionylation, butyrylation, 2-hydroxyisobutyrylation, succinylation, malonylation, glutarylation, crotonylation, and β-hydroxybutyrylation (Sabari et al., 2017). Notably, there are acylation modifications that are not exclusively restricted to histones. Deglutarylation of G6PD by deacylasesirtuin 5 increases its enzymatic activity (Zhou et al., 2016). Moreover, alterations in H4K8 2-hydroxyisobutyrylation can affect intracellular glucose metabolism (Huang et al., 2017), but whether G6PD is capable of 2-hydroxyisobutyrylation requires further investigation. On the other hand, the lactylation modification of histone lysine residues has been widely studied (Zhang D. et al., 2019). Existing studies have shown that P300 and HDAC1/3 act as lactylation modification "writers" or "erasers" to add or remove lactic acid groups on lysine residues of histones in macrophages, respectively (Zhang D. et al., 2019; Moreno-Yruela et al., 2022). Consistent with glutarylation modifications, lactylation modifications also occur in non-histone proteins. Glycolysis-derived lactate has been found to increase high mobility group box protein 1 lactylation to induce its ectopic transfer from the nucleus to the cytoplasm, enhancing its release from macrophages via exosomes (Yang et al., 2022). In conclusion, these newly identified post-translational modifications are not only restricted to histones (Sabari et al., 2017), but also other proteins (Yang et al., 2022), including G6PD (Zhou et al., 2016). Location and/or enzymatic activity of these targets are hence regulated through these post-translational modifications (Figure 2).

Post-Translational modifications modify G6PD structure

The G6PD protein is composed of approximately 515 amino acid polypeptides and has an apparent molecular mass of approximately 59 kD. G6PD exist as an inactive monomer

and active dimer as well as a tetramer (Hilf et al., 1975). Various factors, including pH value and ionic strength, affect the formation of dimers and tetramers. High values of pH and ion concentration promote the conversion of tetramers to dimers. Conversely, mild oxidative treatment results in the accumulation of tetramers with a corresponding decrease in dimers. Thus, there is an equilibrium between the dimers and tetramers (Hilf et al., 1975). In addition to factors regulating the structure of G6PD, NADPH converts dimers, but not tetramers, to monomers (Bonsignore et al., 1971). Therefore, NADPH is considered a potent inhibitor of G6PD. Depletion of $NADP^+$, a G6PD coenzyme, results in the conversion of the G6PD dimers into monomers; reincubation of $NADP^+$ with the dissociated protein restores dimer expression. This indicates that dimers and monomers can be reversibly converted into each other (Cancedda et al., 1973; Au et al., 1999).

PTM modification of G6PD is involved in the regulation of dimerization. The G6PD molecule has two $NADP^+$ binding sites including a structural $NADP^+$ binding site and a coenzyme $NADP^+$ binding site (Kotaka et al., 2005). Structural $NADP^+$ sites are closer to the dimeric interface of G6PD than those of coenzyme G6PD sites, thus structural $NADP^+$ binding sites are more important in regulating G6PD enzymatic activity and structural integrity than coenzyme structural sites (Au et al., 2000). In G6PD class I mutants, mutations located at the dimer interface and close to the $NADP^+$ structural site lead to a 90% loss of function (Horikoshi et al., 2021), which further suggests that the $NADP^+$ structural site is involved in the regulation of enzyme activity. A total of 57 amino acids have been identified at the dimer interface of G6PD, three of which are involved in dimer and monomer conversions, with the remaining sites in need of further investigation. In addition, mutations in T406, K403, and Y401 proteins, located at the dimer interface, promote the conversion of G6PD dimers to monomers. Specifically, FYN and Plk1 are directly phosphorylated to activate G6PD K401 and K406, promoting dimer formation and increasing enzyme activity, respectively (Ma et al., 2017; Mattè et al., 2020). In addition, KAT9-mediated acetylation of G6PD (K403) inhibits dimer formation of G6PD (Wang et al., 2014) (Figure 3).

G6PD-Rrgulated downstream signalings

G6PD inhibits ferroptosis

Ferroptosis is a novel type of iron-dependent regulated cell death (Dixon et al., 2012). Morphologically, ferroptosis is characterized by an increase in mitochondrial membrane density, reduction or disappearance of mitochondrial cristae, and rupture of the external mitochondrial membrane. Mechanistically, the accumulation of lipid peroxidation by the Fenton reaction between iron ions and ROS in cells leads to ferroptosis. NADPH is an important intracellular reducing equivalent to neutralize ROS and maintain redox homeostasis. According to the MetaCyc database (Caspi et al., 2020), there are at least 143 reactions for the conversion of NADP to NADPH, but only a limited number of these reactions are considered to be contributed significantly from NADP to NADPH conversion. The major source of NADPH in mammals is folate metabolism (methylenetetrahydrofolate dehydrogenase), glutaminolysis (malic enzymes), and oxPPP (G6PD, 6-Phosphogluconate dehydrogenase; 6PGD), of which G6PD is the largest contributor to NADPH production (Chen et al., 2019). Activation of PPP produces NADPH, which promotes resistance of clear cell renal cell carcinoma to ROS and ferroptosis (Zheng et al., 2021). In addition, it has been shown that the expression of cytochrome P450 oxidoreductase (POR), a positive regulator of ferroptosis, is significantly increased in G6PD knockdown hepatocellular carcinoma (HCC) cells, which suggests that G6PD may inhibit ferroptosis through POR (Cao et al., 2021). Thus, G6PD may regulate ferroptosis in an NADPH-dependent manner.

G6PD-mediated metabolites regulate amp-activated protein kinase

Most studies have shown that alterations in signaling pathways can affect metabolites in PPP. Notably, G6PD-mediated metabolites can also regulate signaling molecules. G-6-phosphogluconolactone, a catalytic product of G6PD, can directly bind to Src to enhance the recruitment of protein phosphatase 2A and inhibit the activation of AMPK (Gao et al., 2019). In addition, Ru-5-P, the main metabolite of oxPPP, inactivates AMPK by inhibiting the formation of liver kinase B1 (Lin et al., 2015).

Role of G6PD In Non-Neoplastic diseases

G6PD and virus infection

Pathogen infections are more likely to occur in G6PD-deficient subjects because they have a decreased ability to activate the innate immune response (Yen et al., 2020). The Zika virus (ZIKV) genome is made up of a single-strand, positive-sense RNA with only 10 genes bordered by two untranslated sections (Savidis et al., 2016). ZIKV infection elicits a glycolytic response, as shown by increased extracellular acidification rate and expression of key glycolytic genes (*GLUT1*, *HK2*, *TPI*, and *MCT4*), according to bioinformation studies (Tiwari et al., 2017; Singh et al., 2020). Furthermore, infection with ZIKV leads to metabolic reprogramming and diversion of glycolytic carbon to PPP

(Yau et al., 2021). Therefore, it suggest that ZIKV may increase the flow of PPP by upregulating enzymes including G6PD. In addition, it has been shown that activation of AMPK, a switch in energy metabolism, attenuates ZIKV infection of host cells (Singh et al., 2020). Indeed, pharmacological inhibition or knockdown of AMPK reduces G6PD expression (Shan et al., 2019). Thus, a potential regulatory mechanism for ZIKV virus infection of host cells may be mediated through the AMPK-G6PD axis. Similarly, during Kaposi's sarcoma-associated herpesvirus (KSHV) infection of the human dermal microvascular endothelial, the metabolic pathway shifts from glycolysis to PPP, which is accompanied by a KSHV-induced increase in G6PD and transketolase expression (Sriram et al., 2008). The enhancement in PPP provides KSHV with a supply of nucleotides for the synthesis of host genes necessary for infection or for the synthesis of viral genes during early cellular bursts of the virus. Conversely, it is worth noting that the influenza virus reduces *G6PD* expression and enzyme activity, leading to an increase in oxidative stress and virus replication (De Angelis et al., 2021). Consistent with influenza virus infection, HIV, influenza A, respiratory syncytial virus, and enterovirus 71 induce oxidative stress and are usually suppressed by antioxidants like N-acetyl cysteine (Jain et al., 2020). In conclusion, the above studies that G6PD plays different roles in different types of viral infections.

Since 2020, the coronavirus disease (COVID-19) was declared as global pandemic, with hundreds of millions of people infected worldwide and increasing numbers of people becoming infected to date. However, no specific antiviral medications are currently available. There have been clinical trials using chloroquine and hydroxychloroquine (CQ/HCQ) to treat COVID-19. Several studies have shown that COVID-19 patients with G6PD deficiency show severe hemolysis during treatment with CQ/HCQ, which increase intracellular ROS in therapeutic dosages (da Rocha et al., 2021). Therefore, it is necessary to check the G6PD status of patients if CQ/HCQ is used to treat COVID-19.

Virus induces global changes of PTMs in host cell during infection to facilitate its successful infection and dissemination (Hu et al., 2020). To generate progeny virus, influenza virus replication requires a substantial number of nucleic acids for the synthesis of viral RNA (vRNA), complementary RNA (cRNA), and messenger RNA (mRNA). Vast amounts of energy are also required in the process of generating large amounts of RNAs. Pyruvate kinase M2 (PKM2), which catalyzes the production of ATP in glycolysis, becomes more acidic due to increased phosphorylation after influenza virus infection, and phosphorylated PKM2, which is active as a protein kinase, binds to RNA-dependent RNA polymerase involved in vRNA replication. Therefore, inhibition of PKM2 may be an effective strategy to attenuate viral replication. Whether G6PD phosphorylation is activated after infection to promote viral replication still needs further study (Miyake et al., 2017).

G6PD and vascular diseases

Vascular remodeling is an important pathological phenotypic change in cardiovascular diseases, including hypertension and atherosclerosis, in which vascular smooth muscle plays an important role (Gong et al., 2021). Vascular smooth muscle cells (SMCs) undergo several alterations during biological processes, including phenotypic transformation, proliferation, and apoptosis during disease progression. Multiple studies have shown that G6PD deficiency increases the risk of cardiovascular disease, which implies that G6PD may act as a regulator of SMCs (Pes et al., 2019; Parsanathan and Jain, 2020). Differentiated SMCs located in the middle layer of the vessel wall can contract and relax to regulate blood flow through the circulatory system. SMCs-restricted gene (*Myocd*, *Tagln*, *Myh11*, and *Cnn1*) expression maintains SMCs in a differentiated state; in contrast, downregulation of SMCs-restricted gene expression leads to SMCs cell dedifferentiation causing vascular remodeling. Pharmacological inhibition of G6PD or knockdown of G6PD promotes SMCs-restricted gene expression to maintain vascular function (Dhagia et al., 2021). Therefore, G6PD maintains the dedifferentiated state of SMCs cells to avoid impaired vascular function. In addition, G6PD regulates the relaxation and contraction of vascular smooth muscle by altering the opening and closure of ion channels. G6PD can be activated by protein kinase C to elicit intracellular free Ca^{2+} and thus enhance the contraction of vascular smooth muscle (Ata et al., 2011). Conversely, pharmacological inhibition of G6PD relaxes vascular smooth muscle by opening potassium channels (Farrukh et al., 1998). G6PD-mediated metabolites are also involved in the regulation of vascular smooth muscle contraction. NADPH, the metabolite catalyzed by G6PD, relaxes vascular smooth muscle by inhibiting the dimer formation of PKG1α (Neo et al., 2013; Patel et al., 2014).

Inhibitors

Small molecule inhibitors are useful tools for studying the function of metabolic enzymes. To date, there are 265 compounds that could be potential G6PD inhibitors according to data from BRENDA (https://www.brenda-enzymes.org). However, no details of the specific inhibitors of G6PD are yet available. In the following section, we review the G6PD inhibitors that are widely used in basic research and summarize their concentration and duration of application in different cells and animal models (Tables 1, 2).

Dehydroepiandrosterone (DHEA) was identified as a non-competitive G6PD inhibitor in 1960 (Marks and Banks, 1960). DHEA sulfate (DHEAs) is an androgen produced by the adrenal glands. Humans have the highest levels of circulating DHEAs of all the primates with levels that are generally higher in males

TABLE 1 The effective dosages and durations or the application of G6PD inhibitors in cancer cells.

Inhibitors	Cell lines	Cancer type	Dose (μM)	Duration (H)	References
6-An	H1944	Lung cancer	56.37 ± 2.93	48	Sun et al., (2022)
	H1299	Lung cancer	202.40 ± 39.21	48	Sun et al., (2022)
	H1975	Lung cancer	6.91 ± 0.77	48	Sun et al., (2022)
	A549	Lung cancer	56.27 ± 2.72	48	Sun et al., (2022)
	A549/H460/H358/H441	Lung cancer	62.5	72	Best et al., (2019)
	A549	Lung cancer	500	18–24	Budihardjo et al., (1998)
	T98G	Brain glioblastoma	250	18–24	Budihardjo et al., (1998)
	MCF-7	Breast cancer	125	18–24	Budihardjo et al., (1998)
	OVCAR	Ovarian cancer	31	18–24	Budihardjo et al., (1998)
	U251	Brain glioblastoma	1,000	-	Sun et al., (2021b)
	786-O	kidney cancer	1,000	24	Zhang et al., (2020)
	PC3	Prostate cancer	100	24	Whitburn et al., (2022)
	LNCaP	Prostate cancer	100	24	Whitburn et al., (2022)
	MOLM-14/OCI-AML2/L60/OCI-AML3	-	100	48	Poulain et al., (2017)
	VSMCs	-	1,000	12	Dong et al., (2015)
	HEAC	-	100	12	Dong et al., (2015)
	PASM	-	1,000	72	Chettimada et al., (2015)
	Rat/Mouse neuronglia	-	10	24	Tu et al., (2019)
	Primary hepatocytes cell	-	5,000	0.2	Gupte et al., (2009)
DHEA	231-C3/231-M1	Breast cancer	200	12	Luo et al., (2022)
	HeLa	Cervical cancer	200	0.1	Roshanzadeh et al., (2019)
	WSU - HN6	Oral carcinoma	50	-	Wang et al., (2020)
	CAL27	Tongue carcinoma	50	-	Wang et al., (2020)
	GM00558	-	100	0.2	Cosentino et al., (2011)
	Human red blood cells	-	200	24	Handala et al., (2017)
	MEF	-	100	7	Heiss et al., (2013)
	Rat/Mouse neuronglia	-	100	24	Tu et al., (2019)
	Primary hepatocytes cell	-	100	10	Gupte et al., (2009)
	Pulmonary artery smoot muscle cell	-	100	72	Chettimada et al. (2015)
	Human aortic endothelial cell	-	100	12	Parsanathan and Jain, (2020)
Polydatin	HESCC	Esophageal carcinoma	100–300	24	Su et al., (2021)
	MCF-7	Breast cancer	30	24	Mele et al., (2019)
	HNSCC	Head and neck squamous cell carcinoma	22	24	Mele et al., (2018)
	HNSCC	Head and neck squamous cell carcinoma	17	48	Mele et al., (2018)
NEOU	H446	Lung cancer	10	48	Wang et al., (2022)
	SMCs	-	1	48	Dhagia et al., (2021)
Epi	A7r5	-	50	24	Dhagia et al., (2021)
DP20	Primary bone marrow cells	-	0.9	24	Hashimoto et al., (2020)

(3,200 ng/ml) than those in females (2000 ng/ml) (Nyce, 2021). DHEAs is an ineffective inhibitor of G6PD and is only transported into cells via organic anion transport protein (OATP), which is subsequently desulfated by sulfate esterase (SS) to eventually produce DHEA that inhibits G6PD activity (Klinge et al., 2018). Compared with hydrophilic DHEAs, lipophilic DHEA can function freely across cell membranes. Therefore, DHEA is widely used in cancer research to block G6PD enzyme activity and inhibit the proliferation and migration of cancer cells (Wang et al., 2020; Luo et al., 2022). Moreover, DHEA decreases intracellular NADPH levels by inhibiting G6PD, of which the effect is more pronounced under glucose deprivation (Roshanzadeh et al., 2019). However, Ghergurovich et al. showed that DHEA inhibited the enzymatic activity of G6PD in HepG2 cells, but this effect was not sustained (Ghergurovich et al., 2020). In addition to its

TABLE 2 The effective dosages and therapeutic durations of G6PD inhibitors in animal models of cancer.

Inhibitors	Organism	Dose	Duration	Injection type	References
6-An	Mouse	4 mg/kg/3d	-	Intraperitoneal injection	Sun et al., (2021b)
	Mouse	23 mg/kg/d	-	Intraperitoneal injection	Zhang et al., (2020)
	Mouse	20 mg/kg/10d	40d	Intraperitoneal injection	Best et al., (2019)
	Mouse	5 mg/kg/day	23d	Intraperitoneal injection	Poulain et al., (2017)
DHEA	Mouse	80 mg/kg/3d	20d	Intraperitoneal injection	Wang et al., (2020)
NEOU	Mouse	1.5 mg/kg/d	21d	Intraperitoneal injection	Kitagawa et al., (2021)
	Mouse	1.5 mg/kg/d	28d	Intraperitoneal injection	Joshi et al., (2020)
Epi	Rats	30 mg/kg/d	28d	Intraperitoneal injection	Dhagia et al., (2021)
Polydatin	Mouse	5 mg/kg/d	14d	Intraperitoneal injection	Su et al., (2021)
	Mouse	100 mg/kg	-	Intraperitoneal injection	Mele et al., (2018)

role in cancer therapy, DHEA can be potentially beneficial in the treatment of pulmonary hypertension and protecting against ribavirin antiviral therapy-induced hemolysis (Patel et al., 2014; Handala et al., 2017). Some men in the United States take oral DHEA to boost their androgen levels to prevent aging, but no scientific proof has been obtained. DHEA can significantly inhibit G6PD enzyme activity, resulting in increased susceptibility to COVID-19 (Nyce, 2021).

6-Aminonicotinamide (6-An) is a competitive non-specific G6PD inhibitor that competitively binds to $NADP^+$, to inhibit G6PD enzyme activity (Köhler et al., 1970). G6PD and 6PGD can generate NADPH from $NADP^+$, which suggests that 6-An can also bind competitively with 6PGD to inhibit its activity during oxPPP. The concentrations of 6-An thus should be considered when it is used to inhibit G6PD enzyme activity. 6-An does not affect G6PD, but instead, blocks 6PGD(Aurora et al., 2022). Earlier *in vivo* studies revealed that 6-An inhibits the carbon-atom transfer from glucose to ribose and suppresses oxPPP (Köhler et al., 1970). In addition, 6-An selectively enhances the toxicity of cisplatin, melphalan, and nitrogen mustard to promote apoptosis of tumor cells *in vitro* (Budihardjo et al., 1998).

Additional drugs have been identified to inhibit the enzymatic activity of G6PD. Polydatin, an active ingredient extracted from the traditional Chinese medicine *Polygonum multiflorum*, was identified to inhibit the activity of G6PD enzymes and NADPH in a dose-dependent manner thus suppress the growth and metastasis of tumor cells (Mele et al., 2018). Additionally, (N-ethyl-N = -[(3β,5α)-17-oxoandrostan-3-yl]urea, NEOU) has been reported to inhibit G6PD activity (Joshi et al., 2020).

Summary and perspectives

G6PD is the rate-limiting enzyme of the PPP. Along with serving as biosynthetic substrates, the G6PD-mediated metabolitesRu-5-P and NADPH regulate downstream signaling cascades and induce tumorigenesis (Lin et al., 2015). Lactatemay be employed as a substrate for lactylation modifications to regulate the expression of downstream genes. Lactylation modifications of non-histone proteins may be of great interest for future research, even if no relevant reports are currently available (Sun L. et al., 2021).

In addition, we reviewed the role of G6PD in tumorigenesis and related non-neoplastic diseases, of which we mainly focused on the role of post-translational modifications of G6PD. Post-translational modifications of histones, transcription factors, and other upstream multiple signals are involved in regulating the expression of G6PD. Glycosylation and phosphorylation modifications of G6PD promote dimer formation and increase enzyme activity (Rao et al., 2015; Zeng et al., 2016; Ma et al., 2017). Conversely, acetylation modifications promote dimer to monomer conversion and inhibit enzyme activity G6PD (Wang et al., 2014; Zhang et al., 2021). G6PD not only plays a role in tumorigenesis, but also in the process of viral infection. Briefly, viruses may inhibit intracellular metabolism and reduce the enzymatic activity of G6PD to promote viral infection during the early stages. Furthermore, viruses may activate metabolic pathways, including PPP, to promote viral replication at later stages. Finally, inhibitors of G6PD were summarized and the potential of G6PD as a clinical therapeutic target was evaluated.

Multiple post-translational modification sites of G6PD were identified by mass spectrometry. Serine at position 84 of G6PD could be glycosylated to increase the enzyme activity (Rao et al., 2015). In contrast, the enzyme activity was abolished by acetylation modification of lysine at 403. However, the reasons that changes in modifications affect enzyme activity need further investigation. In addition, serine is widely known to be phosphorylation modified, but no phosphorylation modification was identified at serine 84. Although a variety of G6PD modifications have been identified, there are still many questions that deserve further investigation. Based on this review, two questions were subsequently raised 1) Is there a prior order of post-translational modifications that occur in G6PD? 2) How do the various post-translational modifications collaborate? In

summary, we highlight the role of post-translational modifications of G6PD in regulating structure, enzyme activity, and function. Therefore, targeting post-translational modifications of G6PD may serve as a novel therapeutic strategy.

Author contributions

Z-XX, YW, and HZ constructed the outline of the review. QM wrote the draft. YZ, HS, BL, and HS revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (No: 82020108024), International Cooperation project of the Department of Science and Technology of Jilin Province (No: 20210402005GH), Health Commission of Jilin Province (No: 2020J033) and The Department of Finance of Jilin Province (Nos: JLSWSRCZX2020-020).

Acknowledgments

We would like to thank Editage (www.editage.cn) for English language editing.

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

Ai, G., Dachineni, R., Kumar, D. R., Alfonso, L. F., Marimuthu, S., and Bhat, G. J. (2016). Aspirin inhibits glucose6phosphate dehydrogenase activity in HCT 116 cells through acetylation: Identification of aspirin-acetylated sites. *Mol. Med. Rep.* 14 (2), 1726–1732. doi:10.3892/mmr.2016.5449

Ata, H., Rawat, D. K., Lincoln, T., and Gupte, S. A. (2011). Mechanism of glucose-6-phosphate dehydrogenase-mediated regulation of coronary artery contractility. *Am. J. Physiol. Heart Circ. Physiol.* 300 (6), H2054–H2063. doi:10.1152/ajpheart.01155.2010

Au, S. W., Gover, S., Lam, V. M., and Adams, M. J. (2000). Human glucose-6-phosphate dehydrogenase: The crystal structure reveals a structural NADP(+) molecule and provides insights into enzyme deficiency. *Structure* 8 (3), 293–303. doi:10.1016/s0969-2126(00)00104-0

Au, S. W., Naylor, C. E., Gover, S., Vandeputte-Rutten, L., Scopes, D. A., Mason, P. J., et al. (1999). Solution of the structure of tetrameric human glucose 6-phosphate dehydrogenase by molecular replacement. *Acta Crystallogr. D. Biol. Crystallogr.* 55 (4), 826–834. doi:10.1107/s0907444999000827

Aurora, A. B., Khivansara, V., Leach, A., Gill, J. G., Martin-Sandoval, M., Yang, C., et al. (2022). Loss of glucose 6-phosphate dehydrogenase function increases oxidative stress and glutaminolysis in metastasizing melanoma cells. *Proc. Natl. Acad. Sci. U. S. A.* 119 (6), e2120617119. doi:10.1073/pnas.2120617119

Best, S. A., Ding, S., Kersbergen, A., Dong, X., Song, J.-Y., Xie, Y., et al. (2019). Distinct initiating events underpin the immune and metabolic heterogeneity of KRAS-mutant lung adenocarcinoma. *Nat. Commun.* 10 (1), 4190. doi:10.1038/s41467-019-12164-y

Boguslawska, J., Poplawski, P., Alseekh, S., Koblowska, M., Iwanicka-Nowicka, R., Rybicka, B., et al. (2019). MicroRNA-mediated metabolic reprograming in renal cancer. *Cancers (Basel)* 11 (12), E1825. doi:10.3390/cancers11121825

Bonsignore, A., Cancedda, R., Nicolini, A., Damiani, G., De Flora, A., and De FlorA, A. (1971). Metabolism of human erythrocyte glucose-6-phosphate dehydrogenase. VI. Interconversion of multiple molecular forms. *Arch. Biochem. Biophys.* 147 (2), 493–501. doi:10.1016/0003-9861(71)90406-1

Budihardjo, I. I., Walker, D. L., Svingen, P. A., Buckwalter, C. A., Desnoyers, S., Eckdahl, S., et al. (1998). 6-Aminonicotinamide sensitizes human tumor cell lines to cisplatin. *Clin. Cancer Res.* 4 (1), 117–130.

Cancedda, R., Ogunmola, G., and Luzzatto, L. (1973). Genetic variants of human erythrocyte glucose-6-phosphate dehydrogenase. Discrete conformational states stabilized by NADP + and NADPH. *Eur. J. Biochem.* 34 (1), 199–204. doi:10.1111/j.1432-1033.1973.tb02746.x

Cao, F., Luo, A., and Yang, C. (2021). G6PD inhibits ferroptosis in hepatocellular carcinoma by targeting cytochrome P450 oxidoreductase. *Cell. Signal.* 87, 110098. doi:10.1016/j.cellsig.2021.110098

Caspi, R., Billington, R., Keseler, I. M., Kothari, A., Krummenacker, M., Midford, P. E., et al. (2020). The MetaCyc database of metabolic pathways and enzymes - a 2019 update. *Nucleic Acids Res.* 48 (D1), D445–D453. doi:10.1093/nar/gkz862

Chen, L., Zhang, Z., Hoshino, A., Zheng, H. D., Morley, M., Arany, Z., et al. (2019). NADPH production by the oxidative pentose-phosphate pathway supports folate metabolism. *Nat. Metab.* 1 (3), 404–415. doi:10.1038/s42255-019-0043-x

Chettimada, S., Gupte, R., Rawat, D., Gebb, S. A., McMurtry, I. F., and Gupte, S. A. (2015). Hypoxia-induced glucose-6-phosphate dehydrogenase overexpression and -activation in pulmonary artery smooth muscle cells: Implication in pulmonary hypertension. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 308 (3), L287–L300. doi:10.1152/ajplung.00229.2014

Coda, D., Lingua, M., Morena, D., Foglizzo, V., Bersani, F., Ala, U., et al. (2015). SMYD1 and G6PD modulation are critical events for miR-206-mediated differentiation of rhabdomyosarcoma. *Cell cycleGeorget. Tex.)* 14 (9), 1389–1402. doi:10.1080/15384101.2015.1005993

Cosentino, C., Grieco, D., and Costanzo, V. (2011). ATM activates the pentose phosphate pathway promoting anti-oxidant defence and DNA repair. *EMBO J.* 30 (3), 546–555. doi:10.1038/emboj.2010.330

da Rocha, J. E. B., Othman, H., Tiemessen, C. T., Botha, G., Ramsay, M., Masimirembwa, C., et al. (2021). G6PD distribution in sub-Saharan Africa and potential risks of using chloroquine/hydroxychloroquine based treatments for COVID-19. *Pharmacogenomics J.* 21 (6), 649–656. doi:10.1038/s41397-021-00242-8

De Angelis, M., Amatore, D., Checconi, P., Zevini, A., Fraternale, A., Magnani, M., et al. (2021). Influenza virus down-modulates G6PD expression and activity to induce oxidative stress and promote its replication. *Front. Cell. Infect. Microbiol.* 11, 804976. doi:10.3389/fcimb.2021.804976

Deng, P., Li, K., Gu, F., Zhang, T., Zhao, W., Sun, M., et al. (2021). LINC00242/miR-1-3p/G6PD axis regulates Warburg effect and affects gastric cancer proliferation and apoptosis. *Mol. Med.* 27 (1), 9. doi:10.1186/s10020-020-00259-y

Dhagia, V., Kitagawa, A., Jacob, C., Zheng, C., D'Alessandro, A., Edwards, J. G., et al. (2021). G6PD activity contributes to the regulation of histone acetylation and

gene expression in smooth muscle cells and to the pathogenesis of vascular diseases. *Am. J. Physiol. Heart Circ. Physiol.* 320 (3), H999–H1016. doi:10.1152/ajpheart.00488.2020

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al. (2012). Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell* 149 (5), 1060–1072. doi:10.1016/j.cell.2012.03.042

Dong, L.-H., Li, L., Song, Y., Duan, Z.-L., Sun, S.-G., Lin, Y.-L., et al. (2015). TRAF6-Mediated SM22α K21 ubiquitination promotes G6PD activation and NADPH production, contributing to GSH homeostasis and VSMC survival *in vitro* and *in vivo*. *Circ. Res.* 117 (8), 684–694. doi:10.1161/CIRCRESAHA.115.306233

Du, W., Jiang, P., Mancuso, A., Stonestrom, A., Brewer, M. D., Minn, A. J., et al. (2013). TAp73 enhances the pentose phosphate pathway and supports cell proliferation. *Nat. Cell Biol.* 15 (8), 991–1000. doi:10.1038/ncb2789

Farrukh, I. S., Peng, W., Orlinska, U., and Hoidal, J. R. (1998). Effect of dehydroepiandrosterone on hypoxic pulmonary vasoconstriction: A Ca(2+)-activated K(+)-channel opener. *Am. J. Physiol.* 274 (2), L186–L195. doi:10.1152/ajplung.1998.274.2.L186

Gao, X., Zhao, L., Liu, S., Li, Y., Xia, S., Chen, D., et al. (2019). γ-6-Phosphogluconolactone, a byproduct of the oxidative pentose phosphate pathway, contributes to AMPK activation through inhibition of PP2A. *Mol. Cell* 76 (6), 857–871. doi:10.1016/j.molcel.2019.09.007

Ghergurovich, J. M., Garcia-Canaveras, J. C., Wang, J., Schmidt, E., Zhang, Z., TeSlaa, T., et al. (2020). A small molecule G6PD inhibitor reveals immune dependence on pentose phosphate pathway. *Nat. Chem. Biol.* 16 (7), 731–739. doi:10.1038/s41589-020-0533-x

Gomez-Manzo, S., Marcial-Quino, J., Vanoye-Carlo, A., Serrano-Posada, H., Ortega-Cuellar, D., Gonzalez-Valdez, A., et al. (2016). Glucose-6-Phosphate dehydrogenase: Update and analysis of new mutations around the world. *Int. J. Mol. Sci.* 17 (12), E2069. doi:10.3390/ijms17122069

Gong, C., Qiao, L., Feng, R., Xu, Q., Zhang, Y., Fang, Z., et al. (2020). IL-6-induced acetylation of E2F1 aggravates oxidative damage of retinal pigment epithelial cell line. *Exp. Eye Res.* 200, 108219. doi:10.1016/j.exer.2020.108219

Gong, X., Tian, M., Cao, N., Yang, P., Xu, Z., Zheng, S., et al. (2021). Circular RNA circEsyt2 regulates vascular smooth muscle cell remodeling via splicing regulation. *J. Clin. Invest.* 131 (24), e147031. doi:10.1172/JCI147031

Gu, M., Zhou, X., Sohn, J. H., Zhu, L., Jie, Z., Yang, J.-Y., et al. (2021). NF-κB-inducing kinase maintains T cell metabolic fitness in antitumor immunity. *Nat. Immunol.* 22 (2), 193–204. doi:10.1038/s41590-020-00829-6

Gupte, R. S., Floyd, B. C., Kozicky, M., George, S., Ungvari, Z. I., Neito, V., et al. (2009). Synergistic activation of glucose-6-phosphate dehydrogenase and NAD(P)H oxidase by Src kinase elevates superoxide in type 2 diabetic, Zucker fa/fa, rat liver. *Free Radic. Biol. Med.* 47 (3), 219–228. doi:10.1016/j.freeradbiomed.2009.01.028

Handala, L., Domange, B., Ouled-Haddou, H., Garçon, L., Nguyen-Khac, E., Helle, F., et al. (2017). DHEA prevents ribavirin-induced anemia via inhibition of glucose-6-phosphate dehydrogenase. *Antivir. Res.* 146, 153–160. doi:10.1016/j.antiviral.2017.09.002

Hashimoto, R., Lanier, G. M., Dhagia, V., Joshi, S. R., Jordan, A., Waddell, I., et al. (2020). Pluripotent hematopoietic stem cells augment α-adrenergic receptor-mediated contraction of pulmonary artery and contribute to the pathogenesis of pulmonary hypertension. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 318 (2), L386–L401. doi:10.1152/ajplung.00327.2019

Heiss, E. H., Schachner, D., Zimmermann, K., and Dirsch, V. M. (2013). Glucose availability is a decisive factor for Nrf2-mediated gene expression. *Redox Biol.* 1, 359–365. doi:10.1016/j.redox.2013.06.001

Hilf, R., Ickowicz, R., Bartley, J. C., and Abraham, S. (1975). Multiple molecular forms of glucose-6-phosphate dehydrogenase in normal, preneoplastic, and neoplastic mammary tissues of mice. *Cancer Res.* 35 (8), 2109–2116.

Horikoshi, N., Hwang, S., Gati, C., Matsui, T., Castillo-Orellana, C., Raub, A. G., et al. (2021). Long-range structural defects by pathogenic mutations in most severe glucose-6-phosphate dehydrogenase deficiency. *Proc. Natl. Acad. Sci. U. S. A.* 118 (4), e2022790118. doi:10.1073/pnas.2022790118

Hu, J., Zhang, L., and Liu, X. (2020). Role of post-translational modifications in influenza A virus life cycle and host innate immune response. *Front. Microbiol.* 11, 517461. doi:10.3389/fmicb.2020.517461

Huang, J., Luo, Z., Ying, W., Cao, Q., Huang, H., Dong, J., et al. (2017). 2-Hydroxyisobutyrylation on histone H4K8 is regulated by glucose homeostasis in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* 114 (33), 8782–8787. doi:10.1073/pnas.1700796114

Jain, S. K., Parsanathan, R., Levine, S. N., Bocchini, J. A., Holick, M. F., and Vanchiere, J. A. (2020). The potential link between inherited G6PD deficiency, oxidative stress, and vitamin D deficiency and the racial inequities in mortality associated with COVID-19. *Free Radic. Biol. Med.* 161, 84–91. doi:10.1016/j.freeradbiomed.2020.10.002

Jiang, A., Dong, C., Li, B., Zhang, Z., Chen, Y., Ning, C., et al. (2019). MicroRNA-206 regulates cell proliferation by targeting G6PD in skeletal muscle. *FASEB J. official Publ. Fed. Am. Soc. Exp. Biol.* 33 (12), 14083–14094. doi:10.1096/fj.201900502RRRR

Joshi, S. R., Kitagawa, A., Jacob, C., Hashimoto, R., Dhagia, V., Ramesh, A., et al. (2020). Hypoxic activation of glucose-6-phosphate dehydrogenase controls the expression of genes involved in the pathogenesis of pulmonary hypertension through the regulation of DNA methylation. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 318 (4), L773–L786. doi:10.1152/ajplung.00001.2020

Ju, H.-Q., Lin, J.-F., Tian, T., Xie, D., and Xu, R.-H. (2020). NADPH homeostasis in cancer: Functions, mechanisms and therapeutic implications. *Signal Transduct. Target. Ther.* 5 (1), 231. doi:10.1038/s41392-020-00326-0

Kitagawa, A., Jacob, C., Jordan, A., Waddell, I., McMurtry, I. F., and Gupte, S. A. (2021). Inhibition of glucose-6-phosphate dehydrogenase activity attenuates right ventricle pressure and hypertrophy elicited by VEGFR inhibitor + hypoxia. *J. Pharmacol. Exp. Ther.* 377 (2), 284–292. doi:10.1124/jpet.120.000166

Klinge, C. M., Clark, B. J., and Prough, R. A. (2018). Dehydroepiandrosterone research: Past, current, and future. *Vitam. Horm.* 108, 1–28. doi:10.1016/bs.vh.2018.02.002

Köhler, E., Barrach, H. J., and Neubert, D. (1970). Inhibition of NADP dependent oxidoreductases by the 6-aminonicotinamide analogue of NADP. *FEBS Lett.* 6 (3), 225–228. doi:10.1016/0014-5793(70)80063-1

Kotaka, M., Gover, S., Vandeputte-Rutten, L., Au, S. W., Lam, V. M., and Adams, M. J. (2005). Structural studies of glucose-6-phosphate and NADP+ binding to human glucose-6-phosphate dehydrogenase. *Acta Crystallogr. D. Biol. Crystallogr.* 61 (5), 495–504. doi:10.1107/S0907444905002350

Li, W., Kou, J., Qin, J., Li, L., Zhang, Z., Pan, Y., et al. (2021a). NADPH levels affect cellular epigenetic state by inhibiting HDAC3–Ncor complex. *Nat. Metab.* 3, 75–89. doi:10.1038/s42255-020-00330-2

Li, Z., He, Y., Li, Y., Li, J., Zhao, H., Song, G., et al. (2021b). NeuroD1 promotes tumor cell proliferation and tumorigenesis by directly activating the pentose phosphate pathway in colorectal carcinoma. *Oncogene* 40, 6736–6747. doi:10.1038/s41388-021-02063-2

Lin, R., Elf, S., Shan, C., Kang, H.-B., Ji, Q., Zhou, L., et al. (2015). 6-Phosphogluconate dehydrogenase links oxidative PPP, lipogenesis and tumour growth by inhibiting LKB1-AMPK signalling. *Nat. Cell Biol.* 17 (11), 1484–1496. doi:10.1038/ncb3255

Liu, B., Fang, M., He, Z., Cui, D., Jia, S., Lin, X., et al. (2015). Hepatitis B virus stimulates G6PD expression through HBx-mediated Nrf2 activation. *Cell Death Dis.* 6, e1980. doi:10.1038/cddis.2015.322

Lu, C., Yang, D., Klement, J. D., Colson, Y. L., Oberlies, N. H., Pearce, C. J., et al. (2022). H3K9me3 represses G6PD expression to suppress the pentose phosphate pathway and ROS production to promote human mesothelioma growth. *Oncogene* 41, 2651–2662. doi:10.1038/s41388-022-02283-0

Luo, M., Fu, A., Wu, R., Wei, N., Song, K., Lim, S., et al. (2022). High expression of G6PD increases doxorubicin resistance in triple negative breast cancer cells by maintaining GSH level. *Int. J. Biol. Sci.* 18 (3), 1120–1133. doi:10.7150/ijbs.65555

Lv, Y., Lv, X., Zhang, J., Cao, G., Xu, C., Zhang, B., et al. (2022). BRD4 targets the KEAP1-nrf2-G6PD Axis and suppresses redox metabolism in small cell lung cancer. *Antioxidants (Basel)* 11 (4), 661. doi:10.3390/antiox11040661

Ma, H., Zhang, F., Zhou, L., Cao, T., Sun, D., Wen, S., et al. (2021). c-Src facilitates tumorigenesis by phosphorylating and activating G6PD. *Oncogene* 40, 2567–2580. doi:10.1038/s41388-021-01673-0

Ma, X., Wang, L., HuangLi, Y., Yang, D., Li, T., Li, F., et al. (2017). Polo-like kinase 1 coordinates biosynthesis during cell cycle progression by directly activating pentose phosphate pathway. *Nat. Commun.* 8 (1), 1506. doi:10.1038/s41467-017-01647-5

Makarona, K., Caputo, V. S., Costa, J. R., Liu, B., O'Connor, D., Iskander, D., et al. (2014). Transcriptional and epigenetic basis for restoration of G6PD enzymatic activity in human G6PD-deficient cells. *Blood* 124 (1), 134–141. doi:10.1182/blood-2014-02-553792

Marks, P. A., and Banks, J. (1960). Inhibition of mammalian glucose-6-phosphate dehydrogenase by steroids. *Proc. Natl. Acad. Sci. U. S. A.* 46 (4), 447–452. doi:10.1073/pnas.46.4.447

Mattè, A., Lupo, F., Tibaldi, E., Di Paolo, M. L., Federti, E., Carpentieri, A., et al. (2020). Fyn specifically Regulates the activity of red cell glucose-6-phosphate-dehydrogenase. *Redox Biol.* 36, 101639. doi:10.1016/j.redox.2020.101639

Mele, L., la Noce, M., Paino, F., Regad, T., Wagner, S., Liccardo, D., et al. (2019). Glucose-6-phosphate dehydrogenase blockade potentiates tyrosine kinase inhibitor

effect on breast cancer cells through autophagy perturbation. *J. Exp. Clin. Cancer Res.* 38 (1), 160. doi:10.1186/s13046-019-1164-5

Mele, L., Paino, F., Papaccio, F., Regad, T., Boocock, D., Stiuso, P., et al. (2018). A new inhibitor of glucose-6-phosphate dehydrogenase blocks pentose phosphate pathway and suppresses malignant proliferation and metastasis *in vivo*. *Cell Death Dis.* 9 (5), 572. doi:10.1038/s41419-018-0635-5

Miyake, Y., Ishii, K., and Honda, A. (2017). Influenza virus infection induces host pyruvate kinase M which interacts with viral RNA-dependent RNA polymerase. *Front. Microbiol.* 8, 162. doi:10.3389/fmicb.2017.00162

Moreno-Yruela, C., Zhang, D., Wei, W., Baek, M., Liu, W., Gao, J., et al. (2022). Class I histone deacetylases (HDAC1-3) are histone lysine delactylases. *Sci. Adv.* 8 (3), eabi6696. doi:10.1126/sciadv.abi6696

Neo, B. H., Patel, D., Kandhi, S., and Wolin, M. S. (2013). Roles for cytosolic NADPH redox in regulating pulmonary artery relaxation by thiol oxidation-elicited subunit dimerization of protein kinase G1α. *Am. J. Physiol. Heart Circ. Physiol.* 305 (3), H330–H343. doi:10.1152/ajpheart.01010.2011

Ni, Y., Yang, Z., Agbana, Y. L., Bai, H., Wang, L., Yang, L., et al. (2021). Silent information regulator 2 promotes clear cell renal cell carcinoma progression through deacetylation and small ubiquitin-related modifier 1 modification of glucose 6-phosphate dehydrogenase. *Cancer Sci.* 112 (10), 4075–4086. doi:10.1111/cas.15085

Nie, H., Huang, P. Q., Jiang, S. H., Yang, Q., Hu, L. P., Yang, X. M., et al. (2021). The short isoform of PRLR suppresses the pentose phosphate pathway and nucleotide synthesis through the NEK9-Hippo axis in pancreatic cancer. *Theranostics* 11 (8), 3898–3915. doi:10.7150/thno.51712

Nyce, J. (2021). Alert to US physicians: DHEA, widely used as an OTC androgen supplement, may exacerbate COVID-19. *Endocr. Relat. Cancer* 28 (2), R47–R53. doi:10.1530/ERC-20-0439

Pan, S., World, C. J., Kovacs, C. J., and Berk, B. C. (2009). Glucose 6-phosphate dehydrogenase is regulated through c-Src-mediated tyrosine phosphorylation in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 29 (6), 895–901. doi:10.1161/ATVBAHA.109.184812

Parsanathan, R., and Jain, S. K. (2020). Glucose-6-Phosphate dehydrogenase deficiency activates endothelial cell and leukocyte adhesion mediated via the TGFβ/NADPH oxidases/ROS signaling pathway. *Int. J. Mol. Sci.* 21 (20), E7474. doi:10.3390/ijms21207474

Patel, D., Kandhi, S., Kelly, M., Neo, B. H., and Wolin, M. S. (2014). Dehydroepiandrosterone promotes pulmonary artery relaxation by NADPH oxidation-elicited subunit dimerization of protein kinase G 1α. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 306 (4), L383–L391. doi:10.1152/ajplung.00301.2013

Pes, G. M., Parodi, G., and Dore, M. P. (2019). Glucose-6-phosphate dehydrogenase deficiency and risk of cardiovascular disease: A propensity score-matched study. *Atherosclerosis* 282, 148–153. doi:10.1016/j.atherosclerosis.2019.01.027

Poulain, L., Sujobert, P., Zylbersztejn, F., Barreau, S., Stuani, L., Lambert, M., et al. (2017). High mTORC1 activity drives glycolysis addiction and sensitivity to G6PD inhibition in acute myeloid leukemia cells. *Leukemia* 31 (11), 2326–2335. doi:10.1038/leu.2017.81

Rao, X., Duan, X., Mao, W., Li, X., Li, Z., Li, Q., et al. (2015). O-GlcNAcylation of G6PD promotes the pentose phosphate pathway and tumor growth. *Nat. Commun.* 6, 8468. doi:10.1038/ncomms9468

Raza, H., John, A., and Benedict, S. (2011). Acetylsalicylic acid-induced oxidative stress, cell cycle arrest, apoptosis and mitochondrial dysfunction in human hepatoma HepG2 cells. *Eur. J. Pharmacol.* 668 (1-2), 15–24. doi:10.1016/j.ejphar.2011.06.016

Roshanzadeh, A., Kang, H., You, S., Park, J., Khoa, N., Lee, D., et al. (2019). Real-time monitoring of NADPH levels in living mammalian cells using fluorescence-enhancing protein bound to NADPHs. *Biosens. Bioelectron.* 146, 111753. doi:10.1016/j.bios.2019.111753

Sabari, B. R., Zhang, D., Allis, C. D., and Zhao, Y. (2017). Metabolic regulation of gene expression through histone acylations. *Nat. Rev. Mol. Cell Biol.* 18 (2), 90–101. doi:10.1038/nrm.2016.140

Savidis, G., McDougall, W. M., Meraner, P., Perreira, J. M., Portmann, J. M., Trincucci, G., et al. (2016). Identification of zika virus and dengue virus dependency factors using functional genomics. *Cell Rep.* 16 (1), 232–246. doi:10.1016/j.celrep.2016.06.028

Shan, C., Lu, Z., Li, Z., Sheng, H., Fan, J., Qi, Q., et al. (2019). 4-hydroxyphenylpyruvate dioxygenase promotes lung cancer growth via pentose phosphate pathway (PPP) flux mediated by LKB1-AMPK/HDAC10/G6PD axis. *Cell Death Dis.* 10 (7), 525. doi:10.1038/s41419-019-1756-1

Singh, S., Singh, P. K., Suhail, H., Arumugaswami, V., Pellett, P. E., Giri, S., et al. (2020). AMP-activated protein kinase restricts zika virus replication in endothelial cells by potentiating innate antiviral responses and inhibiting glycolysis. *J. Immunol.* 204, 1810–1824. doi:10.4049/jimmunol.19013107

Sriram, G., Rahib, L., He, J.-S., Campos, A. E., Parr, L. S., Liao, J. C., et al. (2008). Global metabolic effects of glycerol kinase overexpression in rat hepatoma cells. *Mol. Genet. Metab.* 93 (2), 145–159. doi:10.1016/j.ymgme.2007.09.008

Su, Z., Gao, A., Li, X., Zou, S., He, C., Wu, J., et al. (2021). DNA polymerase iota promotes esophageal squamous cell carcinoma proliferation through erk-OGT-induced G6PD overactivation. *Front. Oncol.* 11, 706337. doi:10.3389/fonc.2021.706337

Sun, L., Zhang, H., and Gao, P. (2021a). Metabolic reprogramming and epigenetic modifications on the path to cancer. *Protein Cell* 13, 877–919. doi:10.1007/s13238-021-00846-7

Sun, M., Sheng, H., Wu, T., Song, J., Sun, H., Wang, Y., et al. (2021b). PIKE-A promotes glioblastoma growth by driving PPP flux through increasing G6PD expression mediated by phosphorylation of STAT3. *Biochem. Pharmacol.* 192, 114736. doi:10.1016/j.bcp.2021.114736

Sun, S., Zhang, Y., Xu, W., Yang, R., Yang, Y., Guo, J., et al. (2022). Plumbagin reduction by thioredoxin reductase 1 possesses synergy effects with GLUT1 inhibitor on KEAP1-mutant NSCLC cells. *Biomed. Pharmacother.* 146, 112546. doi:10.1016/j.biopha.2021.112546

Teesalu, M., Rovenko, B. M., and Hietakangas, V. (2017). Salt-inducible kinase 3 provides sugar tolerance by regulating NADPH/NADP(+) redox balance. *Curr. Biol.* 27 (3), 458–464. doi:10.1016/j.cub.2016.12.032

Tiwari, S. K., Dang, J., Qin, Y., Lichinchi, G., Bansal, V., and Rana, T. M. (2017). Zika virus infection reprograms global transcription of host cells to allow sustained infection. *Emerg. Microbes Infect.* 6 (4), e24. doi:10.1038/emi.2017.9

Tu, D., Gao, Y., Yang, R., Guan, T., Hong, J. S., and Gao, H. M. (2019). The pentose phosphate pathway regulates chronic neuroinflammation and dopaminergic neurodegeneration. *J. Neuroinflammation* 16 (1), 255. doi:10.1186/s12974-019-1659-1

Wang, B., Moya, N., Niessen, S., Hoover, H., Mihaylova, M. M., Shaw, R. J., et al. (2011). A hormone-dependent module regulating energy balance. *Cell* 145 (4), 596–606. doi:10.1016/j.cell.2011.04.013

Wang, M., Hu, J., Yan, L., Yang, Y., He, M., Wu, M., et al. (2019). High glucose-induced ubiquitination of G6PD leads to the injury of podocytes. *FASEB J.* 33 (5), 6296–6310. doi:10.1096/fj.201801921R

Wang, S., Zeng, F., Liang, S., Wang, Q., Wen, Y., Wang, Q., et al. (2022). lncRNA Linc00173 modulates glucose metabolism and multidrug chemoresistance in SCLC: Potential molecular panel for targeted therapy. *Mol. Ther.* 30 (4), 1787. doi:10.1016/j.ymthe.2022.03.013

Wang, Y., Li, Q., Niu, L., Xu, L., Guo, Y., Wang, L., et al. (2020). Suppression of G6PD induces the expression and bisecting GlcNAc-branched N-glycosylation of E-Cadherin to block epithelial-mesenchymal transition and lymphatic metastasis. *Br. J. Cancer* 123 (8), 1315–1325. doi:10.1038/s41416-020-1007-3

Wang, Y. P., Zhou, L. S., Zhao, Y. Z., Wang, S. W., Chen, L. L., Liu, L. X., et al. (2014). Regulation of G6PD acetylation by SIRT2 and KAT9 modulates NADPH homeostasis and cell survival during oxidative stress. *EMBO J.* 33 (12), 1304–1320. doi:10.1002/embj.201387224

Whitburn, J., Rao, S. R., Morris, E. V., Tabata, S., Hirayama, A., Soga, T., et al. (2022). Metabolic profiling of prostate cancer in skeletal microenvironments identifies G6PD as a key mediator of growth and survival. *Sci. Adv.* 8 (8), eabf9096. doi:10.1126/sciadv.abf9096

Wu, S., Wang, H., Li, Y., Xie, Y., Huang, C., Zhao, H., et al. (2018). Transcription factor YY1 promotes cell proliferation by directly activating the pentose phosphate pathway. *Cancer Res.* 78 (16), 4549–4562. doi:10.1158/0008-5472.CAN-17-4047

Xu, S.-N., Wang, T.-S., Li, X., and Wang, Y.-P. (2016). SIRT2 activates G6PD to enhance NADPH production and promote leukaemia cell proliferation. *Sci. Rep.* 6, 32734. doi:10.1038/srep32734

Xu, Y., Osborne, B. W., and Stanton, R. C. (2005). Diabetes causes inhibition of glucose-6-phosphate dehydrogenase via activation of PKA, which contributes to oxidative stress in rat kidney cortex. *Am. J. Physiol. Ren. Physiol.* 289 (5), F1040–F1047. doi:10.1152/ajprenal.00076.2005

Yang, K., Fan, M., Wang, X., Xu, J., Wang, Y., Tu, F., et al. (2022). Lactate promotes macrophage HMGB1 lactylation, acetylation, and exosomal release in polymicrobial sepsis. *Cell Death Differ.* 29 (1), 133–146. doi:10.1038/s41418-021-00841-9

Yau, C., Low, J. Z. H., Gan, E. S., Kwek, S. S., Cui, L., Tan, H. C., et al. (2021). Dysregulated metabolism underpins Zika-virus-infection-associated impairment in fetal development. *Cell Rep.* 37 (11), 110118. doi:10.1016/j.celrep.2021.110118

Yen, W.-C., Wu, Y.-H., Wu, C.-C., Lin, H.-R., Stern, A., Chen, S.-H., et al. (2020). Impaired inflammasome activation and bacterial clearance in G6PD deficiency due to defective NOX/p38 MAPK/AP-1 redox signaling. *Redox Biol.* 28, 101363. doi:10.1016/j.redox.2019.101363

Yin, X., Tang, B., Li, J.-H., Wang, Y., Zhang, L., Xie, X.-Y., et al. (2017). ID1 promotes hepatocellular carcinoma proliferation and confers

chemoresistance to oxaliplatin by activating pentose phosphate pathway. *J. Exp. Clin. Cancer Res.* 36 (1), 166. doi:10.1186/s13046-017-0637-7

Zeng, Q., Zhao, R. X., Chen, J., Li, Y., Li, X. D., Liu, X. L., et al. (2016). O-linked GlcNAcylation elevated by HPV E6 mediates viral oncogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 113 (33), 9333–9338. doi:10.1073/pnas.1606801113

Zhang, D., Tang, Z., Huang, H., Zhou, G., Cui, C., Weng, Y., et al. (2019a). Metabolic regulation of gene expression by histone lactylation. *Nature* 574 (7779), 575–580. doi:10.1038/s41586-019-1678-1

Zhang, H.-S., Zhang, Z.-G., Du, G.-Y., Sun, H.-L., Liu, H.-Y., Zhou, Z., et al. (2019b). Nrf2 promotes breast cancer cell migration via up-regulation of G6PD/HIF-1α/Notch1 axis. *J. Cell. Mol. Med.* 23 (5), 3451–3463. doi:10.1111/jcmm.14241

Zhang, Q., Yang, Z., Ni, Y., Bai, H., Han, Q., Yi, Z., et al. (2020). NF-κB and pSTAT3 synergistically drive G6PD overexpression and facilitate sensitivity to G6PD inhibition in ccRCC. *Cancer Cell Int.* 20, 483. doi:10.1186/s12935-020-01576-2

Zhang, R., Tao, F., Ruan, S., Hu, M., Hu, Y., Fang, Z., et al. (2019c). The TGFβ1-FOXM1-HMGA1-TGFβ1 positive feedback loop increases the cisplatin resistance of non-small cell lung cancer by inducing G6PD expression. *Am. J. Transl. Res.* 11 (11), 6860–6876.

Zhang, X., Gao, F., Ai, H., Wang, S., Song, Z., Zheng, L., et al. (2021). TSP50 promotes hepatocyte proliferation and tumour formation by activating glucose-6-phosphate dehydrogenase (G6PD). *Cell Prolif.* 54, e13015. doi:10.1111/cpr.13015

Zheng, Q., Li, P., Zhou, X., Qiang, Y., Fan, J., Lin, Y., et al. (2021). Deficiency of the X-inactivation escaping gene KDM5C in clear cell renal cell carcinoma promotes tumorigenicity by reprogramming glycogen metabolism and inhibiting ferroptosis. *Theranostics* 11 (18), 8674–8691. doi:10.7150/thno.60233

Zhou, L., Wang, F., Sun, R., Chen, X., Zhang, M., Xu, Q., et al. (2016). SIRT5 promotes IDH2 desuccinylation and G6PD deglutarylation to enhance cellular antioxidant defense. *EMBO Rep.* 17 (6), 811–822. doi:10.15252/embr.201541643

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 15 September 2022
DOI 10.3389/fonc.2022.971288

OPEN ACCESS

EDITED BY
Yong Li,
Western Michigan University,
United States

REVIEWED BY
Ling-Zhi Liu,
Thomas Jefferson University,
United States
Zhe-Sheng Chen,
St. John's University, United States

*CORRESPONDENCE
Chitra Thakur
Chitra.Thakur@
stonybrookmedicine.edu
Fei Chen
Fei.Chen@stonybrookmedicine.edu

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

RECEIVED 16 June 2022
ACCEPTED 26 August 2022
PUBLISHED 15 September 2022

CITATION
Thakur C, Qiu Y, Fu Y, Bi Z, Zhang W, Ji H and Chen F (2022) Epigenetics and environment in breast cancer: New paradigms for anti-cancer therapies.
Front. Oncol. 12:971288.
doi: 10.3389/fonc.2022.971288

Epigenetics and environment in breast cancer: New paradigms for anti-cancer therapies

Chitra Thakur[1,2*], Yiran Qiu[1], Yao Fu[1], Zhuoyue Bi[1], Wenxuan Zhang[1], Haoyan Ji[1] and Fei Chen[1,2*]

[1]Department of Pathology, Stony Brook Cancer Center, Stony Brook, NY, United States, [2]Department of Pathology, Renaissance School of Medicine, Stony Brook University, Stony Brook, NY, United States

Breast cancer remains the most frequently diagnosed cancer in women worldwide. Delayed presentation of the disease, late stage at diagnosis, limited therapeutic options, metastasis, and relapse are the major factors contributing to breast cancer mortality. The development and progression of breast cancer is a complex and multi-step process that incorporates an accumulation of several genetic and epigenetic alterations. External environmental factors and internal cellular microenvironmental cues influence the occurrence of these alterations that drives tumorigenesis. Here, we discuss state-of-the-art information on the epigenetics of breast cancer and how environmental risk factors orchestrate major epigenetic events, emphasizing the necessity for a multidisciplinary approach toward a better understanding of the gene-environment interactions implicated in breast cancer. Since epigenetic modifications are reversible and are susceptible to extrinsic and intrinsic stimuli, they offer potential avenues that can be targeted for designing robust breast cancer therapies.

KEYWORDS

breast cancer, epigenetics, DNA methylation, chromatin modification, metabolism, environment, therapies

Breast cancer overview

Cancers of the breast are the most prevalent malignancy observed in women worldwide. In the year 2022 alone, it is estimated that in the United States, nearly 287,850 new cases of invasive breast cancer and 51,400 new cases of ductal carcinoma *in situ* (DCIS) would be diagnosed, while 43,250 breast cancer deaths would occur (1). Breast cancers if diagnosed at an early stage, can significantly enhance the effective treatment strategies and improve the survival. The five-year survival rate for early detection is more than 90%, whereas it is reduced to 25% for patients diagnosed at the advanced stages (2).

Breast cancer is a highly heterogeneous disease and research is still ongoing to clearly understand its origin and the underlying mechanisms. The breast consists of milk producing glands and the connective tissues comprising the fibrous and fatty tissues. Lobules are the milk producing glands, and ducts carry the milk to the nipples, Figures 1A, B. Most breast cancers begin in the ducts or the lobules and based on the metastatic spread, they can either be benign or invasive. Ductal carcinoma *in situ* (DCIS) is considered as non-invasive and early-stage breast cancer confined to the milk ducts. If cancer originates in the ducts or lobules and metastasizes, they are considered invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) respectively. Almost, 80% of breast cancers belong to the IDC category (4, 5).

With the emergence of new high-throughput technologies and gene expression profiling, breast cancer has been molecularly characterized into distinct subtypes based on the expression of hormone receptors and proliferation statuses. Activation of human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), progesterone receptor (PR), proliferation marker Ki67, and/or mutations in the Breast Cancer (BRCA) gene, has been utilized in the histological and molecular characterization of breast cancer. These molecular subtypes are clinically divided into major forms that include Luminal A, Luminal B, HER2-enriched, and basal/triple negative breast cancer (TNBC). Luminal A cancer can either be ER and/or PR positive (+) or HER2 negative (-). Luminal B tumor can either be ER+ and/or PR+ or PR- and/or HER2+/-. HER2 overexpressed tumors constitute the HER2 enriched group, while TNBC lacks the ER, PR, and HER2 statuses. Luminal A tumors have low Ki 67 levels, are of low grade, and have the best prognosis, compared to Luminal B which have high Ki 67 levels and are usually high grade. Among all, TNBCs, have the worst prognosis and are aggressive due to high metastatic behavior (6–8). Such an existence of multiple subtypes of breast cancer is associated with distinct clinical behaviors/responses and has significant implications in breast cancer therapies (9, 10), Figure 1C.

Genetic predisposition or family history constitutes almost 10% of all breast cancer cases. Mutations in the BRCA gene, *BRCA1* and *BRCA2* is the most common germline aberrations associated with breast cancer having a collective 70% lifetime risk of developing breast cancer (11, 12). In fact, 15 to 20% of all TNBC cases are linked with the germline mutations in *BRCA1* or *BRCA2* (13) and in US, 12% of breast cancers are contributed by TNBCs with a 5 year survival rate of 8 to 16 percent only (14). Studying a series of early breast cancers revealed that the most frequently amplified genes in the tumors are the *p53, Myc, PTEN, PIK3CA, ERBB2, CCND1, GATA 3* and *FGFR1* (15). The risk of developing breast cancer is high in patients harboring mutations in the *BRCA1*, *BRCA2*, *TP53*, and *PTEN* genes (16). In addition to the genetic factors, breast cancer microenvironment plays a major role in its development and progression where the immune cell repertoire is cardinal (17).

Heightened or prolonged exposure to estrogen contributes to the major risk factor for breast cancer development. The occurrence of sporadic breast cancers is associated with exposure to estrogen, which is a substantial risk factor for the development of such cancers (18). Other risk factors include old age, obesity, high breast density, alcohol intake, smoking, hormonal therapy, and pregnancy associated factors (19–24). Additionally, early menarche/late menopause, usage of oral contraceptives, hormone replacement therapy, benign lesions, and radiation therapy are some of the known risk factors (25–28). Few of them are modifiable risk factors such as lifestyle and physical activity if adopted successfully, can offer reduction in the disease burden (29).

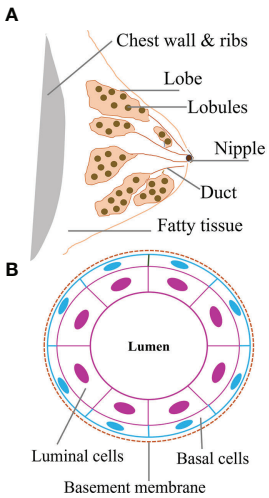
Epigenetic players in breast cancer

Dynamic and heritable modifications occurring to the genome independently of DNA sequence, is a phenomenon referred to as the "epigenetics". Interestingly, cancer was the first disease linked to epigenetic changes (30). For the onset of cancer, the activation of oncogenes and/or the suppression of tumor suppressor genes are the key events that are always accompanied with epigenetic changes. These epigenetic changes include DNA methylation, histone posttranslational modifications, expression of micro-RNA, and long non-coding RNA (31, 32).

Breast cancer development is a complex and multistep process involving the synergistic crosstalk between genetic and epigenetic alterations which are influenced by a plethora of internal and external factors. Such factors include but not limited to the cell's intrinsic microenvironment, nutrient supply, cellular stress as well as external environmental exposures to agents that are endocrine disrupters or are of carcinogenic nature. Altogether, critical genes involved in proliferation, apoptosis, cell motility, invasion, etc. are influenced by the epigenetic changes that are implicated in breast cancer development and progression (Figure 2).

DNA methylation

One of the most well-known and major epigenetic mechanisms is DNA methylation, which involves the covalent addition of a methyl group (CH_3) to the 5′-position of cytosine that resides before the guanine in the DNA sequence. Such methylation within the CpG dinucleotides which are concentrated in large clusters also called the CpG islands, regulates gene expression thereby governing the major biological process implicated in cancer



C

Molecular subtypes	Biomarkers	Frequency of cases (%)	Histological grade	Prognosis	Response to therapies
Luminal A	ER+ HER2- PR+ Ki67 low	40-50	Well differentiated (Grade I)	Good	Endocrine
Luminal B [HER 2+]	ER+ HER2+ PR+/- Ki67 low/high	20-30	Moderately differentiated (Grade II)	Intermediate	Endocrine Chemotherapy Target therapy
Luminal B [HER 2-]	ER+ HER2- PR- Ki67 high				Endocrine Chemotherapy
HER 2+	ER- HER2+ PR- Ki67 high	15-20	Poorly differentiated (Grade III)	Poor	Chemotherapy Target therapy
Triple negative	ER- HER2- PR- Ki67 high	10-20	Poorly differentiated (Grade III)	Poor	Chemotherapy PARP inhibitors

ER: estrogen receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor 2

FIGURE 1
Classification of Breast Cancer **(A)** Breast showing the different tissue types consisting of duct, lobe, lobules, nipples, and fatty tissue. **(B)** Cross-sectional view of mammary duct, consisting of basal cells and luminal cells. Breast cancer arising from the luminal or basal cells can be further characterized based on the expression of different hormone receptors. **(C)** Based on the expression of ER, PR, HER2, and proliferation status as assessed by Ki67, different molecular subtypes of breast cancer have been identified that have distinct prognostic features and response to therapies (3).

(33, 34). As a result of methylation, a 5-methylcytosine (5mC) structure is formed that can either block the access of transcription factors to the binding sites of the DNA or engage methyl binding domain proteins (MBDs) in conjunction with the modification of histone proteins, so that the expression of methylated genes is prevented. In such a scenario when the promoters of key tumor suppressor genes are densely methylated, leads to their silencing and if oncogenes are less methylated, leads to their aberrant activation (35, 36).

DNA methylation is a reversible process where a specific group of enzymes called the DNA methyltransferase (DNMTs) govern the process. DNMT1, DNMT3a, and DNMT3b are the three active DNA methyltransferases. Demethylation of DNA is catalyzed by an enzyme family belonging to the Ten-eleven translocation methylcytosine dioxygenases also known as ten-

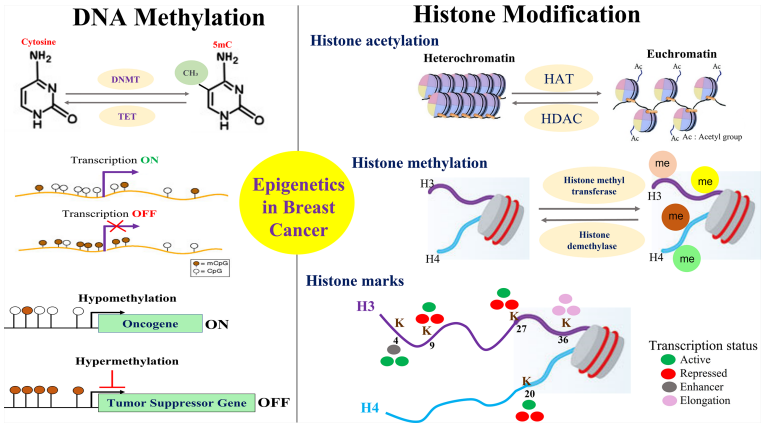


FIGURE 2
Overview of Key Epigenetic Events in Breast Cancer. Mechanisms for epigenetic alterations in breast cancer are shown focusing on two major players that include the methylation of DNA and the modification of histone proteins. Hypomethylation of oncogenes and hypermethylation of tumor suppressor genes is an important epigenetic phenomenon in breast cancer that affects various cellular processes of proliferation, apoptosis, migration, invasion, drug resistance, etc. Post translation modifications made to histone proteins impact gene expression by altering the chromatin structure towards open or closed conformation. Histone methylation of lysine is implicated in both transcriptional activation and repression depending on the methylation site that constitutes the various histone marks/code.

eleven translocations (TETs), which can turn 5mC to 5-hydroxymethylcytosine (5-hmC) by the process of hydroxymethylation. TET1, TET2, and TET3 are three such enzymes involved in DNA demethylation thereby recovering the silenced genes that are once affected by the DNMTs. Together, this entire process influences the transcriptional activation of important genes involved in carcinogenesis and genomic stability (37–41). Several other proteins that have DNA demethylase activities and are implicated in breast cancer include the growth arrest and DNA-damage inducible protein (GADD45) and the cytidine deaminases family of proteins, Activation-induced cytidine deaminase (AID) and Apolipoprotein B mRNA editing catalytic polypeptide-like family (APOBEC). GADD45A has compelling associations between DNA repair and epigenetic gene regulation (42, 43). In breast cancer, the interaction between GADD45 and BRCA1 gene has been suggested to influence the pathogenesis of the disease most likely *via* triggering the nucleotide excision repair mechanisms (44). Interestingly, GADD45A is abnormally methylated in breast cancer (45). AID proteins have important roles in the active DNA demethylation, where its engagement in the deamination of 5-mC to thymine has been reported (40, 46). Also, AID is known to facilitate DNA demethylation and is essential for the EMT in non-transformed mammary epithelial cells (47). Furthermore, while, APOBEC1 possesses DNA demethylase activity (48–50), APOBEC mutagenesis influencing the tumor evolution in ER+/HER2-breast cancer has been reported (51). Most recently it was shown that the APOBEC mutagenesis prohibited the growth of breast tumors by eliciting immunogenic responses (52).

Several genes in breast cancer exhibit CpG island hypermethylation (53) and in several instances, abnormal activity of DNA methyltransferases led to the hypermethylation and silencing of *HOXA5, TMS1, p16, RASSF1A*, and *BRCA1* genes of tumor suppressor behavior (54–56). Additionally, genes that are silenced due to promoter hypermethylation include *E-cadherin, TMS1*, *GSTP1*, and *p16 (*57–59). These genes are involved in major biological processes such as estrogen signaling, pro-apoptosis (*HOXA5, TMS1*), cell cycle check points (*RASSF1A, p16*) and DNA repair mechanisms (*BRCA1*). While one of the best examples of a breast cancer susceptibility gene that is frequently silenced in sporadic breast tumors is the *BRCA1* gene, CpG hypermethylation of *BRCA1* associated with DNMT 3b overexpression has been reported (60). Early stages of sporadic breast cancer exhibit the loss of cell cycle checkpoint gene *p16INK4a via* aberrant CpG promoter methylation (61) and nearly 80% of breast tumors also exhibit a decreased expression of another cell cycle inhibitor gene *p21/CIP1*$^{/WAF1}$ via elevated methylation of *p21/CIP1*$^{/WAF1}$ gene (62).

DNA methylation also follows a distinct pattern that is displayed in different subtypes of breast cancer. For example, a high frequency of DNA methylation has been shown in ER +/luminal breast cancer compared to ER−/basal-like tumors (63, 64). Also, well-differentiated tumors have less methylated CpG islands in comparison to poorly differentiated breast tumors which exhibits a greater degree of methylated CpG islands (65). Similarly, increased promoter hypermethylation of the progesterone receptor gene has been observed in the PR negative breast tumors (66). Such a differential methylation pattern in the ER or PR or HER2 gene may affect the expression of these receptors on the breast tumor and hence can significantly impact the responsiveness of such tumors to relevant endocrine/hormonal therapies. In an attempt to study the DNA methylation profiles of the well-known expression subtypes of breast cancer i.e. luminal A, luminal B, and Basal like, 807 cancer associated genes were analyzed and it was revealed that there is variability in the methylation profiles of each of the three breast cancer subtypes and that the profiles are different from each other (64).

DNA methylation alterations in normal breast tissue or normal tissues adjacent to cancer can also give clues towards the likelihood of the occurrence of breast cancer. Interestingly, it is suggested that the detectable methylation variabilities in some of the cancer related genes in normal breast tissues can predate the occurrence of breast cancer (67). Moreover, distinct types of breast cancer can be tracked down back to the specific progenitor population, deploying their unique methylation profiles, thereby addressing the issues owing to their cell of origin or biological heterogeneity as observed in breast cancer (68). More recently, by comparing breast cancer to normal breast, seven breast cancer-specific methylation biomarkers have been identified, while six CpG sites are suggested to predict patient survival (69). Using a genome wide approach to analyze the DNA methylation and expression patterns in breast cancer and normal breast, *PRAC2*, *TDR10*, and *TMEM132C* genes have been identified that can serve as novel DNA methylation-gene markers of diagnostic and prognostic significance in breast cancer (70). Large scale integrative analysis of the DNA methylation profiles across 1538 METABRIC breast tumors with respect to transcriptional, genetic, and clinical aspects, revealed six global trends that affect the DNA methylation profiles of the breast. These trends consist of "contamination of immune and stromal cells", "replication linked hypomethylation clock", "X chromosome dosage compensation", and "epigenetic instability at CpG islands". Most importantly, this study identified X inactivation as a strong dosage compensation machinery, which can be the causative reason behind the methylation of attained X-associated loci in ER negative tumors (71).

Chromatin modification

DNA is wrapped around histone proteins so that it can fit into the nucleus. Individual histone octamer consists of two copies of H2A/H2B dimer cores and H3/H4 tetramers, that wrap

around 146 base pairs of the DNA. Nucleosomes comprise repeating histone units that ultimately make up the chromatin (72, 73). Histone octamer harbors an unstructured N terminal tail of differing lengths that protrudes outward from the nucleosome. This protruding amino terminal tail can be subjected to various kinds of modifications where chemical moieties are added. The addition of various chemical moieties or tags determines whether the DNA wrapped around histones is available for transcription. In case, when the chromatin is tightly folded, the DNA remains inaccessible to the transcription factors and hence the structure is transcriptionally silent, also called heterochromatin. Whereas when the structure is less condensed, more relaxed, and hence more accessible to the transcription factors and thereby remains transcriptionally active, also called euchromatin (74). There are at least four amino acid residues that are subjected to modifications, these include lysine, serine, tyrosine and arginine, and there are more than six kinds of modifications that can occur. These include methylation, acetylation, phosphorylation, ubiquitination, biotinylation, sumoylation, and proline isomerization. The different patterns of histone modifications, also famously referred as the histone code, influences the transition of the chromatin states between the euchromatin and heterochromatin eventually regulating gene expression (75, 76).

Histone acetylation

Post translational modifications made to histone proteins impact gene expression by altering the chromatin structure. Histone acetylation involves the addition of acetyl groups to the lysine residues of histones H3 and H4 by the group of enzymes known as the histone acetyltransferases (HATs) also called as "writers". As a part of the gene regulatory machinery, such modifications disrupt histone-DNA interactions resulting in the unwinding of the nucleosome. HATs utilize acetyl CoA as a cofactor and catalyze the reaction, and in doing so they neutralize the positive charge on the lysine, thereby weakening the interaction between the histones and the negatively charged phosphate groups of the DNA. As a result, the condensed chromatin is now a more open and relaxed structure that is associated with a higher degree of gene transcription.

Acetylation is a dynamic and reversible process, where the acetyl groups can be removed by the group of enzymes called histone deacetylases (HDACs) also called "erasers", resulting in the deacetylation of the histone lysine residues thereby making the chromatin more condensed and transcriptionally repressed (74, 76, 77). Acetylation of histone H3 on lysine 9 residue [H3K9], lysine 14 [H3K14], lysine 27 [H3K27], and lysine 122 [H3K122] has been associated with active transcription (78–80). It is interesting to note that DNA methyltransferases can directly interact with the HDACs and the methyl CpG binding domain family of proteins at their promoter regions and ultimately build a complex that is transcriptionally repressive. This repressive complex is critical for the conversion of acetylated histones that is transcriptionally active, to the deacetylated transcriptionally silent form (81).

Enzymes belonging to the category of histone acetylation "writers", e.g., enzyme harboring the histone acetylation domains P300 is implicated in breast cancer where it is overexpressed and bestow towards an elevated risk of cancer occurrence and lower survival (82). P300/CBP, also modulate several processes associated with proliferation, cell death, epithelial mesenchymal transition (EMT), and metastasis in breast cancer (83–86).

There are important roles exerted by the histone deacetylases "erasers" where they regulate the cell growth, EMT, angiogenesis, and metastasis of breast cancer (87–95). For e.g., Sirtuins, a class III histone deacetylase family regulates the oncogenes and tumor suppressor genes thereby affecting the breast carcinogenesis in a dual fashion. In this context, SIRT1 hindered the TNBC tumorigenesis, whereas fostered the tumorigenesis of luminal subtypes (96, 97). Interestingly, SIRT1 functions downstream of the *BRCA1* gene and negatively regulate *Survivin*, an anti-apoptotic gene. Such transcriptional repression of *Survivin* is mediated *via* the deacetylation of histone H3 on lysine 9 on its promoter. Therefore, ablation of *BRCA1 via* lessened SIRT1 resulted in an upregulation of *Survivin* that facilitated the growth of breast tumors (98). Other Sirtuin family members are also implicated in breast cancer. For e.g., in TNBC cells, SIRT2 upregulation facilitated the deacetylation of histone H4 at the tumor suppressor gene *ARRDC3* and this rendered the aggressiveness of breast cancer (99). Also, SIRT7 is elevated in human breast cancers (100).

Histone methylation

Histone methylation mainly occurs on the side chains of lysine and arginine residues. Unlike acetylation, histone methylation does not alter the charge of the histone protein but involves the addition of the methyl groups. Depending upon the number of methyl moiety added, lysine can be mono, di, or tri methylated whereas arginine can be symmetrically or asymmetrically methylated (101, 102). A special group of enzymes called histone methyltransferases (KMTs) catalyze the transfer of a methyl group from the S-adenosylmethionine (SAM) to a lysine's ϵ-amino group. Methylation is also a dynamic and reversible process where the removal of the methyl groups is carried out by demethylases (histone demethylases, KDMs). The consequences of histone methylation are more complicated and largely dependent upon the targeted residues. For example, methylation of lysine H3K4, H3K36, and H3K79 at histone H3 contributes to transcriptional activation, while methylation of lysine at H3K9, H3K27 on histone H3 and, H4K20 on histone H4 is associated with transcriptional repression and are considered repressive

epigenetic marks (103). Some of the methylated lysine histone marks have a role in DNA repair e.g., H3K36me3 is important for the homologous recombinational repair of the DNA double strand breaks, and H4K20me3 aids the repair *via* non-homologous end joining process (104). The resulting balance between methyltransferases (also called "writer") and demethylases (also referred to as "eraser") determines the methylation status of the cell (105), where DNA methylation and histone acetylation act in coordination to govern the overall gene transcriptional regulation. The balance between the histone acetyltransferases (HATs "writer") and histone deacetylases (HDACs "eraser") control the overall chromatin states/structures, hence regulating the gene expression. Histone modifications offer novel targets that can be exploited in breast cancer therapies (106).

In breast cancer, luminal A subtypes are found to exhibit increased global acetylation and methylation of the histone protein in comparison to the basal subtype (107). By measuring the relative levels of seven modified histones proteins including H3K18ac, H3K9ac, H4R3me2, H3K4me2, H4K12ac, H4K16ac, and H4K20me3 in 880 invasive breast cancer patients, it was revealed that the expressions of all seven markers were negatively correlated with tumor grade. While the loss of H4K16ac was suggestive to be an early event in the pathogenesis of invasive breast cancer, reduced levels of H4R3me2, H3K9ac, and H4K16ac were significantly associated with large tumor size. High levels of H4R3me2 and H3K9ac correlated with low lymph node stage (107). Interestingly, the metastatic behavior of breast cancer was correlated to an increased H3K4 histone mark where the dynamics of H3K4 acetylation and methylation exemplify the different breast cancer subtypes. While breast cancer cells representing both early and late cancer cell phenotypes are associated with a genome-wide gain of H3K4ac; late-stage cancer cells exhibited a gain of H3K4me3 (108). PI3K/AKT signaling cascade plays a significant role in breast cancer progression and this signaling was found to regulate the methylation of H3K4 in breast cancer, where an elevated level of H3K4me3 was linked with breast tumors (109). Another histone mark, H3K27ac has an important role in breast cancer progression and is found to regulate the EMT process (110, 111). The loss of a repressive epigenetic mark, the H3K27me3 has been identified as a negative prognostic indicator in breast cancer (112). Strikingly, enrichment of H3K27me3 within the promoter of genes *FOXC1, RAD51, CDH1*, and *RUNX3*, resulted in enhanced cell growth and metastasis of breast cancer (113). Loss of Cadherin 1 due to its hypermethylation *via* DNA methylation and trimethylation of H3K27 has been reported during metastasis (114), where it is important to note that *Cadherin 1* is one of the key genes that inhibits metastasis and progression of breast cancer cells. Another mark, H4K20me3 is found to be significantly decreased in breast cancer and, importantly, it was an independent predictor of poor prognosis of the disease. This specific methylation of H4K20 is carried by the KMT5 family of enzymes that ultimately represses the transcription process (115, 116).

Among the enzymes implicated in gene regulation *via* epigenetic mechanisms, the enhancer of zeste homolog 2 (EZH2) is an important histone methyltransferase that methylates H3K27 leading to the transcriptional silencing of the target genes in breast cancer. Notably, in breast cancer, EZH2 has been found to be upregulated and promoted the EMT process (117, 118). Moreover, the level of EZH2 was gradually increased in breast cancer progression scenarios ranging from normal epithelium to epithelial hyperplasia, DCIS, IDC, and distant metastasis; and the expression of EZH2 was an independent predictor of breast cancer recurrence (119).

Members of the histone methyltransferases family, such as lysine methyltransferase 2 (KMT2) are also involved in the growth and spread of breast cancer cells, where they mediate the active histone methylation of H3K4 at the enhancer and the promoter regions of oncogenes and pro-metastatic genes, thereby facilitating the activation of genes that are estrogen dependent (120–123).

One of the only known histone 3 lysine 79 (H3K79) methyltransferases, is the histone methylase disruptor silencing 1 like (DOT1L) which has critical role in the development of breast cancer and is a potential therapeutic target for invasive breast cancer (124, 125). DOT1L is known to facilitate the aggressiveness of tumors by elevating the metastatic behavior of cancer cells (126) and is implicated in lymph node metastasis of breast cancer (127). In fact, targeting DOT1L by pharmacological interventions inhibited the growth and metastasis of TNBC cancer (128).

Among histone demethylases (erasers) family members are the prominent enzymes that are Fe^{2+}/oxoglutarate-dependent containing a JumonjiC (JmjC) domain (129). Histone demethylase protein LSD1, a non JmjC demethylase has been found to negatively regulate the expression of cell growth and motility genes in breast cancer (130–133). Other JmjC KDMs involved in breast cancer are KDM4A, KDM4B and, KDM4C. Increased levels of KDM4A and KDM4B have been observed in ERα positive breast cancer cells, while TNBC cells showed an increased level of KDM4C (134). KDM4B regulates the cell cycle progression of breast cancer cells and is a direct target of ERα (135). While an increase of KDM3A is concomitant with a reduced H3K9me2/3 during breast tumorigenesis, KDM3A facilitated the activation of genes implicated in breast cancer as *MYC*, *PAX3*, *Cyclin D1*, *MMP-9*, *S100A4*, and *JUN*, thereby enhancing the proliferation and motility of breast cancer cells (136–138). KDM3A also promotes the growth of mammary gland ducts and tumors by positively affecting the proliferation *via* cyclin D1 (138). KDM4C is also necessary for breast cancer growth and, metastasis, where it serves as a co-activator of HIF-1α, with the underlying epigenetic mechanism of demethylating the H3K9me3 (139). Another histone demethylase PHF8

promoted EMT and breast tumorigenesis (140). PHF20L1, a methyl lysine reader protein containing a TUDOR domain, plays important role in breast cancer metastasis (141). Studies suggested its oncogenic role in response to hypoxic conditions, where it facilitated glycolysis, cell growth and metastasis of breast cancer cells by exerting its direct inhibitory activities on certain genes of tumor suppressive nature like *HIC1*, *KISS1*, and *BRCA1* (142).

Non-coding RNAs

Functional RNA molecules that cannot be translated into proteins also referred to as non-coding RNA possess important regulatory effects and influence the expression of certain genes implicated in breast cancer. Among these are the long non-coding RNAs (lncRNAs) and micro-RNAs (miR). Micro-RNAs have been widely studied for its epigenetic regulation where they either activate or repress critical biological pathways and mechanisms important for breast tumorigenesis. Interestingly the let-7 family of micro RNAs has a significant role in breast cancer where its silencing has been associated with the development of metastasis and high-grade hormone negative breast tumors (143–145). Other micro-RNAs have important roles too. For. e.g., miR-9-3 activated apoptosis and miR-148a & miR-152 inhibited cell growth and angiogenesis (146, 147). Micro-RNAs involved in invasion and metastasis includes miR-125b, miR-126 and, miR-31 respectively (148–150). Some of the microRNAs whose aberrant hypermethylation has been reported in primary breast tumors include mir-663, mir-148, mir-9-1, mir-152, and mir-124a3 (151). Aberrant hypermethylation of H19, a lncRNA has been observed in invasive breast carcinoma when compared to normal breast tissues, where tumor suppressive functions of H19 have been suggested (152). HOTAIR, is another lncRNA where studies reported the recruitment of several writer proteins such as MLL1, MLL3, and P300/CBP to the HOTAIR's promoter region thereby resulting in an enrichment of histone acetylation and elevation of H3K4me3, further driving the progression of breast cancer by suppressing the apoptosis (153).

Therefore, epigenetic mechanisms offer many modalities that can be exploited for breast cancer therapies. Considering the fact that epigenetic changes induced by DNMTs and HDACs are transient and reversible, a number of studies are currently ongoing to establish effective, optimal dose and the treatment schedules for several epigenetic agents implicated in breast cancer, Figure 3. Data adapted from (154).

Environmental triggers of epigenetic aberrations in breast cancer

In addition to family history and genetic predisposition, epidemiological studies unraveled the influence of environmental exposures to hormonal agents and other factors that can increase the risk for breast cancer development. Exposure to endocrine disrupters, indoor and outdoor air pollution, polycyclic aromatic hydrocarbons (PAHs) etc. can induce epigenetic changes in an exposure or disease relation fashion. Xenobiotics such as activators of the aryl hydrocarbon receptor (AHR), dioxin, phthalates, polychlorinated biphenyls (PCB), PAHs, bisphenol A (BPA), arsenic etc. prevalent in the environment, dietary items, soil, water, and other consumable products, are likely to contribute to the epigenetic dysregulation of oncogenes and tumor suppressor genes in breast cancer.

Target	Epigenetic Agent	Clinical Trial Identifier	Phase
HDAC	Entinostat*	NCT03538171	III
HDAC	Romidepsin*	NCT00098397 (metastatic BC)	II
DNMT plus HDAC	Azacitidine* Entinostat*	NCT01349959	II
HDAC plus chemotherapy	Entinostat* Capecitabine	NCT03473639	I
HDAC plus Anti-PD-L1 antibody, anti-VEGF antibody, aromatase inhibitor, AKT kinase inhibitor, estrogen receptor inhibitor	Entinostat* Atezolizumab, Bevacizumab, Exemestane, Fulvestrant, Tamoxifen, Ipatasertib	NCT03280563	I/II
HDAC plus HER1/HER2 kinase inhibitor, anti-HER2 antibody	Entinostat* Lapatinib, Trastuzumab	NCT01434303	I
HDAC plus Anti-PD-L1 antibody	Entinostat* Atezolizumab	NCT02708680 (for TNBC)	I/II
HDAC plus Anti-PD-1 antibody, chemotherapy	Romidepsin*, Nivolumab Cisplatin	NCT02393794	I/II
BET inhibitor plus Estrogen receptor inhibitor	Molibresib* Fulvestrant	NCT02964507	II

FIGURE 3
Epigenetic Targets and other combined inhibitors for breast cancer therapies under clinical trial. Data adapted from (154). Star (*) represents the specific epigenetic agent.

AHR is a well-known sensor and a regulator of toxic and carcinogenic responses to environmental insults (155, 156). In advanced malignant breast carcinomas, AHR is shown to be constitutively active (157) and several studies reveal that targeting AHR can offer a potential treatment option for breast cancer patients (158, 159). Industrial xenobiotics, dietary metabolites etc., serve as agonists of AHR and are ubiquitously present in the environment. AHR-mediated epigenetic repression has been found in the *BRCA1* gene which is also a direct target for AHR (160). In fact, CpG hypermethylation, deacetylation of H3K9, upregulation of H3K9me3, DNMT-1, DNMT-3a, DNMT-3b, and methyl-binding protein (MBD)-2 are some of the epigenetic changes linked with AHR mediated repression of *BRCA1* gene (161, 162).

BPA is yet another endocrine disrupter and is an epigenetically active xenoestrogen prevalent in plastic and food cans (163, 164) whose exposure has been linked with an increased risk of breast cancer (165). While overexpression of EZH2 is linked to breast cancer, *in-utero* exposure to BPA is able to alter the EZH2 expression in mammary tissues (166). In fact, exposure of normal breast cells to the environmentally relevant doses of BPA caused the ERα to internalize into the nucleus and also changed the DNA methylation status of a lysosomal associated membrane protein (LAMP3) (167). LAMP3 protein is implicated in metastasis and breast cancer cell motility and is of prognostic significance (168–170).

A very prevalent environmental contaminant of soil, food, and water is arsenic which has been studied widely for its carcinogenic effect. Exposure to arsenic and the risk of developing breast cancer has been reviewed extensively (171). Arsenic is able to transform the normal mammary epithelial cells that were subjected to chronic treatment with low levels. Moreover arsenic facilitated the growth of breast cancer cells that were ERα-positive (172, 173). The involvement of arsenic in the carcinogenesis process comes from the fact that it induces genomic instability mediated by disrupting the Fanconi anemia (FA) and/or breast cancer (BRCA) pathway (174). The epigenetic influence of arsenic has been established in studies reporting that arsenic influences DNA methylation by affecting the pool of available methyl groups. This is because the detoxification of arsenic utilizes methyl group from S-adenosyl-homocysteine (SAM) (175). Therefore, exposure to arsenic and its subsequent metabolism within the cells, impart towards a global hypomethylation owing to the usage of existing methyl stores available from SAM (176). Strikingly, maternal exposure to arsenic not only altered the DNA methylation but also increased the DNA methylation in children (177, 178).

The source of PAHs is myriad, which includes combustion products, automobile exhaust, cigarette smoke, indoor and outdoor air pollution, waste incinerators etc. (179). Tobacco smoking represents one of the important risk factors for breast cancer (180–182). Smoking not only affects the DNA methylation pattern of breast tumors, but it has been a critical factor linking DNA methylation and breast cancer for ER positive cancer subtypes (183, 184). Aberrant methylation alterations have also been observed in breast cancer cells exposed to benzo(*a*)pyrene, which resulted in the generation of DNA adducts at the CpG dinucleotides, ultimately affecting the epigenetic landscape of the methylation process (185).

External factors are not just limited to toxicants or environmental agents. The cellular microenvironment is sensitive to cues such as nutrient availability, hypoxia and, extracellular pH, and can epigenetically reprogram the metabolic behavior of cancer cells to adapt to the changing environment (186). The fact that metabolic profiles of cancer cells differ from the normal cells, gives us a clear indication of the underlying genetic and epigenetic machinery that are altered in the carcinogenesis process, thereby bestowing growth advantage to cancer cells for their survival. Hence metabolic reprogramming is indispensable for breast cancer and has many therapeutic ramifications (187). Cellular metabolites shuffling from the different cellular compartments such as cytoplasm, mitochondria, nucleus, etc., has the potential to regulate gene expression by altering the availability of enzymatic substrates and co-factors required for the metabolic reactions mediated epigenetic processes, such as DNA and histone modifications. Glucose remains one of the most important metabolites shaping the metabolic profiles of breast cancer by shifting the energy generating mechanisms from glycolysis to oxidative phosphorylation or vice versa. In this context, the availability of glucose affects the estrogen which facilitates glycolysis in a high glucose state but urged oxidative phosphorylation under the conditions of low glucose to meet the energy demands of the breast cancer cells (188). It is noteworthy that in adipose tissues, a major component of the breast, ERα is the vital regulator of a glucose transporter protein expression GLUT4 (189). Glycolysis can also be influenced by ERα, during the conditions of hypoxic stress. Hypoxia inducible factor-1α (HIF-1α) which is an oxygen-dependent transcriptional activator that carries out cellular adaptation to low oxygen and nutrient starved environment, is implicated in the ERα mediated activation of the glycolysis process in breast cancer (190). However, under normoxia and hypoxia conditions, both ERα and HIF-1α regulate histone demethylase JMJD2B and orchestrate breast cancer cell growth by epigenetically regulating the genes implicated in the cell cycle. Moreover, knocking down ERα can compromise the HIF-1α function even under hypoxic circumstances (135). One of the important transcription factors that aid cancer cells in metabolic adaption in a nutrient deprived environment, oxidative or xenobiotic stress is the nuclear factor erythroid 2-related factor 2 (NRF2) (191). Epigenetic modifications including DNA methylation are crucial for the regulation of NRF2 and its adaptor protein KEAP1 (192, 193). In breast cancer patients, elevated NRF2 expression led to decreased overall survival and disease-free survival (194). Elevated NRF2 enhanced the growth and

motility of breast cancer cells by upregulating a pivotal enzyme of the pentose phosphate pathway, i.e., the glucose-6-phosphate dehydrogenase (G6PD) (195). In fact, Estradiol (E2) can stimulate NRF2 transcription, leading to an elevation in mitochondrial biogenesis (196).

Mdig, an environment regulated gene in breast cancer

To ascertain the kind of risks and exposures affecting breast carcinogenesis, it is essential to gain an understanding of gene-environment interaction and the genes that are induced and manifested in breast cancer. Since a fraction of breast cancer cases is also sporadic, studying the genetic and epigenetic mechanisms that regulates breast tumor development under environmental and occupational settings, will undoubtedly offer new targets for chemoprevention and therapies.

We have identified one such environmentally induced gene named the Mineral dust-induced gene (mdig), also called MINA53, RIOX2, or NO52. Certain environmental agents such as mineral dust, tobacco smoke, arsenic, silica, etc. induced the expression of mdig (197–200). Mdig has oncogenic and epigenetic roles in a variety of human cancers, where it exhibits elevated expression (201, 202). Mdig promoted cell proliferation, cell cycle transition, and anti-apoptotic behaviors in different cell types, further corroborating its oncogenic role (198, 203). Mdig played key roles in the pathogenesis of arsenic induced lung cancer, where JNK-STAT3 signaling and mi-RNA21 mediate the processes. Further, we found that arsenic exposure induces the phosphorylation of EZH2 at serine 21 *via* JNK- and STAT3-dependent Akt activation (199, 204). Mdig is also upregulated in smokers in a pack-year dependent fashion, where it predicted poor overall survival in smokers that had lung cancer (205).

More recently, our studies on mdig and environmental factor arsenic revealed crosstalk between mdig and a master regulator of oxidative stress, NRF2, where together they contribute to arsenic induced generation of cancer stem like cells. Normal lung cells treated with arsenic showed an enhancement of HIF1α in the promoter of mdig, which was somehow accredited by activated NRF2 in response to arsenic (206). Since HIF1α is a direct transcriptional target of NRF2 (206) and considering the important role of NRF2 and HIF1 in tumorigenesis, our research further potentiates the importance of mdig on regulating the stress response activities implicated in genomic instability relying on metabolic reprogramming and cancer stem cells (207).

In breast cancer, we have identified that the expression level of mdig predicts the survival outcomes depending upon the different status of lymph node metastasis. A higher level of mdig predicted poor overall survival of patients who had no lymph node metastasis, whereas, in those patients who were positive for lymph node metastasis, high mdig expression predicted better overall survival (208). Dwelling further to assess the role of mdig in breast cancer, our studies revealed a negative correlation of mdig on the migration, invasion, and DNA methylation of breast cancer cells. Mdig not only regulated the chromatin accessibility of the migration/invasion genes but also exhibited a context dependent expression, where its expression was downregulated in invasive and triple negative breast cancer. This supported the notion that mdig is inhibitory for cell motility and spread and that's why its high expression predicts favorable outcomes in lymph node metastasis positive cases of breast cancer (209). Since mdig is transcriptionally governed by an upstream regulator c-myc (210), which has both tumor accelerator and suppressive roles and can inhibit cancer metastasis (211), our studies are suggestive of the dual roles of mdig in breast cancer, where it is essential for the early stages of cancer development due to its pro-proliferative feature but is inhibitory in the later stages owing to its metastasis inhibitory features.

Mdig protein contains a conserved JmjC domain. Since JmjC domain has been identified as a signature motif of the JmjC family of histone demethylases (129), mdig's involvement in the epigenetic process of histone modifications is inevitable. Recent studies provide evidence that the oncogenic activity of mdig is presumably achieved *via* its regulation on the demethylation of histone proteins. Our studies showed a demethylase like activity of mdig towards the repressive histone methylation markers that include H3K9me3, H3K27me3, and H4K20me3. Using the CRISPR-Cas9 gene editing approach coupled with chromatin immunoprecipitation sequencing (ChIP) in human lung epithelial cell line BEAS 2B, lung cancer cell line A549, and breast cancer cell line MDA-MB-231, an antagonistic effect of mdig on repressive histone trimethylation marks were revealed where mdig favored the open conformation of chromatin and permitted active gene transcription. Knocking down mdig resulted in a pronounced enrichment of these repressive trimethylation markers on the genes that are implicated in cell growth, stemness, inflammation, and metastasis (212). With the loss of mdig, there also occurred an increase in the levels of the polycomb repressive complex (PRC2) proteins EZH2 and RBBP4. Strikingly, these proteins are known to catalyze H3K27me3, and our previous studies identified a direct protein-protein interaction between mdig and CBX3, CBX5, RBBP4, and RBBP7 proteins. While RBBP4 and RBBP7 are the regulatory subunits of the PRC2 complex, CBX3 and CBX5 can recognize and bind to H3K9me3 (213).

In breast cancer cells, loss of mdig also enhanced an epigenetic mark of transcription elongation H3K36me3, in addition to H4K20me3 and H3K9me3. In this view, H4K20me3 being a marker for closed chromatin status in the somatic and embryonic stem cells (214), it is suggested that an elevation of H4K20me3 can contribute to growth inhibitory activities in the somatic cells. This notion is further supported by our previous studies where reduced mdig resulted in a decline of the S phase cells (198). It is also

indicated that mdig acts as DNA demethylase or indirectly controls DNA methylation *via* the Tet family of DNA methylases (202). Additionally, a negative correlation was also observed between mdig and H3K9me3 in cellular studies (209, 215, 216). One of the consequences of enriched histone repressive marks H3K9me3 and H3K27me3, is on the transcription of genes implicated in glycan metabolism. Mdig exerted a positive regulatory role on the glycosylation process by inhibiting the repressive histone methylation marks (217).

Altogether, our research on mdig provided a much-needed rationale to explore its activities in several aspects of inflammation, stemness, metabolism, cell growth, metastasis, and epigenetic reprogramming orchestrating the carcinogenesis machinery in breast cancer.

Perspectives

Despite tremendous progress being made in breast cancer research, some challenges still prevail. Metabolic plasticity, epigenetic reprogramming, and altered receptor repertoire lead to the issues of drug resistance and treatment failure. It is yet not fully clear as to what are the remarkable mechanistic programs that are critical for the breast tumor to become metastatic. Although our understanding of the heterogeneity of breast cancers has improved that has led to the generation of novel anti-cancer therapies exploiting the hormone receptor status, epigenetic marks, and other biological machineries, yet, when it comes to the general population there has been very limited success owing to the individual differences among the patients. An efficient personalized therapy would offer rescue to some extent towards combating the setbacks originated due to the heterogeneity and plasticity issues as observed in breast cancer therapies under clinical settings.

Environmental exposure to risk factors for breast cancer require particular attention, where relevant biomarkers related to such exposure need to be identified. Epigenetic mechanisms particularly DNA and histone methylation are involved in the onset of carcinogenesis by modulating the expression of potent oncogenes and tumor suppressors. Thus, dissecting the epigenetic elements would widen our knowledge towards better understanding the causative factors as well as the different routes that cancer cells adopt to attain heterogeneity. Moreover, studying maternal, *in utero* or pre-conception exposures and unraveling an association between the agents exposed and the different epigenetic repertoires correlating with the disease outcome, will be a promising avenue to explore. Such a strategy would assist in adopting modifiable approaches that can have significant implications in reducing the risk factors as a part of chemoprevention tactics. This demands a multidisciplinary effort that would integrate genomics, proteomics, and metabolomics in examining the different epigenomic profiles and pattern that drive the breast carcinogenesis under the conditions of sporadic and environmental settings. In this context, research on environmentally modulated genes engaged in breast cancer such as mdig, is warranted.

Author contributions

CT and FC conceived the idea and wrote the article. YQ, YF, ZB, WZ, and HJ participated in conducting systemic review of the literature. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by National Institutes of Health (NIH) grants R01 ES031822, R01 ES028335, R01 ES028263, and Research Start-up fund of the Stony Brook University to FC.

Acknowledgments

We would like to thank all the authors and researchers whose work has been cited here.

Conflict of interest

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

Publisher's note

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA: A Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708

2. Cardoso F, Senkus E, Costa A, Papadopoulos E, Aapro M, André F, et al. 4th ESO–ESMO international consensus guidelines for advanced breast cancer (ABC 4)††These guidelines were developed by the European school of oncology (ESO) and the European society for medical oncology (ESMO). *Ann Oncol* (2018) 29 (8):1634–57. doi: 10.1093/annonc/mdy192

3. Nascimento R, Otoni K. Histological and molecular classification of breast cancer: what do we know? *Mastology* (2020) 30:1–8. doi: 10.29289/25945394202020200024

4. Henry NL, Cannon-Albright LA. Breast cancer histologic subtypes show excess familial clustering. *Cancer* (2019) 125(18):3131–8. doi: 10.1002/cncr.32198

5. Tan PH, Ellis I, Allison K, Brogi E, Fox SB, Lakhani S, et al. The 2019 world health organization classification of tumours of the breast. *Histopathology* (2020) 77(2):181–5. doi: 10.1111/his.14091

6. Fragomeni SM, Sciallis A, Jeruss JS. Molecular subtypes and local-regional control of breast cancer. *Surg Oncol Clinics North America* (2018) 27(1):95–120. doi: 10.1016/j.soc.2017.08.005

7. Jones RL, Constantinidou A, Reis-Filho JS. Molecular classification of breast cancer. *Surg Pathol Clin* (2012) 5(3):701–17. doi: 10.1016/j.path.2012.06.008

8. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* (2007) 13(15 Pt 1):4429–34. doi: 10.1158/1078-0432.CCR-06-3045

9. Zardavas D, Irrthum A, Swanton C, Piccart M. Clinical management of breast cancer heterogeneity. *Nat Rev Clin Oncol* (2015) 12(7):381–94. doi: 10.1038/nrclinonc.2015.73

10. Malhotra GK, Zhao X, Band H, Band V. Histological, molecular and functional subtypes of breast cancers. *Cancer Biol Ther* (2010) 10(10):955–60. doi: 10.4161/cbt.10.10.13879

11. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, et al. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. *Jama* (2017) 317(23):2402–16. doi: 10.1001/jama.2017.7112

12. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. *J Clin Oncol* (2007) 25(11):1329–33. doi: 10.1200/JCO.2006.09.1066

13. Pohl-Rescigno E, Hauke J, Loibl S, Möbus V, Denkert C, Fasching PA, et al. Association of germline variant status with therapy response in high-risk early-stage breast cancer: A secondary analysis of the GeparOcto randomized clinical trial. *JAMA Oncol* (2020) 6(5):744–8. doi: 10.1001/jamaoncol.2020.0007

14. Howard FM, Olopade OI. Epidemiology of triple-negative breast cancer: A review. *Cancer J* (2021) 27(1):8–16. doi: 10.1097/PPO.0000000000000500

15. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature* (2016) 534(7605):47–54. doi: 10.1038/nature17676

16. Walsh T, King MC. Ten genes for inherited breast cancer. *Cancer Cell* (2007) 11(2):103–5. doi: 10.1016/j.ccr.2007.01.010

17. Ruffell B , Au A, Rugo HS, Esserman LJ, Hwang ES, Coussens LM. Leukocyte composition of human breast cancer. *Proc Natl Acad Sci U.S.A.* (2012) 109(8):2796–801. doi: 10.1073/pnas.1104303108

18. Mitrunen K, Hirvonen A. Molecular epidemiology of sporadic breast cancer. the role of polymorphic genes involved in oestrogen biosynthesis and metabolism. *Mutat Res* (2003) 544(1):9–41. doi: 10.1016/S1383-5742(03)00016-4

19. Nur U , El Reda D, Hashim D, Weiderpass E. A prospective investigation of oral contraceptive use and breast cancer mortality: findings from the Swedish women's lifestyle and health cohort. *BMC Cancer* (2019) 19(1):807. doi: 10.1186/s12885-019-5985-6

20. Del Pup L, Codacci-Pisanelli G, Peccatori F. Breast cancer risk of hormonal contraception: Counselling considering new evidence. *Crit Rev Oncol Hematol* (2019) 137:123–30. doi: 10.1016/j.critrevonc.2019.03.001

21. Kehm RD, Genkinger JM, MacInnis RJ, John EM, Phillips KA, Dite GS, et al. Recreational physical activity is associated with reduced breast cancer risk in adult women at high risk for breast cancer: A cohort study of women selected for familial and genetic risk. *Cancer Res* (2020) 80(1):116–25. doi: 10.1158/0008-5472.CAN-19-1847

22. Barrios-Rodríguez R, Toledo E, Martinez-Gonzalez MA, Aguilera-Buenosvinos I, Romanos-Nanclares A, Jiménez-Moleón JJ. Adherence to the 2018 world cancer research Fund/American institute for cancer research recommendations and breast cancer in the SUN project. *Nutrients* (2020) 12 (7):1–12. doi: 10.3390/nu12072076

23. Hamajima N, Hirose K, Tajima K, Rohan T, Calle EE, Heath CW, Jr. , et al, et al. Alcohol, tobacco and breast cancer–collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. *Br J Cancer* (2002) 87(11):1234–45.doi: 10.1038/sj.bjc.6600596

24. Seitz HK , Pelucchi C, Bagnardi V, La Vecchia C. Epidemiology and pathophysiology of alcohol and breast cancer: Update 2012. *Alcohol Alcohol* (2012) 47(3):204–12. doi: 10.1093/alcalc/ags011

25. Collaborative Group on Hormonal Factors in Breast CancerBreast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *Lancet* (1996) 347(9017):1713–27. doi.org/10.1016/s0140-6736(96)90806-5

26. Collaborative Group on Hormonal Factors in Breast. C., menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *Lancet Oncol* (2012) 13(11):1141–51. doi.org/10.1016/s1470-2045(12)70425-4

27. Goldberg M , D'Aloisio AA, O'Brien KM, Zhao S, Sandler DP. Pubertal timing and breast cancer risk in the sister study cohort. *Breast Cancer Res* (2020) 22 (1):112. doi: 10.1186/s13058-020-01326-2

28. Doody MM, Freedman DM, Alexander BH, Hauptmann M, Miller JS, Rao RS, et al. Breast cancer incidence in U.S. radiologic technologists. *Cancer* (2006) 106(12):2707–15. doi: 10.1002/cncr.21876

29. Chlebowski RT, Luo J, Anderson GL, Barrington W, Reding K, Simon MS, et al. Weight loss and breast cancer incidence in postmenopausal women. *Cancer* (2019) 125(2):205–12. doi: 10.1002/cncr.31687

30. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* (1983) 301(5895):89–92. doi: 10.1038/301089a0

31. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. *Nat Rev Cancer* (2011) 11(10):726–34. doi: 10.1038/nrc3130

32. Esteller M. Epigenetics in cancer. *New Engl J Med* (2008) 358(11):1148–59. doi: 10.1056/NEJMra072067

33. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* (2003) 349(21):2042–54. doi: 10.1056/NEJMra023075

34. Kulis M, Esteller M. 2 - DNA methylation and cancer. In: Z Herceg and T Ushijima, editors. *Advances in genetics*. (Barcelona, Catalonia, Spain: Cancer Epigenetics and Biology Program (2010). 27–56.

35. Jones PA, Baylin SB. The epigenomics of cancer. *Cell* (2007) 128(4):683–92. doi: 10.1016/j.cell.2007.01.029

36. Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. *Nature* (2007) 447(7143):433–40. doi: 10.1038/nature05919

37. Kareta MS, Botello ZM, Ennis JJ, Chou C, Chédin F. Reconstitution and mechanism of the stimulation of de novo methylation by human DNMT3L. *J Biol Chem* (2006) 281(36):25893–902. doi: 10.1074/jbc.M603140200

38. Okano M , Bell DW, Haber DA, Li E. DNA Methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* (1999) 99(3):247–57. doi: 10.1016/S0092-8674(00)81656-6

39. Robertson KD. DNA Methylation and human disease. *Nat Rev Genet* (2005) 6(8):597–610. doi: 10.1038/nrg1655

40. Scourzic L, Mouly E, Bernard OA. TET proteins and the control of cytosine demethylation in cancer. *Genome Med* (2015) 7(1):9. doi: 10.1186/s13073-015-0134-6

41. Williams K, Christensen J, Helin K. DNA Methylation: TET proteins-guardians of CpG islands? *EMBO Rep* (2011) 13(1):28–35. doi: 10.1038/embor.2011.233

42. Chandramouly G. Gadd45 in DNA demethylation and DNA repair. In: MR Zaidi and DA Liebermann, editors. *Gadd45 stress sensor genes*. (Philadelphia, PA, USA: Springer International Publishing: Cham) (2022). 55–67.

43. Schäfer A. Gadd45 proteins: key players of repair-mediated DNA demethylation. *Adv Exp Med Biol* (2013) 793:35–50. doi: 10.1007/978-1-4614-8289-5_3

44. Pietrasik S, Zajac G, Morawiec J, Soszynski M, Fila M, Blasiak J. Interplay between BRCA1 and GADD45A and its potential for nucleotide excision repair in breast cancer pathogenesis. *Int J Mol Sci* (2020) 21(3):1–22. doi: 10.3390/ijms21030870

45. Wang W, Huper G, Guo Y, Murphy SK, Olson JA, Jr. , Marks JR, et al. Analysis of methylation-sensitive transcriptome identifies GADD45a as a frequently methylated gene in breast cancer. *Oncogene* (2005) 24(16):2705–14. doi: 10.1038/sj.onc.1208464

46. Jeschke J, Collignon E, Fuks F. Portraits of TET-mediated DNA hydroxymethylation in cancer. *Curr Opin Genet Dev* (2016) 36:16–26. doi: 10.1016/j.gde.2016.01.004

47. Muñoz DP, Lee EL, Takayama S, Coppé JP, Heo SJ, Boffelli D, et al. Activation-induced cytidine deaminase (AID) is necessary for the epithelial-mesenchymal transition in mammary epithelial cells. *Proc Natl Acad Sci U.S.A.* (2013) 110(32):E2977–86. doi: 10.1073/pnas.1301021110

48. Morgan HD, Dean W, Coker HA, Reik W, Petersen-Mahrt SK. Activation-induced cytidine deaminase deaminates 5-methylcytosine in DNA and is expressed in pluripotent tissues: IMPLICATIONS FOR EPIGENETIC REPROGRAMMING*. *J Biol Chem* (2004) 279(50):52353–60. doi: 10.1074/jbc.M407695200

49. Popp C, Dean W, Feng S, Cokus SJ, Andrews S, Pellegrini M, et al. Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. *Nature* (2010) 463(7284):1101–5. doi: 10.1038/nature08829

50. Guo JU, Su Y, Zhong C, Ming GL, Song H. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. *Cell* (2011) 145(3):423–34. doi: 10.1016/j.cell.2011.03.022

51. Bos MK, Smid M, Sleijfer S, Martens JWM. Apolipoprotein b mRNA-editing catalytic polypeptide-Like-Induced protein changes in estrogen receptor-positive, human epidermal growth factor receptor 2-negative breast cancer throughout disease progression. *JCO Precis Oncol* (2022) 6:e2100190. doi: 10.1200/PO.21.00190

52. DiMarco AV, Qin X, McKinney BJ, Garcia NMG, Van Alsten SC, Mendes EA, et al. APOBEC mutagenesis inhibits breast cancer growth through induction of T cell-mediated antitumor immune responses. *Cancer Immunol Res* (2022) 10 (1):70–86. doi: 10.1158/2326-6066.CIR-21-0146

53. Koboldt DC, Fulton RS, McLellan MD, Schmidt H, Kalicki-Veizer J, McMichael JF, et al. Comprehensive molecular portraits of human breast tumours. *Nature* (2012) 490(7418):61–70. doi: 10.1038/nature11412

54. Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, et al. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. *J Natl Cancer Inst* (2000) 92(7):564–9. doi: 10.1093/jnci/92.7.564

55. Radpour R, Barekati Z, Kohler C, Schumacher MM, Grussenmeyer T, Jenoe P, et al. Integrated epigenetics of human breast cancer: synoptic investigation of targeted genes, microRNAs and proteins upon demethylation treatment. *PloS One* (2011) 6(11):e27355. doi: 10.1371/journal.pone.0027355

56. Fujikane T, Nishikawa N, Toyota M, Suzuki H, Nojima M, Maruyama R, et al. Genomic screening for genes upregulated by demethylation revealed novel targets of epigenetic silencing in breast cancer. *Breast Cancer Res Treat* (2010) 122 (3):699–710. doi: 10.1007/s10549-009-0600-1

57. Shargh SA, Sakizli M, Khalaj V, Movafagh A, Yazdi H, Hagigatjou E, et al. Downregulation of e-cadherin expression in breast cancer by promoter hypermethylation and its relation with progression and prognosis of tumor. *Med Oncol* (2014) 31(11):1–6. doi: 10.1007/s12032-014-0250-y

58. Mirza S, Sharma G, Prasad CP, Parshad R, Srivastava A, Gupta SD, et al. Promoter hypermethylation of TMS1, BRCA1, ERα and PRB in serum and tumor DNA of invasive ductal breast carcinoma patients. *Life Sci* (2007) 81(4):280–7. doi: 10.1016/j.lfs.2007.05.012

59. Esteller M, Corn PG, Baylin SB, Herman JG. A gene hypermethylation profile of human cancer. *Cancer Res* (2001) 61(8):3225–9.

60. Butcher DT, Rodenhiser DI. Epigenetic inactivation of BRCA1 is associated with aberrant expression of CTCF and DNA methyltransferase (DNMT3B) in some sporadic breast tumours. *Eur J Cancer* (2007) 43(1):210–9. doi: 10.1016/j.ejca.2006.09.002

61. Vallian S, Sedaghat M, Nassiri I, Frazmand A. Methylation status of p16 INK4A tumor suppressor gene in Iranian patients with sporadic breast cancer. *J Cancer Res Clin Oncol* (2009) 135(8):991–6. doi: 10.1007/s00432-008-0534-8

62. Askari M, Sobti RC, Nikbakht M, Sharma SC. Aberrant promoter hypermethylation of p21 (WAF1/CIP1) gene and its impact on expression and role of polymorphism in the risk of breast cancer. *Mol Cell Biochem* (2013) 382(1-2):19–26. doi: 10.1007/s11010-013-1696-5

63. Győrffy B, Bottai G, Fleischer T, Munkácsy G, Budczies J, Paladini L, et al. Aberrant DNA methylation impacts gene expression and prognosis in breast cancer subtypes. *Int J Cancer* (2016) 138(1):87–97. doi: 10.1002/ijc.29684

64. Holm K, Hegardt C, Staaf J, Vallon-Christersson J, Jönsson G, Olsson H, et al. Molecular subtypes of breast cancer are associated with characteristic DNA methylation patterns. *Breast Cancer Res* (2010) 12(3):R36. doi: 10.1186/bcr2590

65. Yan PS , Perry MR, Laux DE, Asare AL, Caldwell CW, Huang TH. CpG island arrays: an application toward deciphering epigenetic signatures of breast cancer. *Clin Cancer Res* (2000) 6(4):1432–8.

66. Lapidus RG, Ferguson AT, Ottaviano YL, Parl FF, Smith HS, Weitzman SA, et al. Methylation of estrogen and progesterone receptor gene 5' CpG islands correlates with lack of estrogen and progesterone receptor gene expression in breast tumors. *Clin Cancer Res* (1996) 2(5):805–10.

67. Ennour-Idrissi K, Dragic D, Issa E, Michaud A, Chang S-L, Provencher L, et al. DNA Methylation and breast cancer risk: An epigenome-wide study of normal breast tissue and blood. *Cancers* (2020) 12(11):3088. doi: 10.3390/cancers12113088

68. Dedeurwaerder S, Desmedt C, Calonne E, Singhal SK, Haibe-Kains B, Defrance M, et al. DNA Methylation profiling reveals a predominant immune component in breast cancers. *EMBO Mol Med* (2011) 3(12):726–41. doi: 10.1002/emmm.201100801

69. Zhang M, Wang Y, Wang Y, Jiang L, Li X, Gao H, et al. Integrative analysis of DNA methylation and gene expression to determine specific diagnostic biomarkers and prognostic biomarkers of breast cancer. *Front Cell Dev Biol* (2020) 8. doi: 10.3389/fcell.2020.529386

70. de Almeida BP , Apolónio JD, Binnie A, Castelo-Branco P. Roadmap of DNA methylation in breast cancer identifies novel prognostic biomarkers. *BMC Cancer* (2019) 19(1):219. doi: 10.1186/s12885-019-5403-0

71. Batra RN, Lifshitz A, Vidakovic AT, Chin S-F, Sati-Batra A, Sammut S-J, et al. DNA Methylation landscapes of 1538 breast cancers reveal a replication-linked clock, epigenomic instability and cis-regulation. *Nat Commun* (2021) 12 (1):5406. doi: 10.1038/s41467-021-25661-w

72. Luger K , Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 a resolution. *Nature* (1997) 389 (6648):251–60. doi: 10.1038/38444

73. Bentley GA , Lewit-Bentley A, Finch JT, Podjarny AD, Roth M. Crystal structure of the nucleosome core particle at 16 a resolution. *J Mol Biol* (1984) 176 (1):55–75. doi: 10.1016/0022-2836(84)90382-6

74. Allfrey VG, Faulkner R, Mirsky AE. Acetylation and methylation of histones and their possible role in the regulation of rna synthesis. *Proc Natl Acad Sci U.S.A.* (1964) 51(5):786–94. doi: 10.1073/pnas.51.5.786

75. Audia JE, Campbell RM. Histone Modifications and Cancer *Cold Spring Harb Perspect Biol* (2016) 8(4):a019521. doi: 10.1101/cshperspect.a019521

76. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res* (2011) 21(3):381–95. doi: 10.1038/cr.2011.22

77. Ropero S, Esteller M. The role of histone deacetylases (HDACs) in human cancer. *Mol Oncol* (2007) 1(1):19–25. doi: 10.1016/j.molonc.2007.01.001

78. Koch CM, Andrews RM, Flicek P, Dillon SC, Karaöz U, Clelland GK, et al. The landscape of histone modifications across 1% of the human genome in five human cell lines. *Genome Res* (2007) 17(6):691–707. doi: 10.1101/gr.5704207

79. Creyghton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, et al. Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc Natl Acad Sci U.S.A.* (2010) 107(50):21931–6. doi: 10.1073/pnas.1016071107

80. Pradeepa MM, Grimes GR, Kumar Y, Olley G, Taylor GC, Schneider R, et al. Histone H3 globular domain acetylation identifies a new class of enhancers. *Nat Genet* (2016) 48(6):681–6. doi: 10.1038/ng.3550

81. Lopez-Serra L, Ballestar E, Ropero S, Setien F, Billard LM, Fraga MF, et al. Unmasking of epigenetically silenced candidate tumor suppressor genes by removal of methyl-CpG-binding domain proteins. *Oncogene* (2008) 27(25):3556–66. doi: 10.1038/sj.onc.1211022

82. Xiao XS, Cai MY, Chen JW, Guan XY, Kung HF, Zeng YX, et al. High expression of p300 in human breast cancer correlates with tumor recurrence and predicts adverse prognosis. *Chin J Cancer Res* (2011) 23(3):201–7. doi: 10.1007/s11670-011-0201-5

83. Dong H, Wang W, Mo S, Chen R, Zou K, Han J, et al. SP1-induced lncRNA AGAP2-AS1 expression promotes chemoresistance of breast cancer by epigenetic regulation of MyD88. *J Exp Clin Cancer Res* (2018) 37(1):202. doi: 10.1186/s13046-018-0875-3

84. Chi Y, Xue J, Huang S, Xiu B, Su Y, Wang W, et al. CapG promotes resistance to paclitaxel in breast cancer through transactivation of PIK3R1/P50. *Theranostics* (2019) 9(23):6840–55. doi: 10.7150/thno.36338

85. He H, Wang D, Yao H, Wei Z, Lai Y, Hu J, et al. Transcriptional factors p300 and MRTF-a synergistically enhance the expression of migration-related genes in MCF-7 breast cancer cells. *Biochem Biophys Res Commun* (2015) 467(4):813–20. doi: 10.1016/j.bbrc.2015.10.060

86. Lin C, Liao W, Jian Y, Peng Y, Zhang X, Ye L, et al. CGI-99 promotes breast cancer metastasis via autocrine interleukin-6 signaling. *Oncogene* (2017) 36 (26):3695–705. doi: 10.1038/onc.2016.525

87. Yu S, Gong X, Ma Z, Zhang M, Huang L, Zhang J, et al. Endocrine resistant breast cancer cells with loss of ERα expression retain proliferative ability by reducing caspase7-mediated HDAC3 cleavage. *Cell Oncol (Dordr)* (2020) 43(1):65–80. doi: 10.1007/s13402-019-00439-x

88. Hou MF, Luo CW, Chang TM, Hung WC, Chen TY, Tsai YL, et al. The NuRD complex-mediated p21 suppression facilitates chemoresistance in BRCA-proficient breast cancer. *Exp Cell Res* (2017) 359(2):458–65. doi: 10.1016/j.yexcr.2017.08.029

89. Liu R, Wang L, Chen G, Katoh H, Chen C, Liu Y, et al. FOXP3 up-regulates p21 expression by site-specific inhibition of histone deacetylase 2/histone deacetylase 4 association to the locus. *Cancer Res* (2009) 69(6):2252–9. doi: 10.1158/0008-5472.CAN-08-3717

90. Tang X, Li G, Su F, Cai Y, Shi L, Meng Y, et al. HDAC8 cooperates with SMAD3/4 complex to suppress SIRT7 and promote cell survival and migration. *Nucleic Acids Res* (2020) 48(6):2912–23. doi: 10.1093/nar/gkaa039

91. Lu C , Wang X, Zhao X, Xin Y, Liu C. Long non-coding RNA ARAP1-AS1 accelerates cell proliferation and migration in breast cancer through miR-2110/HDAC2/PLIN1 axis. *Biosci Rep* (2020) 40(4):1–10. doi: 10.1042/BSR20191764

92. Cassandri M, Butera A, Amelio I, Lena AM, Montanaro M, Mauriello A, et al. ZNF750 represses breast cancer invasion via epigenetic control of prometastatic genes. *Oncogene* (2020) 39(22):4331–43. doi: 10.1038/s41388-020-1277-5

93. Ray A, Alalem M, Ray BK. Loss of epigenetic kruppel-like factor 4 histone deacetylase (KLF-4-HDAC)-mediated transcriptional suppression is crucial in increasing vascular endothelial growth factor (VEGF) expression in breast cancer. *J Biol Chem* (2013) 288(38):27232–42. doi: 10.1074/jbc.M113.481184

94. Roy SS, Gonugunta VK, Bandyopadhyay A, Rao MK, Goodall GJ, Sun LZ, et al. Significance of PELP1/HDAC2/miR-200 regulatory network in EMT and metastasis of breast cancer. *Oncogene* (2014) 33(28):3707–16. doi: 10.1038/onc.2013.332

95. Gong C, Qu S, Lv XB, Liu B, Tan W, Nie Y, et al. BRMS1L suppresses breast cancer metastasis by inducing epigenetic silence of FZD10. *Nat Commun* (2014) 5:5406. doi: 10.1038/ncomms6406

96. Rifaï K, Judes G, Idrissou M, Daures M, Bignon YJ, Penault-Llorca F, et al. Dual SIRT1 expression patterns strongly suggests its bivalent role in human breast cancer. *Oncotarget* (2017) 8(67):110922–30. doi: 10.18632/oncotarget.23006

97. Rifaï K, Judes G, Idrissou M, Daures M, Bignon YJ, Penault-Llorca F, et al. SIRT1-dependent epigenetic regulation of H3 and H4 histone acetylation in human breast cancer. *Oncotarget* (2018) 9(55):30661–78. doi: 10.18632/oncotarget.25771

98. Wang R-H, Zheng Y, Kim H-S, Xu X, Cao L, Lahusen T, et al. Interplay among BRCA1, SIRT1, and survivin during BRCA1-associated tumorigenesis. *Mol Cell* (2008) 32(1):11–20. doi: 10.1016/j.molcel.2008.09.011

99. Soung YH, Pruitt K, Chung J. Epigenetic silencing of ARRDC3 expression in basal-like breast cancer cells. *Sci Rep* (2014) 4:3846. doi: 10.1038/srep03846

100. Ashraf N, Zino S, Macintyre A, Kingsmore D, Payne A, George W, et al. Altered sirtuin expression is associated with node-positive breast cancer. *Br J Cancer* (2006) 95(8):1056–61. doi: 10.1038/sj.bjc.6603384

101. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, et al. High-resolution profiling of histone methylations in the human genome. *Cell* (2007) 129 (4):823–37. doi: 10.1016/j.cell.2007.05.009

102. Bedford MT, Clarke SG. Protein arginine methylation in mammals: who, what, and why. *Mol Cell* (2009) 33(1):1–13. doi: 10.1016/j.molcel.2008.12.013

103. Jenuwein T, Allis CD. Translating the histone code. *Science* (2001) 293 (5532):1074–80. doi: 10.1126/science.1063127

104. Wei S, Li C, Yin Z, Wen J, Meng H, Xue L, et al. Histone methylation in DNA repair and clinical practice: new findings during the past 5-years. *J Cancer* (2018) 9(12):2072–81. doi: 10.7150/jca.23427

105. Hyun K , Jeon J, Park K, Kim J. Writing, erasing and reading histone lysine methylations. *Exp Mol Med* (2017) 49(4):e324. doi: 10.1038/emm.2017.11

106. Li W , Wu H, Sui S, Wang Q, X. Targeting histone modifications in breast cancer: A precise weapon on the way. *Front Cell Dev Biol* (2021) 9. doi: 10.3389/fcell.2021.736935

107. Elsheikh SE, Green AR, Rakha EA, Powe DG, Ahmed RA, Collins HM, et al. Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome. *Cancer Res* (2009) 69 (9):3802–9. doi: 10.1158/0008-5472.CAN-08-3907

108. Messier TL, Gordon JA, Boyd JR, Tye CE, Browne G, Stein JL, et al. Histone H3 lysine 4 acetylation and methylation dynamics define breast cancer subtypes. *Oncotarget* (2016) 7(5):5094–109. doi: 10.18632/oncotarget.6922

109. Spangle JM, Dreijerink KM, Groner AC, Cheng H, Ohlson CE, Reyes J, et al. PI3K/AKT signaling regulates H3K4 methylation in breast cancer. *Cell Rep* (2016) 15(12):2692–704. doi: 10.1016/j.celrep.2016.05.046

110. Dong H, Hu J, Zou K, Ye M, Chen Y, Wu C, et al. Activation of LncRNA TINCR by H3K27 acetylation promotes trastuzumab resistance and epithelial-mesenchymal transition by targeting MicroRNA-125b in breast cancer. *Mol Cancer* (2019) 18(1):3. doi: 10.1186/s12943-018-0931-9

111. Segelle A, Núñez-Álvarez Y, Oldfield AJ, Webb KM, Voigt P, Luco RF, et al. Histone marks regulate the epithelial-to-mesenchymal transition via alternative splicing. *Cell Rep* (2022) 38(7):110357. doi: 10.1016/j.celrep.2022.110357

112. Wei Y, Xia W, Zhang Z, Liu J, Wang H, Adsay NV, et al. Loss of trimethylation at lysine 27 of histone H3 is a predictor of poor outcome in breast, ovarian, and pancreatic cancers. *Mol Carcinog* (2008) 47(9):701–6. doi: 10.1002/mc.20413

113. Yoo KH, Hennighausen L. EZH2 methyltransferase and H3K27 methylation in breast cancer. *Int J Biol Sci* (2012) 8(1):59–65. doi: 10.7150/ijbs.8.59

114. Chatterjee A, Rodger EJ, Eccles MR. Epigenetic drivers of tumourigenesis and cancer metastasis. *Semin Cancer Biol* (2018) 51:149–59. doi: 10.1016/j.semcancer.2017.08.004

115. Jørgensen S, Schotta G, Sørensen CS. Histone H4 lysine 20 methylation: key player in epigenetic regulation of genomic integrity. *Nucleic Acids Res* (2013) 41 (5):2797–806. doi: 10.1093/nar/gkt012

116. Yokoyama Y, Matsumoto A, Hieda M, Shinchi Y, Ogihara E, Hamada M, et al. Loss of histone H4K20 trimethylation predicts poor prognosis in breast cancer and is associated with invasive activity. *Breast Cancer Res* (2014) 16(3):R66. doi: 10.1186/bcr3681

117. Gan L, Yang Y, Li Q, Feng Y, Liu T, Guo W, et al. Epigenetic regulation of cancer progression by EZH2: from biological insights to therapeutic potential. *biomark Res* (2018) 6(1):10. doi: 10.1186/s40364-018-0122-2

118. Holm K, Grabau D, Lövgren K, Aradottir S, Gruvberger-Saal S, Howlin J, et al. Global H3K27 trimethylation and EZH2 abundance in breast tumor subtypes. *Mol Oncol* (2012) 6(5):494–506. doi: 10.1016/j.molonc.2012.06.002

119. Ding L, Kleer CG. Enhancer of zeste 2 as a marker of preneoplastic progression in the breast. *Cancer Res* (2006) 66(19):9352–5. doi: 10.1158/0008-5472.CAN-06-2384

120. Kim JH, Sharma A, Dhar SS, Lee SH, Gu B, Chan CH, et al. UTX and MLL4 coordinately regulate transcriptional programs for cell proliferation and invasiveness in breast cancer cells. *Cancer Res* (2014) 74(6):1705–17. doi: 10.1158/0008-5472.CAN-13-1896

121. Su CH, Lin IH, Tzeng TY, Hsieh WT, Hsu MT. Regulation of IL-20 expression by estradiol through KMT2B-mediated epigenetic modification. *PloS One* (2016) 11(11):e0166090. doi: 10.1371/journal.pone.0166090

122. Park UH, Kang MR, Kim EJ, Kwon YS, Hur W, Yoon SK, et al. ASXL2 promotes proliferation of breast cancer cells by linking ERα to histone methylation. *Oncogene* (2016) 35(28):3742–52. doi: 10.1038/onc.2015.443

123. Jeong KW, Kim K, Situ AJ, Ulmer TS, An W, Stallcup MR. Recognition of enhancer element-specific histone methylation by TIP60 in transcriptional activation. *Nat Struct Mol Biol* (2011) 18(12):1358–65. doi: 10.1038/nsmb.2153

124. Lee JY, Kong G. DOT1L: a new therapeutic target for aggressive breast cancer. *Oncotarget* (2015) 6(31):30451–2. doi: 10.18632/oncotarget.5860

125. Alexandrova E, Salvati A, Pecoraro G, Lamberti J, Melone V, Sellitto A, et al. Histone methyltransferase DOT1L as a promising epigenetic target for treatment of solid tumors. *Front Genet* (2022) 13. doi: 10.3389/fgene.2022.864612

126. Cho MH, Park JH, Choi HJ, Park MK, Won HY, Park YJ, et al. DOT1L cooperates with the c-Myc-p300 complex to epigenetically derepress CDH1 transcription factors in breast cancer progression. *Nat Commun* (2015) 6:7821. doi: 10.1038/ncomms8821

127. Nassa G, Salvati A, Tarallo R, Gigantino V, Alexandrova E, Memoli D, et al. Inhibition of histone methyltransferase DOT1L silences ERα gene and blocks proliferation of antiestrogen-resistant breast cancer cells. *Sci Adv* (2019) 5(2): eaav5590. doi: 10.1126/sciadv.aav5590

128. Byun WS, Kim WK, Han HJ, Chung H-J, Jang K, Kim HS, et al. Targeting histone methyltransferase DOT1L by a novel psammaplin a analog inhibits growth and metastasis of triple-negative breast cancer. *Mol Ther - Oncolytics* (2019) 15:140–52. doi: 10.1016/j.omto.2019.09.005

129. Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, et al. Histone demethylation by a family of JmjC domain-containing proteins. *Nature* (2006) 439(7078):811–6. doi: 10.1038/nature04433

130. Zheng Y, Zeng Y, Qiu R, Liu R, Huang W, Hou Y, et al. The homeotic protein SIX3 suppresses carcinogenesis and metastasis through recruiting the LSD1/NuRD (MTA3) complex. *Theranostics* (2018) 8(4):972–89. doi: 10.7150/thno.22328

131. Qiu R, Shi H, Wang S, Leng S, Liu R, Zheng Y, et al. BRMS1 coordinates with LSD1 and suppresses breast cancer cell metastasis. *Am J Cancer Res* (2018) 8 (10):2030.

132. Wang Y, Zhang H, Chen Y, Sun Y, Yang F, Yu W, et al. LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer. *Cell* (2009) 138(4):660–72. doi: 10.1016/j.cell.2009.05.050

133. Yang Y, Huang W, Qiu R, Liu R, Zeng Y, Gao J, et al. LSD1 coordinates with the SIN3A/HDAC complex and maintains sensitivity to chemotherapy in breast cancer. *J Mol Cell Biol* (2018) 10(4):285–301. doi: 10.1093/jmcb/mjy021

134. Berry WL, Janknecht R. KDM4/JMJD2 histone demethylases: epigenetic regulators in cancer cells. *Cancer Res* (2013) 73(10):2936–42. doi: 10.1158/0008-5472.CAN-12-4300

135. Yang J, Jubb AM, Pike L, Buffa FM, Turley H, Baban D, et al. The histone demethylase JMJD2B is regulated by estrogen receptor alpha and hypoxia, and is a key mediator of estrogen induced growth. *Cancer Res* (2010) 70(16):6456–66. doi: 10.1158/0008-5472.CAN-10-0413

136. Ramadoss S, Guo G, Wang CY. Lysine demethylase KDM3A regulates breast cancer cell invasion and apoptosis by targeting histone and the non-histone protein p53. *Oncogene* (2017) 36(1):47–59. doi: 10.1038/onc.2016.174

137. Zhao QY, Lei PJ, Zhang X, Zheng JY, Wang HY, Zhao J, et al. Global histone modification profiling reveals the epigenomic dynamics during malignant transformation in a four-stage breast cancer model. *Clin Epigenet* (2016) 8:34. doi: 10.1186/s13148-016-0201-x

138. Qin L, Xu Y, Yu X, Toneff MJ, Li D, Liao Lc , et al. The histone demethylase Kdm3a is required for normal epithelial proliferation, ductal elongation and tumor growth in the mouse mammary gland. *Oncotarget* (2017) 8(49):84761–75. doi: 10.18632/oncotarget.21380

139. Luo W, Chang R, Zhong J, Pandey A, Semenza GL, et al. Histone demethylase JMJD2C is a coactivator for hypoxia-inducible factor 1 that is required for breast cancer progression. *Proc Natl Acad Sci* (2012) 109(49): E3367–76. doi: 10.1073/pnas.1217394109

140. Shao P, Liu Q, Maina PK, Cui J, Bair TB, Li T, et al. Histone demethylase PHF8 promotes epithelial to mesenchymal transition and breast tumorigenesis. *Nucleic Acids Res* (2017) 45(4):1687–702. doi: 10.1093/nar/gkw1093

141. Sierra-Ramirez JA, Seseña-Mendez E, Godinez-Victoria M, Hernandez-Caballero ME, et al. An insight into the promoter methylation of PHF20L1 and the gene association with metastasis in breast cancer. *Adv Clin Exp Med* (2021) 30 (5):507–15. doi: 10.17219/acem/133426

142. Hou Y, Liu W, Yi X, Yang Y, Su D, Huang W, et al. PHF20L1 as a H3K27me2 reader coordinates with transcriptional repressors to promote breast tumorigenesis. *Sci Adv* (2020) 6(16):eaaz0356. doi: 10.1126/sciadv.aaz0356

143. Lu L, Katsaros D, Zhu Y, Hoffman A, Luca S, Marion CE, et al. Let-7a regulation of insulin-like growth factors in breast cancer. *Breast Cancer Res Treat* (2011) 126(3):687–94. doi: 10.1007/s10549-010-1168-5

144. Yun J, Frankenberger CA, Kuo WL, Boelens MC, Eves EM, Cheng N, et al. Signalling pathway for RKIP and let-7 regulates and predicts metastatic breast cancer. *EMBO J* (2011) 30(21):4500–14. doi: 10.1038/emboj.2011.312

145. Aure MR, Leivonen SK, Fleischer T, Zhu Q, Overgaard J, Alsner J, et al. Individual and combined effects of DNA methylation and copy number alterations on miRNA expression in breast tumors. *Genome Biol* (2013) 14(11):R126. doi: 10.1186/gb-2013-14-11-r126

146. Hsu PY, Deatherage DE, Rodriguez BA, Liyanarachchi S, Weng YI, Zuo T, et al. Xenoestrogen-induced epigenetic repression of microRNA-9-3 in breast epithelial cells. *Cancer Res* (2009) 69(14):5936–45. doi: 10.1158/0008-5472.CAN-08-4914

147. Xu Q, Jiang Y, Yin Y, Li Q, He J, Jing Y, et al. A regulatory circuit of miR-148a/152 and DNMT1 in modulating cell transformation and tumor angiogenesis through IGF-IR and IRS1. *J Mol Cell Biol* (2013) 5(1):3–13. doi: 10.1093/jmcb/mjs049

148. Zhang Y, Yan LX, Wu QN, Du ZM, Chen J, Liao DZ, et al. miR-125b is methylated and functions as a tumor suppressor by regulating the ETS1 proto-oncogene in human invasive breast cancer. *Cancer Res* (2011) 71(10):3552–62. doi: 10.1158/0008-5472.CAN-10-2435

149. Augoff K , McCue B, Plow EF, Sossey-Alaoui K. miR-31 and its host gene lncRNA LOC554202 are regulated by promoter hypermethylation in triple-negative breast cancer. *Mol Cancer* (2012) 11:5. doi: 10.1186/1476-4598-11-5

150. Zhang Y, Yang P, Sun T, Li D, Xu X, Rui Y, et al. miR-126 and miR-126* repress recruitment of mesenchymal stem cells and inflammatory monocytes to inhibit breast cancer metastasis. *Nat Cell Biol* (2013) 15(3):284–94. doi: 10.1038/ncb2690

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

152. Barrow TM, Barault L, Ellsworth RE, Harris HR, Binder AM, Valente AL, et al. Aberrant methylation of imprinted genes is associated with negative hormone receptor status in invasive breast cancer. *Int J Cancer* (2015) 137(3):537–47. doi: 10.1002/ijc.29419

153. Bhan A, Hussain I, Ansari KI, Kasiri S, Bashyal A, Mandal SS, et al. Antisense transcript long noncoding RNA (lncRNA) HOTAIR is transcriptionally induced by estradiol. *J Mol Biol* (2013) 425(19):3707–22. doi: 10.1016/j.jmb.2013.01.022

154. Feng S, De Carvalho DD. Clinical advances in targeting epigenetics for cancer therapy. *FEBS J* (2022) 289(5):1214–39. doi: 10.1111/febs.15750

155. Sherr DH. Another important biological function for the aryl hydrocarbon receptor. *Arterioscler Thromb Vasc Biol* (2011) 31(6):1247–8. doi: 10.1161/ATVBAHA.111.227553

156. Barouki R, Coumoul X, Fernandez-Salguero PM. The aryl hydrocarbon receptor, more than a xenobiotic-interacting protein. *FEBS Lett* (2007) 581 (19):3608–15. doi: 10.1016/j.febslet.2007.03.046

157. Eltom SE, Gasmelseed AA, Saoudi-Guentri D. The aryl hydrocarbon receptor is over-expressed and constitutively activated in advanced breast carcinoma. *Proceedings of the American Association for Cancer Research*, (2006) 47. doi:10.1002/jcb.21630

158. Safe S, Cheng Y, Jin U-H. The aryl hydrocarbon receptor (AhR) as a drug target for cancer chemotherapy. *Curr Opin Toxicol* (2017) 2:24–9. doi: 10.1016/j.cotox.2017.01.012

159. Zhang S, Lei P, Liu X, Li X, Walker K, Kotha L , et al. The aryl hydrocarbon receptor as a target for estrogen receptor-negative breast cancer chemotherapy. *Endocrine-related Cancer* (2009) 16(3):835–44. doi: 10.1677/ERC-09-0054

160. Hockings JK, Thorne PA, Kemp MQ, Morgan SS, Selmin O, Romagnolo DF. The ligand status of the aromatic hydrocarbon receptor modulates transcriptional activation of BRCA-1 promoter by estrogen. *Cancer Res* (2006) 66(4):2224–32. doi: 10.1158/0008-5472.CAN-05-1619

161. Papoutsis AJ , Lamore SD, Wondrak GT, Selmin OI, Romagnolo DF. Resveratrol prevents epigenetic silencing of BRCA-1 by the aromatic hydrocarbon receptor in human breast cancer cells. *J Nutr* (2010) 140(9):1607–14. doi: 10.3945/jn.110.123422

162. Papoutsis AJ, et al. BRCA-1 promoter hypermethylation and silencing induced by the aromatic hydrocarbon receptor-ligand TCDD are prevented by resveratrol in MCF-7 cells. *J Nutr Biochem* (2012) 23(10):1324–32. doi: 10.1016/j.jnutbio.2011.08.001

163. Jorgensen EM, Alderman MH. Taylor, Preferential epigenetic programming of estrogen response after in utero xenoestrogen (bisphenol-a) exposure. *FASEB J* (2016) 30(9):3194–201. doi: 10.1096/fj.201500089R

164. Allard P. Chapter 27 - bisphenol a. In: RC Gupta, editor. *Biomarkers in toxicology*. Boston: Academic Press (2014). p. 459–74.

165. Seachrist DD , Borg JL, Selmin OI, Romagnolo DF. A review of the carcinogenic potential of bisphenol a. *Reprod Toxicol* (2016) 59:167–82. doi: 10.1016/j.reprotox.2015.09.006

166. Doherty LF , Bromer JG, Zhou Y, Aldad TS, Taylor HS. In utero exposure to diethylstilbestrol (DES) or bisphenol-a (BPA) increases EZH2 expression in the mammary gland: An epigenetic mechanism linking endocrine disruptors to breast cancer. *Hormones Cancer* (2010) 1(3):146–55. doi: 10.1007/s12672-010-0015-9

167. Weng YI, Hsu PY, Liyanarachchi S, Liu J, Deatherage DE, Huang YW, et al. Epigenetic influences of low-dose bisphenol a in primary human breast epithelial cells. *Toxicol Appl Pharmacol* (2010) 248(2):111–21. doi: 10.1016/j.taap.2010.07.014

168. Kanao H, Enomoto T, Kimura T, Fujita M, Nakashima R, Ueda Y, et al. Overexpression of LAMP3/TSC403/DC-LAMP promotes metastasis in uterine cervical cancer. *Cancer Res* (2005) 65(19):8640–5. doi: 10.1158/0008-5472.CAN-04-4112

169. Nagelkerke A, Bussink J, Mujcic H, Wouters BG, Lehmann S, Sweep FC, et al. Hypoxia stimulates migration of breast cancer cells via the PERK/ATF4/LAMP3-arm of the unfolded protein response. *Breast Cancer Res* (2013) 15(1):R2. doi: 10.1186/bcr3373

170. Nagelkerke A, Mujcic H, Bussink J, Wouters BG, van Laarhoven HW, Sweep FC, et al. Hypoxic regulation and prognostic value of LAMP3 expression in breast cancer. *Cancer* (2011) 117(16):3670–81. doi: 10.1002/cncr.25938

171. Pullella K, Kotsopoulos J. Arsenic exposure and breast cancer risk: A re-evaluation of the literature. *Nutrients* (2020) 12(11):3305. doi: 10.3390/nu12113305

172. Liu Y, Hock JM, Sullivan C, Fang G, Cox AJ, Davis KT, et al. Activation of the p38 MAPK/Akt/ERK1/2 signal pathways is required for the protein stabilization of CDC6 and cyclin D1 in low-dose arsenite-induced cell proliferation. *J Cell Biochem* (2010) 111(6):1546–55. doi: 10.1002/jcb.22886

173. Xu Y, Tokar EJ, Waalkes MP. Arsenic-induced cancer cell phenotype in human breast epithelia is estrogen receptor-independent but involves aromatase activation. *Arch Toxicol* (2014) 88(2):263–74. doi: 10.1007/s00204-013-1131-4

174. Peremartí J , Ramos F, Marcos R, Hernández A. Arsenic exposure disrupts the normal function of the FA/BRCA repair pathway. *Toxicol Sci* (2014) 142(1):93–104. doi: 10.1093/toxsci/kfu159

175. Ren X, McHale CM, Skibola CF, Smith AH, Smith MT, Zhang L. An emerging role for epigenetic dysregulation in arsenic toxicity and carcinogenesis. *Environ Health Perspect* (2011) 119(1):11–9. doi: 10.1289/ehp.1002114

176. Romagnolo DF , Daniels KD, Grunwald JT, Ramos SA, Propper CR, Selmin OI. Epigenetics of breast cancer: Modifying role of environmental and bioactive food compounds. *Mol Nutr Food Res* (2016) 60(6):1310–29. doi: 10.1002/mnfr.201501063

177. Cardenas A, Houseman EA, Baccarelli AA, Quamruzzaman Q, Rahman M, Mostofa G, et al. In utero arsenic exposure and epigenome-wide associations in placenta, umbilical artery, and human umbilical vein endothelial cells. *Epigenetics* (2015) 10(11):1054–63. doi: 10.1080/15592294.2015.1105424

178. Chou WC, Chung YT, Chen HY, Wang CJ, Ying TH, Chuang CY, et al. Maternal arsenic exposure and DNA damage biomarkers, and the associations with birth outcomes in a general population from Taiwan. *PloS One* (2014) 9(2):e86398. doi: 10.1371/journal.pone.0086398

179. Simon R, Gomez Ruiz JA, von Holst C, Wenzl T, Anklam E. Results of a European inter-laboratory comparison study on the determination of EU priority polycyclic aromatic hydrocarbons (PAHs) in edible vegetable oils. *Anal Bioanal Chem* (2008) 391(4):1397–408. doi: 10.1007/s00216-007-1771-4

180. Gaudet MM, Carter BD, Brinton LA, Falk RT, Gram IT, Luo J, et al. Pooled analysis of active cigarette smoking and invasive breast cancer risk in 14 cohort studies. *Int J Epidemiol* (2017) 46(3):881–93. doi:10.1002/jcb.21630

181. Dossus L, Boutron-Ruault MC, Kaaks R, Gram IT, Vilier A, Fervers B, et al. Active and passive cigarette smoking and breast cancer risk: results from the EPIC cohort. *Int J Cancer* (2014) 134(8):1871–88. doi: 10.1002/ijc.28508

182. Nicolas M, Grandal B, Dubost E, Kassara A, Guerin J, Toussaint A, et al. Breast cancer (BC) is a window of opportunity for smoking cessation: Results of a retrospective analysis of 1234 BC survivors in follow-up consultation. *Cancers (Basel)* (2021) 13(10):1–12. doi: 10.3390/cancers13102423

183. Conway K, Edmiston SN, Parrish E, Bryant C, Tse CK, Swift-Scanlan T, et al. Breast tumor DNA methylation patterns associated with smoking in the Carolina breast cancer study. *Breast Cancer Res Treat* (2017) 163(2):349–61. doi: 10.1007/s10549-017-4178-8

184. Tang H , Yang D, Han C, Mu P. Smoking, DNA methylation, and breast cancer: A mendelian randomization study. *Front Oncol* (2021) 11. doi: 10.3389/fonc.2021.745918

185. Sadikovic B, Andrews J, Rodenhiser DI. DNA Methylation analysis using CpG microarrays is impaired in benzopyrene exposed cells. *Toxicol Appl Pharmacol* (2007) 225(3):300–9. doi: 10.1016/j.taap.2007.08.013

186. Thakur C, Chen F. Connections between metabolism and epigenetics in cancers. *Semin Cancer Biol* (2019) 57:52–8. doi: 10.1016/j.semcancer.2019.06.006

187. Gandhi N, Das GM. Metabolic reprogramming in breast cancer and its therapeutic implications. *Cells* (2019) 8(2):89. doi: 10.3390/cells8020089

188. O'Mahony F, Razandi M, Pedram A, Harvey BJ, Levin ER. Estrogen modulates metabolic pathway adaptation to available glucose in breast cancer cells. *Mol Endocrinol* (2012) 26(12):2058–70. doi: 10.1210/me.2012-1191

189. Barros RP, Gabbi C, Morani A, Warner M, Gustafsson JA. Participation of ERalpha and ERbeta in glucose homeostasis in skeletal muscle and white adipose tissue. *Am J Physiol Endocrinol Metab* (2009) 297(1):E124–33. doi: 10.1152/ajpendo.00189.2009

190. Yang J, AlTahan A, Jones DT, Buffa FM, Bridges E, Interiano RB, et al. Estrogen receptor-α directly regulates the hypoxia-inducible factor 1 pathway associated with antiestrogen response in breast cancer. *Proc Natl Acad Sci U.S.A.* (2015) 112(49):15172–7. doi: 10.1073/pnas.1422015112

191. Robledinos-Antón N. Activators and inhibitors of NRF2: A review of their potential for clinical development. *Oxid Med Cell Longev 2019* (2019) p:9372182. doi: 10.1155/2019/9372182

192. Guo Y , Yu S, Zhang C, Kong AN. Epigenetic regulation of Keap1-Nrf2 signaling. *Free Radic Biol Med* (2015) 88(Pt B):337–49. doi: 10.1016/j.freeradbiomed.2015.06.013

193. Cheng D , Wu R, Guo Y, Kong ANc. Regulation of Keap1-Nrf2 signaling: The role of epigenetics. *Curr Opin Toxicol* (2016) 1:134–8. doi: 10.1016/j.cotox.2016.10.008

194. Almeida M, Soares M, Ramalhinho AC, Moutinho JF, Breitenfeld L, Pereira L. The prognostic value of NRF2 in breast cancer patients: a systematic review with meta-analysis. *Breast Cancer Res Treat* (2020) 179(3):523–32. doi: 10.1007/s10549-019-05494-4

195. Zhang HS, et al. Nrf2 promotes breast cancer cell migration via up-regulation of G6PD/HIF-1α/Notch1 axis. *J Cell Mol Med* (2019) 23(5):3451–63. doi: 10.1111/jcmm.14241

196. Mattingly KA , Ivanova MM, Riggs KA, Wickramasinghe NS, Barch MJ, Klinge CM. Estradiol stimulates transcription of nuclear respiratory factor-1 and increases mitochondrial biogenesis. *Mol Endocrinol* (2008) 22(3):609–22. doi: 10.1210/me.2007-0029

197. Wu K, Li L, Thakur C, Lu Y, Zhang X, Yi Z, et al. Proteomic characterization of the world trade center dust-activated mdig and c-myc signaling circuit linked to multiple myeloma. *Sci Rep* (2016) 6:36305. doi: 10.1038/srep36305

198. Zhang Y, Lu Y, Yuan BZ, Castranova V, Shi X, Stauffer JL, et al. The human mineral dust-induced gene, mdig, is a cell growth regulating gene associated with lung cancer. *Oncogene* (2005) 24(31):4873–82. doi: 10.1038/sj.onc.1208668

199. Sun J, Yu M, Lu Y, Thakur C, Chen B, Qiu P, et al. Carcinogenic metalloid arsenic induces expression of mdig oncogene through JNK and STAT3 activation. *Cancer Lett* (2014) 346(2):257–63. doi: 10.1016/j.canlet.2014.01.002

200. Thakur C, Wolfarth M, Sun J, Zhang Y, Lu Y, Battelli L, et al. Oncoprotein mdig contributes to silica-induced pulmonary fibrosis by altering balance between Th17 and treg T cells. *Oncotarget* (2015) 6(6):3722–36. doi: 10.18632/oncotarget.2914

201. Thakur C, Chen F. Current understanding of mdig/MINA in human cancers. *Genes Cancer* (2015) 6(7-8):288–302. doi: 10.18632/genesandcancer.73

202. Zhang Q, Thakur C, Shi J, Sun J, Fu Y, Stemmer P, et al. New discoveries of mdig in the epigenetic regulation of cancers. *Semin Cancer Biol* (2019) 57:27–35. doi: 10.1016/j.semcancer.2019.06.013

203. Yu M, Sun J, Thakur C, Chen B, Lu Y, Zhao H, et al. Paradoxical roles of mineral dust induced gene on cell proliferation and migration/invasion. *PloS One* (2014) 9(2):e87998. doi: 10.1371/journal.pone.0087998

204. Chen B , Liu J, Chang Q, Beezhold K, Lu Y, Chen F. JNK and STAT3 signaling pathways converge on akt-mediated phosphorylation of EZH2 in bronchial epithelial cells induced by arsenic. *Cell Cycle (Georgetown Tex.)* (2013) 12(1):112–21. doi: 10.4161/cc.23030

205. Shi J, Thakur C, Zhao Y, Li Y, Nie L, Zhang Q, et al. Pathological and prognostic indications of the mdig gene in human lung cancer. *Cell Physiol Biochem* (2021) 55(S2):13–28. doi: 10.33594/000000322

206. Bi Z, Zhang Q, Fu Y, Seno A, Wadgaonkar P, Qiu Y, et al. Cooperation between NRF2-mediated transcription and MDIG-dependent epigenetic modifications in arsenic-induced carcinogenesis and cancer stem cells. *Semin Cancer Biol* (2021) 76:310–8. doi: 10.1016/j.semcancer.2021.03.030

207. Bi Z, Zhang Q, Fu Y, Wadgaonkar P, Zhang W, Almutairy B, et al. Nrf2 and HIF1α converge to arsenic-induced metabolic reprogramming and the formation of the cancer stem-like cells. *Theranostics* (2020) 10(9):4134–49. doi: 10.7150/thno.42903

208. Thakur C , Lu Y, Sun J, Yu M, Chen B, Chen F. Increased expression of mdig predicts poorer survival of the breast cancer patients. *Gene* (2014) 535(2):218–24. doi: 10.1016/j.gene.2013.11.031

209. Thakur C , Chen B, Li L, Zhang Q, Yang Z-Q, Chen F. Loss of mdig expression enhances DNA and histone methylation and metastasis of aggressive breast cancer. *Signal transduction targeted Ther* (2018) 3:25–5. doi: 10.1038/s41392-018-0027-4

210. Tsuneoka M, Koda Y, Soejima M, Teye K, Kimura H, et al. A novel myc target gene, mina53, that is involved in cell proliferation. *J Biol Chem* (2002) 277(38):35450–9. doi: 10.1074/jbc.M204458200

211. Liu H, Radisky DC, Yang D, Xu R, Radisky ES, Bissell MJA, et al. MYC suppresses cancer metastasis by direct transcriptional silencing of αv and β3 integrin subunits. *Nat Cell Biol* (2012) 14(6):567–74. doi: 10.1038/ncb2491

212. Zhang Q, Thakur C, Fu Y, Bi Z, Wadgaonkar P, Xu L, et al. Mdig promotes oncogenic gene expression through antagonizing repressive histone methylation markers. *Theranostics* (2020) 10(2):602–14. doi: 10.7150/thno.36220

213. Wang W, Lu Y, Stemmer PM, Zhang X, Bi Y, Yi Z, et al. The proteomic investigation reveals interaction of mdig protein with the machinery of DNA double-strand break repair. *Oncotarget* (2015) 6(29):28269–81. doi: 10.18632/oncotarget.4961

214. Pannetier M, Julien E, Schotta G, Tardat M, Sardet C, Jenuwein T, et al. PR-SET7 and SUV4-20H regulate H4 lysine-20 methylation at imprinting control regions in the mouse. *EMBO Rep* (2008) 9(10):998–1005. doi: 10.1038/embor.2008.147

215. Lu Y, Chang Q, Zhang Y, Beezhold K, Rojanasakul Y, Zhao H, et al. Lung cancer-associated JmjC domain protein mdig suppresses formation of tri-methyl lysine 9 of histone H3. *Cell Cycle* (2009) 8(13):2101–9. doi: 10.4161/cc.8.13.8927

216. Chen B, Yu M, Chang Q, Lu Y, Thakur C, Ma D, et al. Mdig de-represses H19 large intergenic non-coding RNA (lincRNA) by down-regulating H3K9me3 and heterochromatin. *Oncotarget* (2013) 4(9):1427–37. doi: 10.18632/oncotarget.1155

217. Zhang Q, Wadgaonkar P, Xu L, Thakur C, Fu Y, Bi Z, et al. Environmentally-induced mdig contributes to the severity of COVID-19 through fostering expression of SARS-CoV-2 receptor NRPs and glycan metabolism. *Theranostics* (2021) 11(16):7970–83. doi: 10.7150/thno.62138

Glossary

DCIS	Ductal carcinoma in situ
IDC	Invasive ductal carcinoma
ILC	Invasive lobular carcinoma
HER2	Human epidermal growth factor receptor 2
ER	Estrogen receptor
PR	Progesterone receptor
BRCA	Breast Cancer gene
TNBC	Basal/triple negative breast cancer
lncRNAs	Long non-coding RNAs
miR	Micro-RNAs
MBD	Methyl binding domain
5mC	5-methylcytosine
5-hmC	5-hydroxymethylcytosine
DNMT	DNA methyltransferase
TET	Ten-eleven translocations
HAT	Histone acetyltransferases
HDAC	Histone deacetylases
HKMT	Histone methyltransferases
KDM	Histone demethylases
SAM	Sadenosylmethionine
EMT	Epithelial mesenchymal transition
DOT1L	Histone methylase disruptor silencing 1 like
JmjC	JumonjiC
PAH	Polycyclic aromatic hydrocarbons
AHR	Aryl hydrocarbon receptor
PCB	Polychlorinated biphenyls
BPA	Bisphenol A
LAMP3	Lysosomal associated membrane protein
HIF-1α	Hypoxia inducible factor-1α
GLUT4	Glucose transporter protein expressiona
G6PD	Glucose-6-phosphate dehydrogenase
Mdig	Mineral dust-induced gene
NRF2	Nuclear factor erythroid 2-related factor 2
ChIP	Chromatin immunoprecipitation sequencing
PRC2	Polycomb repressive complex
GADD45	Growth arrest and DNA-damage inducible protein
AID	Activation-induced cytidine deaminase
APOBEC	Apolipoprotein B mRNA editing catalytic polypeptide-like family

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 15 September 2022
DOI 10.3389/fphar.2022.946811

OPEN ACCESS

EDITED BY
Lixiang Xue,
Peking University Third Hospital, China

REVIEWED BY
Sonia Emanuele,
University of Palermo, Italy
Lu-Qiang Zhang,
Inner Mongolia University, China
Pilar Navarrete,
National Institute of Pediatrics (Mexico), Mexico
Yunkai Yang,
Chinese Academy of Medical Sciences and Peking Union Medical College, China
Zeng-quan Yang,
Wayne State University, United States

*CORRESPONDENCE
Xinyue Meng,
mengxinyue88@163.com

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 18 May 2022
ACCEPTED 15 August 2022
PUBLISHED 15 September 2022

CITATION
Feng J and Meng X (2022), Histone modification and histone modification-targeted anti-cancer drugs in breast cancer: Fundamentals and beyond. *Front. Pharmacol.* 13:946811. doi: 10.3389/fphar.2022.946811

Histone modification and histone modification-targeted anti-cancer drugs in breast cancer: Fundamentals and beyond

Jianwei Feng and Xinyue Meng*

Department of Ultrasound, Shengjing Hospital of China Medical University, Shenyang, China

Dysregulated epigenetic enzymes and resultant abnormal epigenetic modifications (EMs) have been suggested to be closely related to tumor occurrence and progression. Histone modifications (HMs) can assist in maintaining genome stability, DNA repair, transcription, and chromatin modulation within breast cancer (BC) cells. In addition, HMs are reversible, dynamic processes involving the associations of different enzymes with molecular compounds. Abnormal HMs (e.g. histone methylation and histone acetylation) have been identified to be tightly related to BC occurrence and development, even though their underlying mechanisms remain largely unclear. EMs are reversible, and as a result, epigenetic enzymes have aroused wide attention as anti-tumor therapeutic targets. At present, treatments to restore aberrant EMs within BC cells have entered preclinical or clinical trials. In addition, no existing studies have comprehensively analyzed aberrant HMs within BC cells; in addition, HM-targeting BC treatments remain to be further investigated. Histone and non-histone protein methylation is becoming an attractive anti-tumor epigenetic therapeutic target; such methylation-related enzyme inhibitors are under development at present. Consequently, the present work focuses on summarizing relevant studies on HMs related to BC and the possible mechanisms associated with abnormal HMs. Additionally, we also aim to analyze existing therapeutic agents together with those drugs approved and tested through pre-clinical and clinical trials, to assess their roles in HMs. Moreover, epi-drugs that target HMT inhibitors and HDAC inhibitors should be tested in preclinical and clinical studies for the treatment of

Abbreviations: BC, breast cancer; DNMTs, DNA methyltransferases; EMs, epigenetic modifications; ERα, estrogen receptor α; ESCs, embryonic stem cells; FOXA1, pioneer factor; GF, growth factor; HATs, histone acetyltransferases; HEPH, hephaestin; HER2, human epidermal growth factor receptor 2; HMs, histone modifications; HMTs, histone methyltransferases; HR, hormone receptors; KATi, KAT inhibitors; KDMs, lysine demethylases; KMTs, lysine methyltransferases; MCP-1, macrocyclic peptidomimetic inhibitors; MTDH, metadherin; NF-κB, nuclear factor-κB; NSCLC, non-small cell lung cancer; ORR, objective response rate; PCa, prostatic cancer; PPIs, protein–protein interactions; PTMs, post-translational modifications; SIRT1, sirtuin; TCGA, The Cancer Genome Atlas; Tip60, Tat-interactive protein; TSSs, transcriptional start sites; VPA, valproic acid.

BC. Epi-drugs that target histone methylation (HMT inhibitors) and histone acetylation (HDAC inhibitors) have now entered clinical trials or are approved by the US Food and Drug Administration (FDA). Therefore, the review covers the difficulties in applying HM-targeting treatments in clinics and proposes feasible approaches for overcoming such difficulties and promoting their use in treating BC cases.

KEYWORDS

epi-drugs, histone modification, tumor suppressor gene, breast cancer, epigenetics

Introduction

Breast cancer (BC) accounts for a highly frequent malignancy in the female population (Winters et al., 2017; Hiatt et al., 2022). According to the statistics from the World Health Organization (WHO), BC occupies 11.7% of the overall cancer patients and takes up 6% of the overall death cases. BC displays highly variable intra-tumor and inter-tumor characteristics, cancer stages when the patient is diagnosed and morphologies; as a result, it remains a challenge to effectively treat cancer and predict patient survival. In the past 10 years, BC survival shows an increasing trend due to early screening and improvement in treatment, but its 10-year survival remains unsatisfactory (80%) (Caplan, 2014). In China, a study finds differences between high-income nations and China, which discovers that the Chinese are associated with a young age at BC onset, low BC screening rate, one-child policy, delayed BC diagnosis inducing late/advanced stage when they present with symptoms, insufficient medical resources, and the low consciousness of BC (Fan et al., 2014). Consequently, it is necessary to develop new treatments. Hormone receptors (HR), in particular, progesterone receptor (PR) and estrogen receptor (ER), have important effects on BC occurrence and development (Trabert et al., 2020). Different BC subtypes are associated with different molecular and histological features, growth rates, and endocrine therapy/chemotherapy responses. Consequently, treatments are selected based on ER/PR/human epidermal growth factor receptor 2 (HER2) expression status, tumor size and grade, lymph node metastasis (LNM), and distant metastasis (DM) (Chlebowski and Anderson, 2012).

Epigenetic and genetic alterations are suggested to have a critical effect on various cell processes such as imprinting, X chromosome inactivation, chromatin remodeling, and tumorigenesis (Han et al., 2016; Yang et al., 2021a). As for epigenetic alterations, their frequently seen subtypes are histone modifications (HMs). Epigenetic alterations can be reversible, which is different from genetic mutations; as a result, they are the safer options for anti-BC treatment (Li et al., 2021). In chromatin-associated processes such as gene modulation, histone post-translational modifications (PTMs) have an essential effect, since hub histones H2A-H2B and H3-H4 are wrapped by the 147-bp DNA fragment, forming the fundamental chromatin unit (Talbert and Henikoff, 2021). HMs have been extensively studied from diverse perspectives, but it is still necessary to understand the aforementioned processes for the sake of clarifying HMs' functions and the related enzymatic mechanisms underlying BC. Currently, over 23 classes of HMs have been identified, but just a low portion of them are associated with BC. Therefore, the present review aims to analyze histone methylation acetylation, the most extensively investigated class. Any dysregulation in the aforementioned processes induces imbalanced gene levels within BC and results in abnormalities in cell growth, migration, invasion, and treatment resistance (Byler et al., 2014; Pasculli et al., 2018).

Multidisciplinary consultation is needed in BC treatment. The most updated treatments are surgical treatment, chemotherapy, radiotherapy, and molecularly-targeted endocrine therapy, which are selected based on the BC subtype. Recently, great efforts have been made to improve targeted therapy, especially for bevacizumab-targeting vascular endothelial growth factor (VEGF) and trastuzumab (herceptin)-targeting HER2, both of which are approved (Robert et al., 2011). Epigenetic alterations have been suggested over gene mutation because of reversibility. Epigenetic modifications (EMs) are established and maintained according to special enzyme activities, histone deacetylases together with histone methyltransferases, and they are the major targets for epigenetic treatment (Qin et al., 2019a). Epigenetic treatments that use the aforementioned enzyme inhibitors can suppress tumorigenesis (Yang et al., 2021b).

The present work aims to summarize the relevant information regarding the importance of highly abundant post-translational modifications within BC, H3Kme, H4Kme, and H3Kac for BC occurrence, migration, and prognosis. Particularly, we highlight the histone marker status within BC subtypes, and the impacts on transcriptionally regulating certain genes, erasers, and writers. We also examine the effect of histone H3K and H3K-specific methyltransferase on BC and analyze the

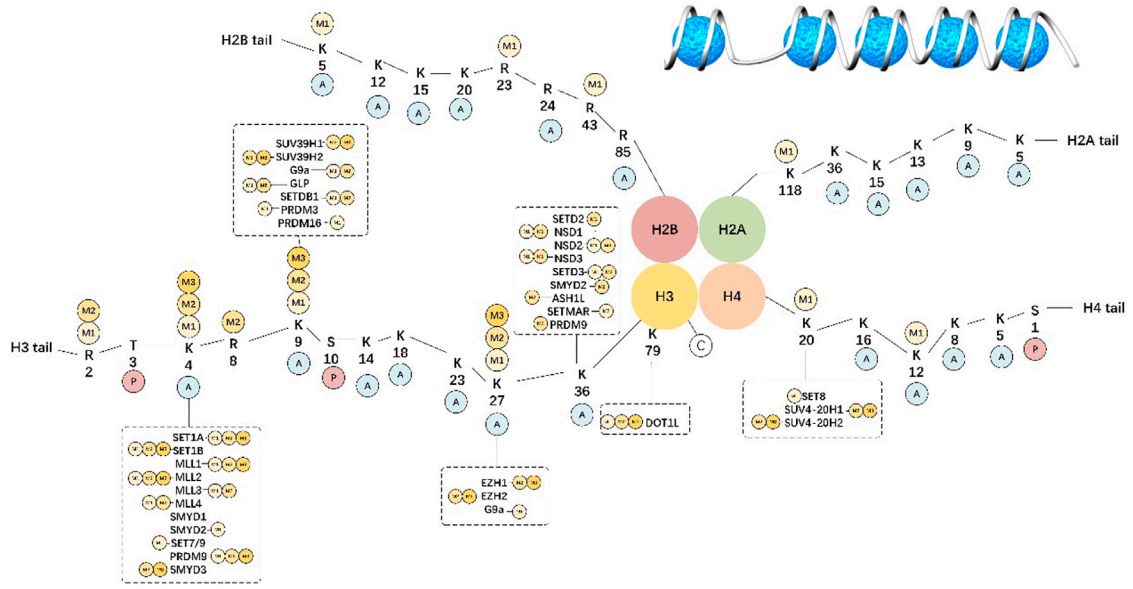


FIGURE 1
Histone methylation-specific targets.

functions of histone methyltransferases (HMTs) and histone acetyltransferases (HATs) in drug-resistant cancer, together with their relevant mechanisms. Methods to diagnose and predict prognosis based on epigenetics make great contributions to precision oncology. Some approaches to diagnose DNA methylation have been applied clinically or entered clinical trials (Cowan et al., 2010). Great efforts have been made to compensate for the abnormal epigenetic mechanisms in precision oncology, which facilitate the development of epi-drugs that target epigenetic modulators. This work collects information regarding inhibitors applied in clinical trials from the ClinicalTrials.gov database maintained by the U.S. National Library of Medicine. At present, just nine epi-drugs have been approved by the FDA, including IDH, EZH2, DNA methyltransferases (DNMTs), and histone deacetylases inhibitors (HDACis). Moreover, numerous other drugs are under clinical trials for the treatment of solid tumors (NCT01928576 and NCT03179943) or hematologic tumors (NCT02717884 and NCT03164057). It is to be noted that ER-positive (ER+) BC phase-II trials (NCT00676663, NCT00828854, and NCT04190056) are conducted to test whether epi-drugs plus conventional treatments are effective, which indicates that more is known about the epigenetic mechanisms governing the development, migration, and drug resistance of ER+ BC. This section will discuss the efficacy and mechanism of action of certain DNMT and HDAC inhibitors in treating cancers.

Histone modifications within BC

Histone methylation in BC

Histone methylation may take place in arginine and lysine residues and involves complicated modifications compared with acetylation. Lysine is mono-, di-, or trimethylated, whereas arginine is asymmetrically or symmetrically methylated (Barski et al., 2007). As a reversible process, histone methylation can be strictly modulated by different demethylases (KDMs) and methyltransferases (KMTs). A portion of such markers (H3K4, H3K36, and H3K79) is related to activation at the transcriptional level, while others (H3K9, H3K27, and H4K20) are linked to suppression at the transcriptional level (Jenuwein and Allis, 2001). Figure 1 summarizes the specific targets identified for diverse HM classes.

In eukaryotic cells, chromatin is the complex formed by DNA and histones. The basic functional unit of chromatin is the nucleosome that contains a histone octamer (H2A, H2B, H3, and H4) wrapped by DNA. Histone tails undergo numerous posttranslational modifications, which are deposited by writers, removed by erasers, and read by readers, and may either loosen or tighten DNA-histone binding with active or silent transcription.

H3K4 methylation

H3K4 methylation shows high enrichment levels at transcriptional start sites (TSSs), promoter regions, and enhancer regions. In addition, H3K4me1 exhibits high

enrichment levels in enhancer regions (Heintzman et al., 2007) and it can bind to H3K27me3 or H3K27ac, thus marking the suppressive or active enhancers, separately (Creyghton et al., 2010). Different from additional H3K4 methylation showing high enrichment levels in intergenic regions, H3K4me2 can mark the 5′-terminal in transcribed genes (Kim and Buratowski, 2009). H3K4me3 is canonically distributed in actively transcribed gene promoters and poised genes related to differentiation (Santos-Rosa et al., 2002). Set1 can form Complex Proteins Associated with Set1 (COMPASS) in yeast and is the unique enzyme related to every H3K4 methylation (Miller et al., 2001).

For mammals, the KMT2 (MLL) family is the main H3K4 HMT, which contains six members (KMT2A–D, KMT2F, and KMT2G). In addition, within human cells, six Set1 homologies (SET1A–SET1B and MLL1–MLL4) together with five additional H3K4 methyltransferases (SMYD1–SMYD3, SET7/9, and PRDM9) have also been identified (Wang et al., 2015). Moreover, the KMT2 family is classified into three categories according to the containing domain type, including KMT2A–KMT2B (MLL1–MLL2), KMT2C–KMT2D (MLL3–MLL4), together with KMT2F–KMT2G (SETD1A–SETD1B) (Shilatifard, 2008). As revealed by *in vitro* research, the core complexes of MLL1–MLL2 display mono-, di-, and low tri-methylation activities in cells (Patel et al., 2008). For instance, MLL1 is suggested to be involved in H3K4 methylation within MCF-7 cells in the estrogen-mediated transcription of ER target genes (Jeong et al., 2011). MLL1 is frequently duplicated or over-expressed within BC cells, and as a result, it may be the therapeutic target for BC treatment (Tate et al., 2019). Additionally, MLL1 can accelerate the transcription of TFF1 (the estrogen-dependent gene) by H3K4me1/2 in the enhancer region's CpG islands and maintains the permissive chromatin architecture to bind to estrogen receptor α (ERα) and the pioneer factor (FOXA1). It results in the relaxation of chromatin for facilitating ERα binding together with its transcription within BC Jeong et al. (2014) H3K4 methyltransferase has been increasingly suggested to participate in BC occurrence. MLL2 shows a certain interaction with ERα and modulates the level of its target, thus mediating BC occurrence (Mo et al., 2006). As reported by Natarajan *et al.*, MLL2 upregulation within BC cells was related to tissue malignancy; meanwhile, MLL2 protein upregulation was also detected in tissues from patients with breast invasive carcinomas (Natarajan et al., 2010). MLL3, a protein with high mutation frequency within BC cells, is also the main factor that regulates the ERα level (Gala et al., 2018). According to recent reports, the upregulation of SETD1A and MLL3 increases the ERα level, thus supporting the growth of tamoxifen-resistant BC. Moreover, according to genome-wide research on histone methylation, MLL3 plays an essential role in H3K4 monomethylation and H3K27 acetylation within the ERα enhancer (Kim et al., 2020). MLL4 and the H3K27 demethylase UTX (KDM6A) synergistically regulate BC growth and migration (Kim et al., 2014). Jin *et al.* analyzed SETD1A's effect on tamoxifen-resistant BC. They suggested that SETD1A increased H3K4 methylation and made the chromatin region accessible to ERα targets within ER+ BC cells to activate the ER+ targets, thereby promoting the recruitment of ERα. They further discovered that SETD1A-regulated genes overlapped with specific tamoxifen-resistant genes within ER+ BC cells, which indicated the possible relation of SETD1A with tamoxifen resistance (Jin et al., 2018). SETD1A protein expression in cells increases in other BC subtypes, such as ER+, HER2+, and triple-negative breast cancer (TNBC) relative to healthy breast cells.

SMYD2 upregulation can modulate TNBC development, which predicts dismal patient survival (Li et al., 2018). SMYD3 can upregulate WNT10B (an oncogene) expression while promoting epithelial–mesenchymal transition (EMT), thus facilitating the metastasis of BC (Hamamoto et al., 2006; Fenizia et al., 2019). SET7/9 stabilizes the ER by methylating ER K302 residue, which then effectively recruits and trans-activates target genes to enhance BC occurrence (Subramanian et al., 2008). Additionally, SET7/9 deficiency promotes the cancer stem cell (CSC) features of BC while accelerating EMT, and it is associated with disease resistance, which indicates the tumor suppressor role of SET7/9 within BC (Montenegro et al., 2016).

As reported by Montenegro et al., SETD7 suppressed EMT by upregulating cadherin-1 while downregulating epidermal growth factor receptor (EGFR) and vimentin protein expression (Montenegro et al., 2016). It was evidenced by the overexpression of SETD7 within triple-negative, metastatic MDA-MB-231 cells, downregulation through siRNAs, and inhibited activity by exposing to 50 μM (R)-PFI-2 for a 3-day period within the non-metastatic estrogen receptor α (ERα/ESR1)-positive MCF-7 cells. Furthermore, SETD7 silencing within MCF-7 cells triggered the CSC phenotype (CD44+/CD24-/low) and mammosphere de-differentiation related to cadherin-1 deficiency. Such results conformed to the greater metastatic ability of MCF-7 xenografts after SETD7 silencing (Takemoto et al., 2016).

H3K9 methylation

H3K9 methylation, in particular H3K9me2 and H3K9me3, is usually related to heterochromatin formation and gene suppression (Bannister et al., 2001). Apart from these, H3K9me1 can be expressed around active gene-related TSSs as well (Vavouri and Lehner, 2012). H3K9me1 and H3K9me2 show nuclear and cytoplasmic localization within mammalian cells, whereas H3K9me3 displays nuclear localization only (Towbin et al., 2012). Additionally, histones can be distributed within the cytoplasm before their chromatin assembly due to the action of histone chaperones. Actually, H3K9 shows co-translational mono- and dimethylation by SETDB1 after it is bound to ribosomes (Rivera et al., 2015).

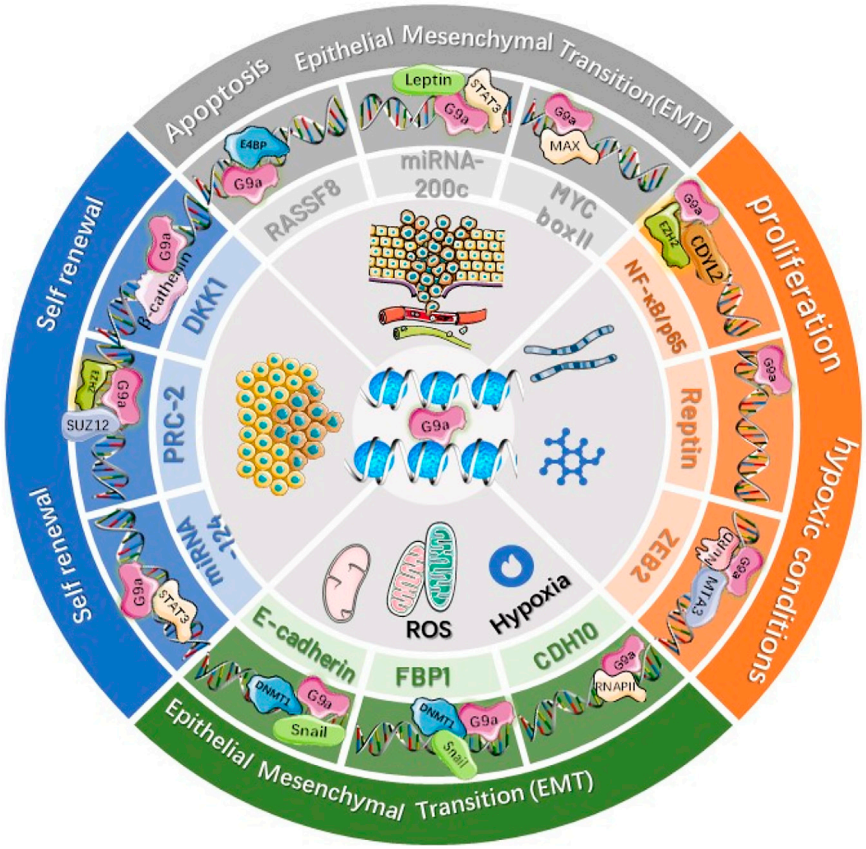


FIGURE 2
Dysregulation of G9a in various breast cancers.

Thereafter, cytoplasmic H3 and K9me1 are assembled in the chromatin, and the product is utilized for reinforcing heterochromatin and H3K9me3 as the substrate.

Proteins belonging to the SUV39 family of human beings, including SUV39H1 (KMT1A), SUV39H2 (KMT1B), SETDB1 (KMT1E), SETDB2 (KMT1F), G9a-like protein (GLP1), and G9A (EHMT2), possess the pre-SET (N-SET) and post-SET (C-SET) domains in addition to the SET domain, which can regulate the methylation of H3K9 (Dillon et al., 2005; Wu et al., 2010). Additionally, G9a may produce homodimers or heterodimers for catalyzing H3K9me1 together with H3K9me2 within the euchromatin (Tachibana et al., 2002). In the heterochromatin, such as the pericentromeric regions, SUV39H1 can catalyze H3K9me2, while SUV39H2 catalyzes H3K9me3 (Rea et al., 2000). G9a plays a critical part in BC occurrence (Jin et al., 2022) as shown in Figure 2. Its activation can suppress anticancer genes, thereby promoting BC cell growth and migration. The overexpression of G9a can inhibit hephaestin (HEPH), thus promoting carcinogenesis of BC (Wang et al., 2017a). Additionally, G9a activation can upregulate T-Box2 (TBX2) within BC cells (Crawford et al., 2019). TBX2 overexpression promotes BC cell growth by decreasing p21WAF1 and Cdkn2a (p14Arf and p19Arf within human beings) gene expression. Suppressing G9a expression can downregulate the TBX2 level while suppressing cancer cell growth. As discovered by Zhang et al., G9a suppression induced autophagy by modulating AMPK/mTOR pathways within BC cells (Zhang et al., 2017).

Upregulation of G9a causes mono- or di-methylation to lysine 9 residue of histone 3 (H3K9), resulting in an increase in the expression of TBX2, FBP1, PRC-2, and NF-κB and a decrease in the expression of DKK1, MYC, CDH10, Reptin, CASP1, ZEB2, RARRES8, and E-cadherin in BC. G9a-mediated up- and downregulation of various genes promotes cell proliferation, invasion, and metastasis and suppresses apoptosis in breast cancers.

Furthermore, SUV39H2 expression is significantly upregulated within basal-like BC, which predicts dismal BC prognostic outcomes (Liu et al., 2015a). Nonetheless, SUV39H2 mutations are detected within BC, suggesting the

TABLE 1 Status of histone methylation marks studied in breast cancer subtypes.

Substrates	Genes	Cooperators	Cell line/Tissue	Targets	H3Kme status	Effects	References
H3K36	MLL1	—	MCF-7 breast cancer cells	↑CpG-rich region of TFF1 enhancer	H3K4me3	↑Proliferation	Jeong et al. (2014)
	MLL2	—	MDA-MB-157 and MDA-MB-231			↑Invasion	Natarajan et al. (2010)
	MLL2	ERα	MCF7 cells	↑IL-20	H3K4me1/2	↑Proliferation	Su et al. (2016)
	MLL2	GCN5	UACC812 cell line, MDA-MB-361, T47D cell lines, BT-474 cell, MCF-HER2 and MCF-Neo cell lines	↑c-Myc	H3K4me3	↑Lapatinib resistance	Matkar et al. (2015)
	MLL2	LSD1	MCF7 cells	↑NCOA3	H3K4me3	↑Proliferation	Park et al. (2016a)
				↑RSP6KB1			
	MLL3	SET1A	Tamoxifen-resistant breast cancer	↑ESR1 gene	H3K4me3	↑ERα expression	Kim et al. (2020)
	MLL3	ER	MCF7 cells	↑HOXB9	H3K4me3	↑Proliferation	Deb et al. (2016)
	MLL3	FOXA1, and ER	MCF7 cells	↑TFF1	H3K4me1	↑Proliferation	Jozwik et al. (2016)
				↑PGR			
				↑MYC			
	MLL3	—	SKBR3, BT-474, Cama-1, T47D, MCF10A HCC1954 and MDA-MB-231 MDA-MB-468 and HCC1806 cell lines	↑AGR3	H3K4me1	↑Proliferation	Gala et al. (2018)
				↑PGR			
				↑CA2			
	MLL3	promoter region of Ras genes	tamoxifen-resistant ER-positive breast cancer cells	↑PI3K/AKT/mTOR signaling pathway	H3K4me1	↑Proliferation	Wu et al. (2020)
					H3K4me3		
	SETD1A	—	MDA-MB231, MCF7, MDA-MB-468	↑SKP2	H3K4me3	↑Proliferation	Tajima et al. (2019)
						↓Senescence	
	SETD1A	—	MDA-MB-231, MCF7, BT549, and SUM159	↑MMPs	H3K4me3	↑Invasion	Salz et al. (2015)
						↑Migration	
	SET7	GATA1	MCF7, ZR75-1 and MDA-MB-231	↑VEGF	H3K4me1	↑Vascular endothelial cell proliferation	Zhang et al. (2016)
						↑Migration	
						↑Tube formation	
	SMYD3	SMAD3	MDA-MB-231 cell line	↑SNAIL1	H3K4me3	↑EMT	Fenizia et al. (2019)
	SMYD3	MRTF-A	MCF7	↑MYL9	H3K4me2/3	↑Migration	Luo et al. (2014)
H3K79	G9a	SNAIL	basal-like breast cancer	↓FBP1	H3K9me2	↑CSCs	Dong et al. (2013a)
		DNMT1					
	G9a	—	Luminal A Type Breast Cancer	↑BMP5 Expression	H3K9me2	↑Smad protein phosphorylation	Jin et al. (2022)
	G9a	EZH2	MCF7, BT474 cells, MCF7 dominant-negative TBX2 cells (MCF7-DN)	↑expression of T-Box2 (TBX2)	H3K9me3	↑NDRG1	Crawford et al. (2019)
	G9a	—	MCF7 cells	↑modulation of AMPK/mTOR pathways	H3K9me1 and H3K9me2	↓autophagy *via* AMPK	Zhang et al. (2017)
	G9a	HDAC1 and YY1	MCF-7, MDA-MB-231, MDA-MB-468, MDA-MB-435, ZR-75-30 and T47D	↑HEPH promoter	H3K9me2	↑iron homeostasis through the repression of ferroxidase hephaestin	Wang et al. (2017a)
	G9a	—		↓CDH10	H3K9me2	↑EMT	Casciello et al. (2020)
	G9a	MYC	MDA-MB-231	↓CDKN1A	H3K9me2	↑Proliferation	Tu et al. (2018)
				↓HMOX1			
				↓VAMP4			
	G9a	—	MCF7 and MDA-MB-231 (MDA231)	↓ARNTL	H3K9me2	↑Proliferation	Casciello et al. (2017)
				↓GATA2		↑Migration	
	G9a	EZH2	MCF7 and MDA-MB-231 cells	↓miR124	H3K9me2	↑Invasion	Siouda et al. (2020)
						↑EMT	
	G9a	TBX2	MCF7 and BT474 cells	↓NDRG1	H3K9me2/3	↑Proliferation	Crawford et al. (2019)
		HP1					

(Continued on following page)

10.3389/fphar.2022.946811

TABLE 1 (*Continued*) Status of histone methylation marks studied in breast cancer subtypes.

Substrates	Genes	Cooperators	Cell line/Tissue	Targets	H3Kme status	Effects	References
		EGR1					
	G9a	E4BP	MCF-7, T47D, and BT-549 cell	↓RASSF8	H3K9me2/3	↑Proliferation	Karthik et al. (2018)
		SUV39H1				↓Apoptosis	
	G9a	HDAC1	MCF-7, MDA-MB-231, S1, SK-BR-3 and MDA-MB-435	↓Hephaestin	H3K9me2	↑Proliferation	Wang et al. (2017a)
		YY1					
	G9a	STAT3	MCF12A, and MCF7	↓miR-200c	H3K9me2	↑EMT	Chang et al. (2015)
						↑CSCs	
	G9a	—	MCF-7, SKBr3, and HCT116 cells	↓LC3-II	H3K9me2	↓Autophagy	Kim et al. (2013a)
				↓GFP-LC3-II			
				↓GFP			
	G9a	SNAIL	BLBC cells and luminal cells	↓E-cadherin	H3K9me2	↑Migration	Dong et al. (2013a)
		DNMT				↑EMT	
	G9a	—	MCF-7 cells	↓Beclin-1	H3K9me2	↓Autophagy	Park et al. (2016b)
	SUV39H1	SNAIL	MCF10A, HMLE and SUM1315 cells	↓E-cadherin	H3K9me3	↑Invasion	Dong et al. (2013b)
						↑Migration	
						↑EMT	
	SUV39H2	LSD1	MDA-MB157 and MDA-MB231 cell		H3K9me3	↑Metastatic biology	Piao et al. (2015)
						↑Poor survival	
	SUV39H2	ERβ represses the expression of SUV39H1/2	MCF7 and MDA-MB-157 cells	↑transcription activated by p53	H3K9me3	↑Proliferation	Lu and Katzenellenbogen, (2017)
						↓Apoptotic activities	
	SUV39H2	Recruited by PR to methylate histone H3K9		Unknown		stabilization of HP1γ binding	Liu et al. (2014)
	SUV39H2	γ-H2AX	MCF-7, SK-BR-3, ZR-75-1, T-47D, MDA-MB-231, and BT-20	Unknown	H3K9me3	↑Chemoresistance of cancer cells	Vougiouklakis et al. (2018)
	SETDB1	SMAD3	NMuMG and MDA-MB-231	↓SNAIL1	H3K9me3	↓Invasion	Du et al. (2018)
						↓Camptothecin resistance	
						↓EMT	
H3K79	EZH2	Unknown	primary human breast cancer samples or xenograft tumors	↓RAD51	H3K27me3	↓HR repair	Chang et al. (2011)
						↑Breast tumor initiating cells expansion	
	EZH2	Unknown	HCC70 and MDA-MB-468 cells	↓FOXO3	H3K27me3	↑Proliferation	Gong et al. (2016)
	EZH2	Unknown		↓ERα	H3K27me3	↑Tamoxifen resistance	Nie et al. (2019)
	EZH2	Unknown	MCF10A, MDA-MB-361, MCF7, MDA-MB-436, MDA-MB-231, BT-20, HCC1937, HCC1395, MDA-MB-468, DU4475, BT-549, SUM-159, CAL-120, CAL-148, MDA-MB-453 and SUM-185	↓GATA3	H3K27me3	↑Fulvestrant resistance	Yomtoubian et al. (2020)
						↑Proliferation	
						↑Invasion	
						↑Migration	
	EZH2	Unknown	MDA-MB-231 and MCF-7	↓KLF2	H3K27me3	↑Proliferation	Taniguchi et al. (2012)
	EZH2	LncRNA UCA1		↓P21	H3K27me3	↑Tamoxifen resistance	Li et al. (2019)
	EZH2	Unknown		↓FOXC1	H3K27me3	↑Invasion	Du et al. (2012)
						↑Migration	
	EZH2	Unknown	H16N2, HME, and MCF10A	↓E-cadherin	H3K27me3	↑Invasion	Cao et al. (2008)
	EZH2	SUZ12	T47D, MCF7, and MDA-MB231	↓RKIP	H3K27me3	↑Invasion	Ren et al. (2012)
	EZH2	Unknown	MCF-7 cells	↓miR-129-5p	H3K27me3	↑EMT	Luan et al. (2016)
						↑Adriamycin resistance	
						↑Vincristine resistance	
						↑Paclitaxel resistance	
	EZH2	Unknown	MDA-MB-231(TCHu227) and MCF7(TCHu74); MDA-MB-436, MDA-MB-453, BT474 and SKBR3	↓TET1	H3K27me3	↑Proliferation	Yu et al. (2019)

(Continued on following page)

10.3389/fphar.2022.946811

TABLE 1 (*Continued*) Status of histone methylation marks studied in breast cancer subtypes.

Substrates	Genes	Cooperators	Cell line/Tissue	Targets	H3Kme status	Effects	References
						↓Senescence	
	EZH2	Unknown	MCF-7	↓RUNX3	H3K27me3	↑Proliferation	Fujii et al. (2008)
	EZH2	Unknown	MDA MB 435	↓CIITA	H3K27me3	↓Tumor immunogenicity	Truax et al. (2012)
	EZH2	Unknown	MCF-7 and ZR-75-1	↓BIK	H3K27me3	↓Apoptosis	Si et al. (2016)
						↑Paclitaxel resistance	
	EZH2	YAP	E0771 and ZR-75-30	↓GDF15	H3K27me3	↑Migration	Wang et al. (2018)
	EZH2	Unknown	MDA-MB-468 and MDA-MB-231	↓TIMP	H3K27me3	↑Invasion	Chien et al. (2018)
						↑Migration	
	EZH2	Unknown	MDA-MB-231 and MCF7	↓WWC1	H3K27me3	↑Proliferation	Liu et al. (2018)
						↑Migration	
	EZH2	Unknown	MCF-10A, and MCF-7	↓Period2	H3K27me3	↑Invasion	Yu et al. (2018)
						↑Colony formation	
						↑Mammosphere formation	
	EZH2	LINC00511	MCF7 cells and UACC-812 and MDA-MB-231 cells	↓CDKN1B	H3K27me3	↑Proliferation	Zhang et al. (2019a)
	EZH2	LncRNA DANCR	MCF10A, MCF7, T47D, MDA-MB-231, and MDA-MB-468	↓SOCS3	H3K27me3	↑Viability	Zhang et al. (2020a)
						↑Invasion	
						↑Migration	
	EZH2	LOXL1-AS1	MDA-MB-231 and MCF7	↓miR-708-5p	H3K27me3	↑Invasion	Dong et al. (2020)
						↑Migration	
	EZH2	Unknown	MCF-7/CDDP and MDA-MB-231/CDDP cells	↓miR-381	H3K27me3	↑Proliferation	Dou et al. (2019)
						↑Cisplatin resistance	
	EZH2	Unknown	MDA-MB-231 and MDA-MB-436 cells	↓FOSB	H3K27me3	↑Proliferation	Zhang et al. (2020b)
	EZH2	YY1	MDA-MB-231 and MDA-MB-453	↓OPB	H3K27me3	↑Cell Viability	Yi et al. (2021)
						↑Migration	
	EZH2	SMYD2	T-47D, Hs 578T and MCF-7 cells	↓SIAH1	H3K27me3	↑Proliferation	Zeng et al. (2019)
				↓RASSF1		↑Invasion	
				↓AXIN2		↑EMT	
	EZH2	DDX21	MDA-MB-231 and MCF-7	↓SNAIL	H3K27me3	↓EMT	Zhang et al. (2018a)
						↓Invasion	
	EZH2	LINC01133	MDA-MB-231, SKBR-3, MDA-MB-468, ZR-75-1, BT474, MCF-7 and T47D	↓SOX4	H3K27me3	↓Invasion	Song et al. (2019)
						↓Migration	
	EZH2	macroH2A1.2	MDA-MB-468, MCF-7, MCF-10–2A, and MDA-MB-231	↓LOX	H3K27me3	↓Bone metastasis	Kim et al. (2018)

HR, hormone receptors; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; ↑, up-regulated; ↓, down-regulated.

10.3389/fphar.2022.946811

TABLE 2 Classification, formal names, and aliases of HATs.

Name	Gene symbol	Alias	Protein groups
Histone acetyltransferase 1	KAT1	HAT1	Writer
K(lysine) acetyltransferase 2A	KAT2A	GCN5, GCN5L2, PCAF-b, hGCN5	Writer/reader
K(lysine) acetyltransferase 2B	KAT2B	CAF, P/CAF, PCAF	Writer/reader
CREB binding protein	KAT3A	CREBBP, CBP, KAT3A, RSTS	Writer/reader
E1A binding protein p300	KAT3B	EP300, RSTS2, p300	Writer/reader
TATA-box binding protein-associated factor 1	TAF1	KAT4, BA2R, CCG1, CCGS, DYT3, DYT3/TAF1, N-TAF1, NSCL2, OF, P250, TAF(II)250, TAF2A, TAFII-250, TAFII250, XDP	Writer/reader
TATA-box binding protein-associated factor 1 like	TAF1L	TAF2A2	Writer/Reader
General transcription factor IIIC	GTF3C4	KAT12, GTF3C4, TFIII90, TFIIIC290, TFIIIC90, TFIIICDELTA	Writer
Activating transcription factor 2	ATF2	CRE-BP1, CREB-2, CREB2, HB16, TREB7	Writer
K(lysine) acetyltransferase 5	KAT5	ESA1, HTATIP, HTATIP1, PLIP, TIP, TIP60, ZC2HC5, cPLA2	Writer
K(lysine) acetyltransferase 6A	KAT6A	MOZ, MRD32, MYST-3, MYST3, RUNXBP2, ZC2HC6A, ZNF220	Writer
K(lysine) acetyltransferase 6B	KAT6B	GTPTS, MORF, MOZ2, MYST4, ZC2HC6B, qkf, querkopf	Writer
K(lysine) acetyltransferase 7	KAT7	HBO1, HBOA, MYST2, ZC2HC7	Writer
K(lysine) acetyltransferase 8	KAT8	MOF, MYST1, ZC2HC8, hMOF	Writer
Elongator acetyltransferase complex subunit 3	KAT9	ELP3	Writer
Nuclear receptor coactivator 1	KAT13A	NCOA1, F-SRC-1, RIP160, SRC1, bHLHe42, bHLHe74	Writer
Nuclear receptor coactivator 3	KAT13B	NCOA3, ACTR, AIB-1, AIB1, CAGH16, CTG26, RAC3, SRC-3, SRC3, TNRC14, TNRC16, TRAM-1, bHLHe42, pCIP	Writer
Clock circadian regulator	KAT13D	CLOCK, bHLHe8	Writer
CSRP2 binding protein	KAT14	CSRP2BP, ATAC2, CRP2BP, PRO1194, dJ717M23.1	Writer
MHC class II transactivator	CIITA	C2TA, CIITAIV, MHC2TA, NLRA	Writer
Testis-specific chromodomain protein Y 1	CDY1	CDY, CDY1A	Writer
Testis-specific chromodomain protein Y 2	CDY2	CDY2A	Writer

possibility of polymorphism within BC (Ozdag et al., 2006). TCGA-based bioinformatics analysis was carried out; as a result, SUV39H2, together with additional new genes (DNMT3B, SUV39H1, AURKB, and EZH2) was remarkably upregulated within TN disorders, which was positively related to Ki67 upregulation, tumor grade, and TN status. SUV39H2 upregulation predicted a poor survival time (Pena-Llopis et al., 2016). In ERα-positive cells, ERβ downregulates SUV39H1 and SUV39H2, and decreases the binding of ERα to p53 to abolish the suppressive heterochromatin. At last, ERβ can produce a p53-ERα transcriptional block while further suppressing proliferation and promoting apoptosis (Lu and Katzenellenbogen, 2017).

H3K27 methylation

H3K27 methylation has been frequently recognized as the gene repression hallmark. H3K27me3 can generate extensive domains within the silenced gene promoters (Margueron and Reinberg, 2011). Additionally, H3K27me3 is enriched at poised enhancers along with a low level of H3K4me1 in mouse and human embryonic stem cells (ESCs) (Rada-Iglesias et al., 2011). Due to the upregulation in enhancers and promoters, H3K27me3 has a critical effect on suppressing development-related genes. Apart from upregulation in poised enhancers, H3K27me2 also shows a relation to promoters in repressive and active genes (Barski et al., 2007). Unlike H3K27me2 and H3K27me3, H3K27me1 is distributed at the actively transcribed gene promoters. The PRC2 complex, which can catalyze H3K27 methylation, has four key subunits (Ezh2, Suz12, EED, and RbAP46/48) and shows the preferential methylation of H3K27. Meanwhile, G9a represents the HMT for H3K9me1/me2, which can promote H3K27 monomethylation (Coward et al., 2018).

Some previous immunohistochemical (IHC) studies have identified the relation of H3K27me3 upregulation with luminal A-like tumors. By contrast, H3K27me3 is in a low level in highly proliferative TNBC, basal-like, ER-positive, and luminal B tumors (Holm et al., 2012; Healey et al., 2014). It is

interesting that H3K27me3 is related to EZH2 upregulation in TNBC and basal-like BC, indicating the role of enhanced EZH2 activity in functions associated with non-H3K27 methylation, such as specifically regulating ubiquitination and transcription factors (TFs), and protein decomposition inducing tumor genesis and development (Park et al., 2021).

H3K36 methylation

H3K36 methylation within human cells can interact with transcriptional elongation and methylation of H3K9 to maintain the repressive chromatin status after gene transcription in a histone acetylation-independent manner (Fang et al., 2010). Additionally, H3K36me3 can recruit DNA methyltransferase 3A (DNMT3A) for achieving DNA methylation, which is the redundant pathway for inhibiting the false initiation of transcription (Dhayalan et al., 2010). H3K36me2, which is located in gene body regions, remains largely unclear. Nonetheless, H3K36me2 upregulation is suggested to be related to aberrant transcription (Kuo et al., 2011). H3K36 methylation is able to suppress the enzymatic activity of the PRC2 complex, thus preventing PRC2-regulated H3K27 methylation (Yuan et al., 2011). Within mammalian cells, nine H3K36 methyltransferases are discovered, among which, SMYD2, NSD1-3, SETMAR, SETD3, and ASH1L directly catalyze H3K36 mono- and dimethylation, while just testis-specific PRDM9 and SETD2 are able to catalyze H3K36me3 (Eram et al., 2014).

Jeong *et al.* reported that NSD3 played an important role in epigenetically regulating BC stemness, metastasis, and EMT, indicating its role as a therapeutic target for metastatic BC (Jeong et al., 2021). Other findings indicate that SETD2 alteration-mediated epigenetic modulation and downstream H3K36me3 are involved in the development of breast phyllodes tumor (PT). In PT pathogenesis, SETD2 mutations possibly take place in the early stage (Tsang et al., 2021).

H3K79 methylation

H3K79 methylation shows a high enrichment level within coding regions, which is related to active transcription. H3K79 methylation occurs in the globular domains of histone H3, which is different from additional histone marks present in unstructured histone tails (Nguyen and Zhang, 2011). The aforementioned three H3K79 methylation types involve yeast Dot1 protein and the mammalian homolog DOT1L (Jones et al., 2008). It is intriguing that H3K79 methylation displays trans-tail histone modification with additional histone marks such as H4K16ac and H2B ubiquitination. In yeast, H2B ubiquitination loss reverses H3K79me2 and H3K79me3 (Ng et al., 2002). As revealed by *in vitro* HMT and structural assays, H2B ubiquitination plays an essential role in DOT1L's methyltransferase activity (McGinty et al., 2008). H4's N-terminal tail is needed for the *in vitro* enzymatic activity of Dot1. Furthermore, H4K16ac upregulation promotes *in vivo* H3K79 methylation (Altaf et al., 2007), and the latter has been suggested to disrupt transcriptional elongation, DNA damage response, and telomeric silencing (Huyen et al., 2004).

H4K20 methylation

H4K20 methylation represents the suppressive hallmark for histone modification. H4K20me1 is located in the coding region of lowly transcribed genes, which can be enriched within parental nucleosomes during cell division (Sato et al., 2016). H4K20me1/me2 can recruit leucine-rich repeats and WD repeat domain containing 1 (LRWD1) and origin recognition complex subunit 1 (ORC1) in the replication origin for regulating DNA replication (Kuo et al., 2012). It is to be noted that H4K20 methylation can directly recruit L3MBTL1 (a chromatin remodeler protein) for inducing chromatin condensation (Boccuni et al., 2003). SET8 contributes to the mono-methylation of H4K20, and later H4K20me1 is methylated into H4K20me2/me3 gradually *via* the action of SUV4-20H1/H2 (Jorgensen et al., 2013). Additionally, H4K20 methylation has been suggested to facilitate DNA damage repair, genomic stability, nucleosome turnover, DNA replication, and chromatin compaction (Tardat et al., 2010; Yang et al., 2016). The status of histone methylation marks were mesmerized in Table 1.

Histone H2A and H2B pathways in breast cancer

Histone H2A and H2B variants are recognized as the mediators of drug resistance and also of drug sensitivity in breast cancer (Nayak et al., 2015). The histone H2A.Z depletion can also be defective in the integrity and stability of the human genome. Rangasamy *et al.* presented the molecular pathways linking H2A.Z to breast cancer and mechanisms were proposed to explain how the altered H2A.Z led to tumorigenesis (Rangasamy, 2010). However, monoubiquitination of histone H2B at lysine 120 (H2Bub1) has been shown to have key roles in transcription, DNA damage response, and stem cell differentiation (Cole et al., 2015). While globally depleted in breast cancer, H2Bub1 is selectively enriched in the coding region of certain highly expressed genes, including p53 target genes in response to DNA damage, functioning to exercise transcriptional control of these loci (Atanassov et al., 2016).

Histone demethylation in BC

Dozens of lysine demethylases (KDMs) have been reported to date that are classified into two main groups (Cloos et al., 2006; Klose et al., 2006): the amine-oxidase type lysine-specific demethylases (LSDs) and the highly conserved Jumonji C (JmjC) domain-containing histone KDMs. KMTs and KDMs have both been implicated in oncogenesis. LSD1 can exhibit either pro-tumor or

TABLE 3 Classification, formal names and aliases of HDACs.

Name	Gene symbol	Alias	Protein groups
Histone deacetylase 1	HDAC1	GON-10, HD1, RPD3, RPD3L1	Eraser
Histone deacetylase 2	HDAC2	HD2, RPD3, YAF1	Eraser
Histone deacetylase 3	HDAC3	HD3, RPD3, RPD3-2	Eraser
Histone deacetylase 4	HDAC4	AHO3, BDMR, HA6116, HD4, HDAC-4, HDAC-A, HDACA	Eraser
Histone deacetylase 5	HDAC5	HD5, NY-CO-9	Eraser
Histone deacetylase 6	HDAC6	CPBHM, HD6, JM21, PPP1R90	Eraser
Histone deacetylase 7	HDAC7	HD7, HD7A, HDAC7A	Eraser
Histone deacetylase 8	HDAC8	CDA07, CDLS5, HD8, HDACL1, MRXS6, RPD3, WTS	Eraser
Histone deacetylase 9	HDAC9	HD9, HDAC, HDAC9B, HDAC9FL, HDRP, MITR	Eraser
Histone deacetylase 10	HDAC10	HD10	Eraser
Histone deacetylase 11	HDAC11	HD11	Eraser
Sirtuin 1	SIRT1	SIR2L1	Eraser
Sirtuin 2	SIRT2	SIR2, SIR2L, SIR2L2	Eraser
Sirtuin 3	SIRT3	SIR2L3	Eraser
Sirtuin 4	SIRT4	SIR2L4	Eraser
Sirtuin 5	SIRT5	SIR2L5	Eraser
Sirtuin 6	SIRT6	SIR2L6	Eraser
Sirtuin 7	SIRT7	SIR2L7	Eraser
ASH1-like histone lysine methyltransferase	ASH1L	ASH1, ASH1L1, KMT2H	Reader
ATPase family, AAA domain containing 2	ATAD2	ANCCA, CT137, PRO2000	Reader
ATPase family, AAA domain containing 2B	ATAD2B	—	Reader
Bromodomain adjacent to zinc finger domain 1A	BAZ1A	ACF1, WALp1, WCRF180, hACF1	Reader
Bromodomain adjacent to zinc finger domain 1B	BAZ1B	WBSCR10, WBSCR9, WSTF	Reader
Bromodomain adjacent to zinc finger domain 2A	BAZ2A	TIP5, WALp3	Reader
Bromodomain adjacent to zinc finger domain 2B	BAZ2B	WALp4	Reader
Bromodomain PHD finger transcription factor	BPTF	FAC1, FALZ, NURF301	Reader
Bromodomain containing 1	BRD1	BRL, BRPF1	Reader
Bromodomain containing 2	BRD2	D6S113E, FSH, FSRG1, NAT, RING3, RNF3	Reader
Bromodomain containing 3	BRD3	ORFX, RING3L	Reader
Bromodomain containing 4	BRD4	CAP, HUNK1, HUNKI, MCAP	Reader
Bromodomain testis-associated	BRDT	BRD6, CT9	Reader
Bromodomain containing 7	BRD7	BP75, CELTIX1, NAG4	Reader
Bromodomain containing 8	BRD8	SMAP, SMAP2, p120	Reader
Bromodomain containing 9	BRD9	LAVS3040, PRO9856	Reader
Bromodomain and PHD finger containing 1	BRPF1	BR140	Reader
Bromodomain and PHD finger containing 3	BRPF3	—	Reader
Bromodomain and WD repeat domain containing 1	BRWD1	C21orf107, N143, WDR9	Reader
Pleckstrin homology domain interacting protein	PHIP	BRWD2, DCAF14, WDR11, ndrp	Reader
Bromodomain and WD repeat domain containing 3	BRWD3	BRODL, MRX93	Reader
CECR2, histone acetyl-lysine reader	CECR2	—	Reader
KIAA2026	KIAA2026	—	Reader
Lysine methyltransferase 2A	KMT2A	ALL-1, CXXC7, HRX, HTRX1, MLL, MLL-AF9, MLL/GAS7, MLL1, MLL1A, TET1-MLL, TRX1, WDSTS	Reader
Polybromo 1	PBRM1	BAF180, PB1	Reader
SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2	SMARCA2	BAF190, BRM, NCBRS, SNF2, SNF2L2, SNF2LA, SWI2, Sth1p, hBRM, hSNF2a	Reader
SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4	SMARCA4	BAF190A, BRG1, MRD16, RTPS2, SNF2, SNF2L4, SNF2LB, SWI2, hSNF2b	Reader

(Continued on following page)

TABLE 3 (*Continued*) Classification, formal names and aliases of HDACs.

Name	Gene symbol	Alias	Protein groups
SP100 nuclear antigen	SP100	lysp100b	Reader
SP110 nuclear body protein	SP110	IFI41, IFI75, IPR1, VODI	Reader
SP140 nuclear body protein	SP140	LYSP100, LYSP100-A, LYSP100-B	Reader
SP140 nuclear body protein-like	SP140L	—	Reader
Tripartite motif containing 24	TRIM24	PTC6, RNF82, TF1A, TIF1, TIF1A, TIF1ALPHA, hTIF1	Reader
Tripartite motif containing 28	TRIM28	KAP1, PPP1R157, RNF96, TF1B, TIF1B	Reader
Tripartite motif containing 33	TRIM33	ECTO, PTC7, RFG7, TF1G, TIF1G, TIF1GAMMA, TIFGAMMA	Reader
Tripartite motif containing 66	TRIM66	C11orf29, TIF1D, TIF1DELTA	Reader
Zinc finger MYND-type containing 8	ZMYND8	PRKCBP1, PRO2893, RACK7	Reader
Zinc finger MYND-type containing 11	ZMYND11	BRAM1, BS69, MRD30	Reader

anti-tumor activity in breast cancer development, highlighting a context-dependent role in regulating different biological processes possibly by using different functional domains (Hu et al., 2019a; Fang et al., 2019). JMJD3 has been associated with breast cancer progression. Xun *et al* showed that ectopic expression of JMJD3 suppresses the stem cell-like characteristics of breast cancer cells (Xun et al., 2017).

Histone acetylation in breast cancer

KATs can be divided into two types according to their cellular localization, namely, cytoplasmic and nuclear KATs (Han et al., 2016; Trisciuoglio et al., 2018). Among them, nuclear KATs can be further divided according to enzyme transfer mechanisms and structural homology (Table 2). There are five different families discovered to have diverse functions and targets, namely, CREB-binding protein and its paralog p300 (p300/CBP), GCN5-related N-acetyltransferase (GNAT), nuclear receptor coactivator factor (NRCF) family, and MYST (Roth et al., 2001; Fiorentino et al., 2018). For p300/CBP, there are about 100 protein substrates detected, contributing to the acetylation of non-histone and histone proteins such as tumor suppressor protein p53 (Bowers et al., 2010; Simon et al., 2016). Among KATs, the MYST family contains the greatest gene number and shows the highest diversity, which is mainly related to gene silencing and DNA repair (Voss and Thomas, 2009), including MOZ (monocytic leukemia zinc finger protein), Tip60 (Tat-interactive protein), Sas2 (something about silencing), YBF2/Sas3, and MOF. They exhibit the features of one conserved 3-terminal histone acetyltransferase (HAT) domain (that contains a binding site for acetyl-CoA), one helix-turn-helix DNA-binding domain, and one C2HC zinc finger related to HAT catalytic performance (Roth et al., 2001; Brown et al., 2016). The KAT family is associated with great variability in structural characteristics, such as chromodomains, zinc fingers, and PHD fingers (Yang and Seto, 2007).

GNAT family

DNA-wrapped surrounding histones can be accessed *via* epigenetic mechanisms such as the acetylation of histone lysine. In each histone, KATs can acetylate 10–20 lysine residues. Histone acetylation will elevate negative charges onto DNA, thereby promoting proteins associated with DNA repair, transcription, and replication to access DNA (Vo and Goodman, 2001; Unnikrishnan et al., 2010). Histone lysine acetylation has been suggested to be related to fundamental transcriptional activation commonly seen in tumor cells, in particular for K9/K11/K18/K56 onto histone H3, and K5/K8/K13K16 onto histone H4 (Berger, 2007). Such acetylation procedure can be regulated *via* lysine acetyltransferases such as p300/CBP, ORC-binding HATs, monocytic leukemia zinc finger protein (MOZ), general control of amino acid synthesis 5-like 2 (GCN5), and MYST2/KAT7 (HBO1) (Kaypee et al., 2016). GCN5 silencing inhibits MDA-MB231 cell invasion, proliferation, and migration, upregulates p21, and downregulates p-AKT, p-STAT3, E2F1, and MMP9 levels within MDA-MB231 cells relative to those treated with TGF-β1. Consequently, GCN5 is the possible downstream target of the TGF-β/Smad pathway responsible for regulating EMT within BC (Zhao et al., 2018).

P300/CEBP family

ERα represents the TF that binds to the growth factor (GF) and hormonal signals to be activated. Actually, ERα is extensively suggested to be acetylated post-translationally *via* the activation of coactivator p300. The persistently activated ERα is related to a higher risk of BC occurrence by promoting aberrant breast tissue development. ERα acetylation can be achieved within hinge/ligand domains in K229, K299, K302, and K303 (Wang et al., 2001). Also, in another research on atypical breast hyperplasia, the ERα acetylation level increases in lysines K266 and K268 *via* p160 and p300 coactivators (Kim et al., 2006). p300/CBP contributes to ERα acetylation and promotes cell growth within BRCA1-mutated BC cells. Cross-talk with CBP and

p300 coactivators within BC cells can decrease the metastatic activity by increasing E-cadherin levels (Liu et al., 2005). H3 acetylation in the promoters of Snail, ZEB1, and ZEB2 promotes the CSC-like characteristics within BC cells (Cho et al., 2015). Metadherin (MTDH) is related to BC cell metastasis and drug resistance, which can interact with CBP, and the latter is thereby translocated into the promoter of the twist family BHLH transcription factor (TWIST) and allows for proximal H3 acetylation in the promoter (Liang et al., 2015). Certain gene mutations have been indicated to upregulate p300/CBP within BC (Tillinghast et al., 2003), which is usually related to disease relapse and chemoresistance (Xiao et al., 2011).

MYST family

Human males absent on the first (hMOF) deficiency can be detected within certain cancer types, and its level is the marker for disease prognosis (Cao et al., 2014). Pfister *et al.* compared the non-transformed control tissues and found that the hMOF protein and mRNA levels were significantly downregulated in primary BC. In addition, the hMOF protein level was closely related to H4K16 acetylation within each tested sample. On the contrary, hMOF expression increases within certain cancer types, which is related to HBO1 acetyltransferase responsible for forming a pre-initiation complex while initiating replication (Iizuka et al., 2006). P53 shows negative regulation on HBO1 while suppressing replication in the case of cell stresses (Iizuka et al., 2008). Moreover, HBO1 expression increases within tumor cells in comparison with healthy cells (Iizuka et al., 2009); meanwhile, its phosphorylated form functions to regulate CSC genesis within BC (Duong et al., 2013). KATs are referred to as MOZ (also known as MYST3 and KAT6A), and they can form tetrameric complexes with their paralog MORF (also known as MYST4 and KAT6B). The as-formed complexes contain two small non-catalytic subunits and bromodomain- and PHD finger-containing protein 1 (BRPF1) (Kaypee et al., 2016). The aforementioned two acetyltransferases are usually mutated within BC (Lynch et al., 2013).

Histone deacetylation in breast cancer

Numerous histone deacetyltransferases are examined in studies to achieve favorable effects (Table 3). Sirtuin (SIRT1)-mediated ERα deacetylation within BC can decrease ERα activity and suppress BC cell growth, which is the effective method for preventing BC progression. Park *et al.* investigated SIRT2 function using $Sirt2^{-/-}$ mammary tumor cell line (MMT) derived from the spontaneous mammary tumors in $Sirt2^{-/-}$ mice, which identified the M2 isoform of pyruvate kinase (PKM2) as a critical target of SIRT2 (Park et al., 2016c). This result was supported by Shi *et al.* who demonstrated that the high expression of SIRT2 by IHC (IHC score >3) was downregulated in tumor tissues compared with the normal adjacent tissues in 296 patients (Shi et al., 2020). In several cell lines and human breast cancer tissues, Nakagawa *et al.* analyzed the expression of class I HDACs, including HDAC1, HDAC2, HDAC 3, and HDAC 8, and investigated which subtypes of class I HDACs were overexpressed in breast cancer. They revealed the high expression levels of these class I HDACs, and IHC results for HDAC1, HDAC2, HDAC3, and HDAC8 were positive in 17 (85%), 20 (100%), 20 (100%), and 17 (85%) of 20 breast cancer cases, respectively (Nakagawa et al., 2007). HDAC6 contributes to cancer metastasis since its upregulation increases cell motility in breast cancer MCF-7 cells and its interaction with cortactin regulates motility. HDAC6 also affects transcription and translation by regulating the heat-shock protein 90 (Hsp90) and stress granules, respectively (Saji et al., 2005). HDAC11 shows different expression levels and biological functions in different systems of the human body and is among the top 1–4% of genes overexpressed in cancers, such as breast cancer (Liu et al., 2020).

Histone methylation-targeted anti-cancer drugs

At present, just a few selective small-molecular substances with the direct inhibition effect of active sites in specific KMT2 family protein enzymes are identified (Chern et al., 2020). Epi-drugs can restore the repressive TSGs or the aberrantly activated oncogenes to suppress BC development. In addition, epi-drugs can prevent drug resistance, increase anti-tumor therapeutic effects, and enhance the radiotherapeutic effect.

Inhibitors that target H3K4-specific HMTs for anticancer therapy

MLL family inhibitors

Chern *et al.* adopted the bisubstrate strategy to prepare a focused library and identified numerous strong MLL methyltransferase inhibitors. It is to be noted that compound 16 (TC-5115) shows the highest strength and displays the 16-nM IC50 value. In the complex of MLL plus another four strong inhibitors, cocrystal structures are observed, revealing the role of such inhibitors in locking the MLL SET domain within the open, inactive conformation. Further optimizing TC-5115 can assist in developing a novel anti-MLL treatment (Chern et al., 2020).

Furthermore, MLL2 expression increases in BC cells and invasive carcinomas (Natarajan et al., 2010). Similarly, MLL4 deficiency reduces H3K4me3 expression while upregulating H3K27me3 expression within SIX1, MMP9, and MMP11 genes of MDA-MD-231 cells (Rabello Ddo et al., 2013). Based on the aforementioned findings, H3K4 methyltransferase possibly connects H3K27 acetylation and H3K4 methylation within BC cells by a certain mechanism, as evidenced by research on MLL4 levels within BC cells. Afterward, the UTX-MLL4 complex

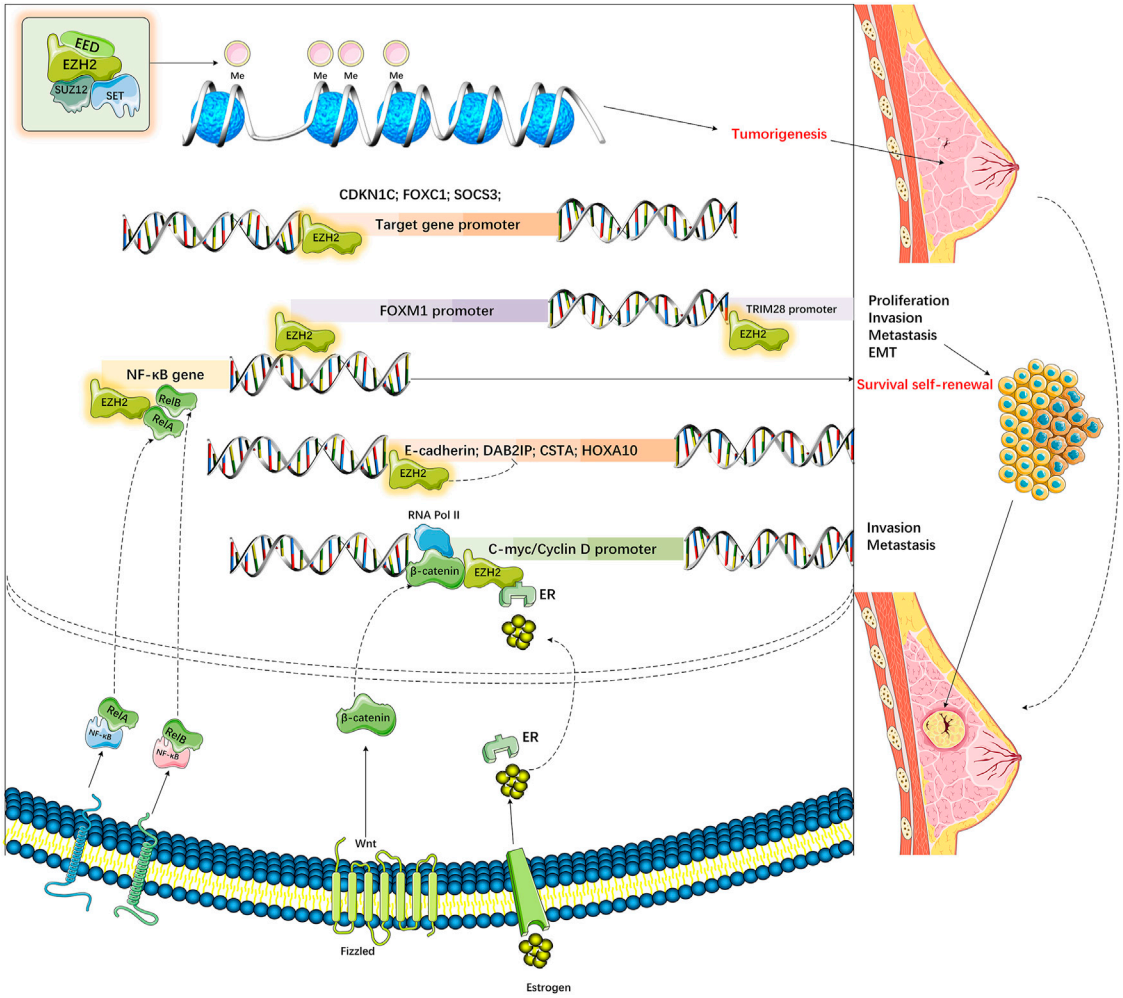


FIGURE 3
Dysregulation of EZH2 in various breast cancers.

significantly promoted H3K27 acetyltransferase p300 to bind to target chromatin regions, thereby additionally increasing H3K27 acetylation while enhancing the gene activation activity of the enhancer (Kim et al., 2014).

Menin-MLL inhibitors

Suppressing the association of menin with HMTs is a possible new treatment. At first, macrocyclic peptidomimetic inhibitors (MCP-1) are prepared for inhibiting the interaction between menin and MLL1. Meanwhile, menin shows direct binding to MI-463 and MI-503 at the low nanomolar binding affinity, which efficiently suppresses the interaction of menin with MLL (Borkin et al., 2015). Small molecules can be used to pharmacologically inhibit the interaction between menin and MLL, which can thereby prevent *in vivo* MLL leukemia progression without affecting healthy hematopoiesis. MI-463 used in combination with auranofin (an inhibitor of thioredoxin reductase) shows synergistic effects on promoting BC cell apoptosis (Kato et al., 2020). Additionally, HO-1 has a strong induction effect, which facilitates synergistically promoting the efficacy of MI-463 and auranofin. Consequently, the combined application of menin-MLL inhibitors, such as MI-463, and auranofin can efficiently treat BC by inducing ferroptosis.

SMYD inhibitors

RANi-induced SMYD2 silencing within TNBC cells or AZ505 (an inhibitor of SMYD2)-mediated SMYD2 inhibition remarkably decreases *in vivo* cancer development (Li et al., 2018). SMYD2 can methylate and activate the new non-histone substrates such as the NF-κB p65 subunit and STAT3 to exert its effect, thus inducing the growth and survival of TNBC cells (Li et al., 2018). As discovered in a recent research study on BC, SMYD3 shows diverse expression levels within T47D and MCF-7 BC cell lines, which enhances cisplatin resistance of MCF-7 cells (Wang et al., 2020). In addition, SMYD3 deficiency

combined with cisplatin exposure suppresses the proliferation and mitochondrial membrane potential (MMP) of cells. Based on the aforementioned findings, SMYD3 has an important effect on analyzing cancer sensitivity and resistance to cisplatin. Consequently, the SMYD3 level has an important effect on BC occurrence, while inhibiting SMYD3 is the new anti-BC treatment.

WDR5 inhibitors

Punzi *et al.* found that WDR5 deficiency decreased cell metastatic ability by abolishing the mesenchymal phenotype of luminal B- and TN-derived cells, thereby promoting the epithelial phenotype. In addition, TGFβ1 regulates the aforementioned process, suggesting that WDR5 is important for inducing EMT by activating TGFβ1. Furthermore, the aforementioned EMT reversion also possibly results from the drug targeting effect of WDR5, which enhances the chemosensitivity of BC cells and the paclitaxel-mediated efficacy (Punzi et al., 2019).

SETD1 inhibitors

As reported in a study, SETD1A activates MMP levels to modulate BC metastasis (Salz et al., 2015), and another study suggests that SETD1A amplification within mixed ductal and lobular breast cancer can upregulate the H3K4me3 marker to modulate mitosis within the mitosis and DNA damage response gene promoters. In addition, SETD1A can modulate some genes regulating DNA damage response, cell cycle, and mitosis by the promoter H3K4 methylation within LC and BC cells. SETD1A loss can trigger the efficacy of aging in suppressing tumors; as a result, SETD1A possibly has a critical effect on maintaining tumor cell growth and mitosis (Tajima et al., 2019). Furthermore, SETD1A can trigger ER+ BC cell growth and invasion by modulating genes related to cell migration and survival independent of ER. Therefore, SETD1A is essential for the development of hormone therapy resistance in BC ER independently (Jin et al., 2018). SETD1B has a similar structure to SETD1A and has a critical effect on the TNBC pathogenic mechanism and survival, no matter whether H3K4 methyltransferase is activated or not, for instance, through the formation of cytoplasmic COMPASS complexes and the regulation of ADIPOR1 *via* the BOD1 interaction (Wang et al., 2017b). A study shows that SET7/9's effect can be achieved through negatively regulating stability *via* E2F1 and DNMT methylation. According to the aforementioned findings, SET7/9 is the biomarker utilized for predicting the invasion and treatment resistance of BC cases.

H3K9 methyltransferase targets anti-BC drugs

G9A inhibitor

Accordingly, the G9A inhibitor treatment efficiently abolishes NDRG1-induced TBX2 suppression and decreases cell growth after functionally inhibiting TBX2. Based on the aforementioned results, TBX2 recruits the huge repressive complex into EGR1-responsive promoters to suppress physical growth control, thus inducing out-of-control BC cell growth (Crawford et al., 2019). A study reported the benzoxazole scaffold by virtual high-throughput screening (HTS), and the design and synthesis of 24 derivatives, which are later utilized to inhibit G9a. Following the repeated screening of anti-proliferative activity and kinase, this work found that GA001, the effective G9a antagonist that had a 1.32 μM IC50 value, triggered autophagy within MCF7 cells through AMPK (Zhang et al., 2017).

H3K27 methylation targeting potential in anti-BC treatment

Using small inhibitory molecules or chromatin-modifying enzyme inhibitors to target tumor epigenome for releasing knockout genes from repressive status is the possible potent method to research cancer and develop new drugs. For erasing the H3K27me3 mark out of gene promoters, some methods have been used, such as directly or indirectly inhibiting EZH2, incorporating H3K27me3-recognizing synthetic TFs, applying natural anti-tumor agents, or combining with known anti-tumor agents. Nonetheless, targeting H3K27 methylation during anti-BC therapy possibly triggers treatment-induced side effects. In particular, apart from the effect of suppressing H3K27 methylation, EZH2 inhibitors can suppress EZH2 activity associated with additional effects. Consequently, applying drugs targeting H3K27 methylation should be properly validated, to overcome non-methylation-associated effects.

EZH2 inhibitors

3-deazaneplanocin A (DZNep) is a known PCR2 inhibitor that can promote tumor cell apoptosis (such as MDA-MB-468, MCF7 BC cells), but it makes no difference to healthy cells (such as MCF-10A) (Tan et al., 2007). DZNep suppresses S-adenosyl-L-homocysteine (SAH) hydrolase activity and upregulates SAH to indirectly inhibit EZH2. SAH is also an antagonist of SAM, which can thereby block HMT activity (Miranda et al., 2009). Additionally, DZNep exerts the lowest effect on DNA methylation-silenced genes (Miranda et al., 2009). DZNep treatment can markedly suppress H3K27 methylation (rather than H3K9 methylation) in diverse tumor cells (such as MB-468 BC cells) by depleting PRC2 component levels in cells (EZH2, SUZ12, and EED) (Tan et al., 2007)) (Figure 3). According to the authors, DZNep treatment reactivates PRC2-suppressed genes in BC. In BRCA1-depleted BC cells with EZH2 upregulation, DZNep further induces apoptosis compared with that in BRCA1-proficient BC cells (Puppe et al., 2009). Although DZNep has aroused wide attention as a possible antitumor therapeutic agent, there is little research on its possible side reactions *in vivo* or in a BC model. Therefore, it is necessary to carry out *in vivo*

experiments to examine its use in BC as an epi-drug. After DZNep, other strong and selective EZH2 inhibitors competing against SAM have also been developed (Verma et al., 2012). Typically, GSK343 and GSK926 are suggested to down-regulate histone H3K27me3 expression while suppressing EZH2 expression within BC (HCC1806 TNBC) and PCa (LNCaP) cells; nonetheless, GSK343 displays certain limitations because it is highly cleared (plasma volume where the drug is completely eliminated per unit of time) in a rat pharmacokinetic study.

Upregulation of EZH2 causes di-methylation (H3K27me2) and H3K27 tri-methylation (H3K27me3) to lysine 27 residue of histone 3 (H3K27). EZH2-mediated up- and downregulation of various genes increases cell proliferation, invasion, and metastasis and decreases the apoptosis of breast cancers.

Interestingly, PARP1, one of the poly(ADP-ribose) polymerase family (PARP) members, is suggested to decrease and interact with EZH2, thus decreasing H3K27me3 expression within MDA-MB-231 cells (Yamaguchi et al., 2018). Upon alkylation and oxidative stress-induced DNA damage, PARP1 contributes to the PARylation of EZH2 while inducing PRC2 complex dissociation, decreasing EZH2 expression, and later downregulating the expression of EZH2-regulated H3K27me3 (Quintayo et al., 2012). On the contrary, PARP inhibitor (PARPi)-mediated PARP suppression can mitigate EZH2 downregulation resulting from alkylating DNA damage, thus, further increasing EZH2-induced gene knockdown and CSC characteristics in comparison with untreated cells. Consequently, the combined application of EZH2i-like GSK343 and PARPi (olaparib) is investigated within BRCA-deficient BC (Yamaguchi et al., 2018). According to results obtained from ovarian cancer (UWB1.289) and BC (HCC38 and SUM149) cells with no response to PARPi alone, adding EZH2i enhances PARPi's efficacy (Yamaguchi et al., 2018). Based on the aforementioned findings, it is necessary to determine whether combination therapy is effective on BRCA-defective tumors in clinical trials. Actually, a phase-II clinical trial is currently recruiting HR+/HER2− advanced BC with endocrine therapy resistance to receive SHR3162 (PARPi) and SHR2554 (EZH2i) treatment (ClinicalTrials.gov Identifier: NCT04355858).

H3K27 methylation inhibitors

Additionally, for BC subtypes that display the lowest H3K27me3 expression and has a dismal prognostic outcome such as TNBC, the chromatin mark may be upregulated for improving patient survival. Some studies have demonstrated the crosstalk between H3K27 methylation and additional chromatin modifications, and the combined application of HDACi (MS275) or DNMTi (guadecitabine/SGI-110) has been examined within the XtMCF and LmMCF cells, and TNBC model cells exhibiting high tumorigenicity and metastasis potentials (Su et al., 2018). The monotherapies of the aforementioned two drugs can upregulate H3K27me3 expression, whereas their combination can synergistically upregulate the H3K27me3 level. Such treatment induces transcriptional reprogramming, which is evidenced by EMT suppression, protein mutant p53 (usually detected within tumor cells), ZEB1 and EZH2 promotion, and induce E-cadherin expression, H3 trimethylation, and apoptosis. Abolishing EMT induces tumor cell proliferation, clone forming, and suppression of their stemness. Additionally, MS275 alone or plus SGI suppresses XtMCF xenograft growth, whereas MS275 decreases the lung metastasis of LmMCF cells within mice (Su et al., 2018). Collectively, the aforementioned data indicate that EMT epigenetic reprogramming, such as H3K27 methylation, inhibits TNBC cells' aggressiveness.

Histone acetylation targeted anti-cancer drugs

Epi-drug can suppress BC progression by abolishing the abnormally suppressed TSGs or abnormally activated oncogenes. Additionally, epi-drugs can prevent treatment resistance, increase antitumor drug efficacy, and enhance radiotherapeutic efficacy. Numerous epi-drugs are examined in clinical studies to achieve favorable effects (Table 3).

HAT inhibitors' effect on anti-BC treatment

Some HAT-targeting inhibitors are investigated; however, none of them has been tested in clinical trials. At present, the existing HATis are library-selected inhibitors, small-molecular HATi (either synthetic or natural), and bi-substrate inhibitors. Of them, bi-substrate mimics, including Lys-CoA, exhibit potent inhibition and are rarely applied in cells due to their great molecular weight (Lau et al., 2000; Cole, 2008). It is to be noted that most potent compound, 1r, the new compound manufactured on the basis of C646, displays potent inhibition, superior drug-like properties, and low cell proliferation after removing the toxic nitro group (Liu et al., 2019). ICG-001 can suppress BC development by targeting protein–protein interactions (PPIs) between beta-catenin and CBP, but not suppressing acetyltransferase activity (Ring et al., 2018; Sulaiman et al., 2018). HATs' acetyltransferase activity and substrate specificity can be measured through the multi-subunit protein complexes. However, the complexities have greatly hindered the translation of *in vitro* experiments to *in vivo* ones. Existing inhibitors are poorly selective and of low efficiency, which have restricted their application, even though they are possibly efficient starting points to develop novel inhibitors.

KAT inhibitors (KATi)

Histone lysine acetylation is related to the occurrence and development of certain disorders, thus indicating that KAT

modulators may be possible therapeutic targets. Nonetheless, it remains a challenge to identify the strong and selective KATi in comparison with modulators for additional epigenetic enzymes such as KDAC inhibitors (Merarchi et al., 2019). Some methods such as computational tools and improved assay techniques are applied in identifying small-molecular KAT inhibitors; however, just a low proportion of them are verified with *in vivo* and *in vitro* activities currently (Krishna et al., 2018; Huang et al., 2019). Such KATi are divided into three categories: *1*) bisubstrate inhibitors, *2*) natural substances and the corresponding derivatives, and *3*) synthesize small molecules.

Bisubstrate inhibitors

Bisubstrate analog mimicking the ternary complex constituted by the lysine substrate and cofactor acetyl-Co is the first KAT inhibitor. Thereafter, some research groups adopted the concept for identifying specific KATi. For instance, lys-CoA is prepared by connecting coenzyme A (CoA) with the single lysine residue by means of the methylene linker (Lau et al., 2000). According to reports, lys-CoA exhibits strong activity to inhibit p300 in comparison with PCAF. Additionally, H3-CoA-20 can specifically bind to PCAF (Lau et al., 2000). Additionally, Boc-C5-CoA is also suggested to occupy two binding pockets in enzyme active sites to suppress p300 (Kwie et al., 2011). Likewise, H4K16-CoA, also a bisubstrate analog, is a strong inhibitor of MYST family enzyme Tip60 and the corresponding yeast homolog Esa1, and its IC50 values are within the micromolar range (Wu et al., 2011).

Nonetheless, bisubstrate inhibitors are poorly permeable into cells and are metabolically unstable, which is ascribed to their partial peptidic structure and polar phosphate group. The aforementioned shortcomings are managed by using cell membrane penetration technologies such as lipid permeabilization and cell micro-injection (Simon et al., 2016). Some possible CoA analog prodrugs targeting p300 are also developed (Cebrat et al., 2003). Modifications including coupling the amino acid backbone of inhibitors into the arginine-abundant peptides or TAT protein transduction domain promote p300 inhibition and transmembrane delivery (Wadia and Dowdy, 2005).

According to one study, polyamine spermidine (Spd) is connected with CoA's S atom in a covalent manner *via* the thioglycolic acid linkage, which thus forms the non-toxic Spd(N1)-CoA that can be internalized in cells by the polyamine transporter (Cullis et al., 1982). Spermidinyl-CoA-based (N1) is able to change pathways related to DNA damage repair and then enhances the chemosensitivity and radiosensitivity of cells (Bandyopadhyay et al., 2009). According to the latest article, the new peptide-CoA conjugate bisubstrate inhibitor is prepared, which displays submicromolar potential to suppress HAT1 (Ngo et al., 2019).

Curcumin

Many articles suggest the effect of some natural substances on inhibiting KATs (Seidel et al., 2012; Kaypee et al., 2016). Curcumin, one of the natural KATi, significantly suppresses different cancers (Shanmugam et al., 2016; Mbese et al., 2019; Tajbakhsh et al., 2018). Curcumin can suppress proliferation and clone-forming abilities of MDA-MB-231 and MCF-7 cells. Based on our results, curcumin's inhibition against BC cells is possibly associated with its resistance to EMT and CSC properties. In line with the aforementioned results, curcumin is an anti-metastatic agent for BC (Hu et al., 2019b). New curcumin preparations have also been under investigation, such as sustained-release capsules and nanoparticles (NPs) to manage inflammation and cancer (Gupta et al., 2013; Di Costanzo et al., 2014). Curcumin can suppress pure p300's acetyltransferase activity by adopting histone H3/p53 to be the substrate. In addition, triggering receptors expressed on myeloid cells 1 (TREM-1) within tumor-associated macrophages (TAMs) cause an inflammatory response initiated by the toll-like receptor (TLR), which shows overexpression within BC cells. TREM-1 is the risk factor for BC (Pullikuth et al., 2021). Curcumin can suppress H3/H4's p300 acetylation to regulate TREM-1 levels within the TREM-1 promoter (Yuan et al., 2012). Moreover, curcumin is also reported to suppress KAT activity within THP-1 cells (human monocytic cell line), inhibit nuclear factor-κB (NF-κB) acetylation at Lys310, and later restrain transcription activation and nuclear translocation of the corresponding downstream targets (Yun et al., 2011). Currently, only curcumin, the KAT with the lowest specificity, is under clinical trials in the treatment of different disease (Manzo et al., 2009; Gupta et al., 2013).

Anacardic acid

First separated in the shell liquid of Anacardium occidentale (cashew nut), anacardic acid (also known as 6-pentadecylsalicylic acid) (Balasubramanyam et al., 2003) is identified as a non-competitive and non-selective inhibitor of PCAF and p300/CBP. It is reported to suppress Tip60 under the same experimental conditions (Ghizzoni et al., 2012). Additionally, it can also affect RelA subunit nuclear localization and acetylation to target the NF-κB pathway, thus suppressing carcinogenesis (Hemshekhar et al., 2012). Because anacardic acid is highly lipophilic and has poor physiochemical characteristics, some new phenoxyacetic acid and 6-alkyl salicylic acid analogs are analyzed to improve KAT inhibition, cell permeability, and solubility. Phenoxyacetic acid analogs have a strong KAT inhibition effect, which is decided by their alkyl chain length and location (Eliseeva et al., 2007). Additionally, anacardic acid can alleviate Tip60-induced DNA damage to augment the radiosensitivity of cancer cells (Sun et al., 2006). Moreover, changes in salicylic acid residue or alkyl chains show specific shifts in MOF suppression of MYST family KATs (Zhang et al., 2018b). Moreover, 4-cyano-3 trifluoromethylphenylbenzamides, the other anacardic acid derivative, is able to suppress KAT3 (Souto et al., 2008). Meanwhile, pentadecylidenemalonate, anacardic acid's simplified analog, is first identified to be a KAT activator/

TABLE 4 Summary of HDAC inhibitors on the BC therapeutic strategy and corresponding clinical trials.

Drug	Therapeutic strategy	Conditions	Phases	NCT
Vorinostat (SAHA)	Monotherapy	BC	I, II (active, not recruiting)	NCT00416130
	Monotherapy	BC	II (completed)	NCT00262834
	Monotherapy	BC	I (Completed)	NCT00788112
	Vorinostat, cyclophosphamide, paclitaxel, trastuzumab, doxorubicin	Locally advanced BC	I, II (completed)	NCT00574587
	Vorinostat, carboplatin, nab-paclitaxel	Operable BC	II (active, not recruiting)	NCT00616967
	Vorinostat, paclitaxel, bevacizumab	Metastatic BC	I, II (completed)	NCT00368875
	Vorinostat, anastrozole, letrozole, exemestane	Stage IV BC	Completed	NCT01720602
	Vorinostat, trastuzumab	Metastatic or locally recurrent BC	I, II (completed)	NCT00258349
	Vorinostat, anastrozole, letrozole, exemestane	Stage IV BC	Completed	NCT01153672
	Vorinostat, radiation	BC patients with brain metastasis	I (completed)	NCT00838929
	Vorinostat, olaparib	Relapsed/refractory and/or metastatic BC	I (recruiting)	NCT03742245
	Vorinostat, tamoxifen, pembrolizumab	BC	II (terminated)	NCT02395627
	Vorinostat, tamoxifen, pembrolizumab	ER-positive BC	II (active, not recruiting)	NCT04190056
	Vorinostat, tamoxifen	Hormone therapy-resistant BC	II (completed)	NCT00365599
	Vorinostat, doxorubicin	BC	I (completed)	NCT00331955
	Vorinostat, ixabepilone	Metastatic BC	I (completed)	NCT01084057
Belinostat (PXD101)	Belinostat, ribociclib	Metastatic BC	I (recruiting)	NCT04315233
	Belinostat, talazoparib	Metastatic BC	I (recruiting)	NCT04703920
	Belinostat, trastuzumab	BC	I (suspended)	NCT03432741
Entinostat (SNDX-275)	Monotherapy	ER-positive BC	II (completed)	NCT00828854
	Monotherapy	TNBC	I (terminated)	NCT03361800
	Entinostat, exemestane	Advanced BC	II (completed)	NCT00676663
	Entinostat, exemestane	ER-positive BC	I (active, not recruiting)	NCT02820961
	Entinostat, atezolizuma	TNBC	I (active, not recruiting)	NCT02708680
	Entinostat, exemestane, atezolizumab	Hormone receptor-positive and HER2-negative BC	I, II	NCT03280563
	Entinostat, exemestane	Advanced or recurrent BC	I (active, not recruiting)	NCT02623751
	Entinostat, exemestane, goserelin	Recurrent hormone receptor-positive BC	E2112 phase III	NCT02115282
	Entinostat, nivolumab, Lpilimumab	Metastatic or locally advanced BC	I (active, not recruiting)	NCT02453620
	Entinostat, Exemestane, erlotinib	BC	I (completed)	NCT01594398
	Entinostat, capecitabine	Metastatic BC, high risk BC after neo-adjuvant therapy	I (recruiting)	NCT03473639
	Entinostat, exemestane	Hormone receptor-positive, locally advanced or metastatic BC	III (active, not recruiting)	NCT03538171
	Entinostat, exemestane	Advanced or recurrent BC	II (active, not recruiting)	NCT03291886
	Entinostat, azactidine	Advanced BC	II (active, not recruiting)	NCT01349959
	Entinostat, lapatinib, trastuzumab	Locally recurrent or distant relapsed metastatic BC	I (completed)	NCT01434303

(Continued on following page)

TABLE 4 (*Continued*) Summary of HDAC inhibitors on the BC therapeutic strategy and corresponding clinical trials.

Drug	Therapeutic strategy	Conditions	Phases	NCT
Panobinostat (LBH-589)	Monotherapy	HER2-negative locally recurrent or metastatic BC	II (completed)	NCT00777049
	Panobinostat, letrozole	Metastatic BC	I, II (completed)	NCT01105312
	Panobinostat, paclitaxel, trastuzumab	HER2-positive or metastatic BC	I (completed)	NCT00788931
	Panobinostat, capecitabine, lapatinib	BC	I (completed)	NCT00632489
Romidepsin	Monotherapy	BC	I (active, not recruiting)	NCT01638533
	Monotherapy	Metastatic BC	II(Completed)	NCT00098397
	Romidepsin, cisplatin, nivolumab	Metastatic TNBC, BRCA mutation locally recurrent or metastatic BC	I, II (suspended)	NCT02393794
	Romidepsin, abraxane	Metastatic inflammatory BC	I, II (terminated)	NCT01938833
Valproic acid (VPA)	Valproate, hydralazine, doxorubicin, cyclophosphamide	BC	II (terminated)	NCT00395655
	Valproic acid, temsirolimus, cetuximab, bevacizuma	Recurrent BC	I (recruiting)	NCT01552434
	Valproic acid, epirubicin, 5-fluorouracil, cyclophosphamide	BC	I (completed)	NCT00246103
Ricolinostat	ACY-1215, nab-paclitaxel	Metastatic BC	I (completed)	NCT02632071
Mocetinostat	MGCD0103, docetaxel	BC	I (terminated)	NCT00511576
CUDC-101	Monotherapy	BC	I (completed)	NCT01171924

inhibitor, which activates PCAF and suppresses recombinant CBP and p300/CBP.

Garcinol

Garcinol (also known as polyisoprenylated benzophenone), a strong non-specific KATi, is extracted from Garcinia indica (Kokum fruit) (Liu et al., 2015b). Its IC50 values for PCAF and p300 are 7 and 5 μM, respectively (Balasubramanyam et al., 2004). Garcinol-induced tumor cell death is related to the inhibition of cell apoptosis and histone acetylation (Arif et al., 2009). For improving garcinol's pharmacokinetic profiles, some derivatives that have a lower toxic effect, higher efficacy, and specificity have been prepared. Isogarcinol is prepared through intramolecular cyclization, and it is adopted to be the template for designing some new KATi. In a recent study, Milite et al. prepared the benzylidene barbituric acid derivative (EML425), which was applied as a factor to selectively block p300/CBP, and had strong inhibition within the low micromolar range (IC50 values of 1.1 and 2.9 μM for CBP and p300, respectively) (Milite et al., 2015). As discovered by Ahmad et al., garcinol exposure promoted β-catenin phosphorylation, and it decreased nuclear localization in BC (Ahmad et al., 2012). Such findings were verified in the xenograft mouse model *in vivo*, in which garcinol suppressed miRNAs, NF-κB, nuclear β-catenin, and vimentin. According to the aforementioned results, garcinol's anti-BC effect is partial because of EMT phenotypic reversal, and this is related to the abnormal levels of let-7s, miR-200s, and Wnt and NF-κB pathways to some extent.

Carnosol

Carnosol, which takes the region binding acetyl-CoA's pantetheine arm, is verified to be the candidate anti-BC target. In addition, it is the new natural p300 inhibitor, which can be listed in the current inhibitor panel (Alsamri et al., 2021).

BET family

In recent years, BET family-targeting small molecules (BRD2–BRD4 and testis-specific BRDT) are novel epi-drugs. The BET family functions to recognize and bind to acetylated lysine by means of bromodomain; in addition, it has critical effects on cell cycle control and transcriptional elongation. At first, BET inhibitors' effects are examined within NUT-midline carcinoma (Filippakopoulos et al., 2010) and hepatological cancers (Dawson et al., 2011; Delmore et al., 2011; Mertz et al., 2011). Thereafter, their anticancer effects are assessed in preclinical studies on additional solid tumors such as prostatic cancer (PCa) (Asangani et al., 2014), non-small cell lung cancer

(NSCLC), pancreatic cancer (Garcia et al., 2016), together with BC (Ocana et al., 2017). BET inhibitors are only effective on TNBC treated combined with PLK1 inhibitors or chemotherapy or traditional treatment-resistant TNBC (Nieto-Jimenez et al., 2017). Nonetheless, as the aforementioned studies are pre-clinical studies at present, more clinical trials are needed to verify BET inhibitors' effect on treating BC.

Effects of HDACs inhibitors on BC therapy

HDACs can be further classified into four main types according to their sequence homology, namely, class I (HDAC1–3, and HDAC8), class II (HDAC4–7 and HDAC9–10), class III (sirtuin 1–7), and class VI (HDAC11) (Dawson and Kouzarides, 2012). The zinc metal ion is necessary for HDACs of class I/II/IV, so HDAC inhibitors can block the catalytic performance of HDACs by chelating zinc ions. Of diverse HDAC inhibitors, romidepsin and vorinostat have been approved by the FDA for the clinical treatment of cutaneous T-cell lymphoma (Olsen et al., 2007; Piekarz et al., 2009). As HDAC inhibitors display anticancer efficacy in different cancers *in vitro* and *in vivo* (Beckers et al., 2007; Kim et al., 2013b), they can be applied in clinical practice for more tumors such as BC.

According to their different structures, HDACis are classified as four types, namely, cyclic peptides, hydroxamic acids, benzamides, and aliphatic fatty acids (Table 4). At present, three HDACis of the hydroxamic acid type are approved by the FDA, which are vorinostat (SAHA), panobinostat (LBH-589), and belinostat (PXD101).

Such agents present antitumor activity in BC as well. For instance, SAHA can suppress cell growth, EMT, migration, and invasion and induce cell apoptosis, differentiation, autophagy, anoikis, and cell cycle arrest (Lee et al., 2016; Wawruszak et al., 2019; Wawruszak et al., 2021). SAHA remarkably promotes response and suppresses the resistance to tamoxifen (Lee et al., 2012), cisplatin (Wawruszak et al., 2015), olaparib (Min et al., 2015), taxol (Shi et al., 2010), epirubicin (Marchion et al., 2004), docetaxel, and trastuzumab (Bali et al., 2005). Also, SAHA efficiently promotes TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis, and this is achieved by triggering anoikis, increasing CD137 receptor expression, and suppressing Apo2L/TRAIL resistance (Bellarosa et al., 2012; Zhou et al., 2016). Nonetheless, SAHA can enhance TNBC cell metastasis and EMT by suppressing HDAC8, indicating that it should be cautious when treating BC with SAHA, since it might accelerate tumor metastasis (Wu et al., 2016). Meanwhile, a study showed that the combination SAHA and epigallocatechin-3-gallate (EGCG) is effective in inducing apoptosis of breast cancer cells and reducing their migratory capacity (Steed et al., 2020). In addition, Carlisi et al. showed that SAHA synergistically sensitized MDA-MB231 cells to the cytotoxic effect of parthenolide (Carlisi et al., 2015). Belinostat (PXD101) can suppress cell growth and promote cell apoptosis by PKC and Wnt/b-catenin pathways; moreover, applying belinostat in combination with the HSP90 inhibitor (17-AAG) can synergistically exert the anti-tumor effect (Lu et al., 2019; Zuo et al., 2020). Panobinostat (LBH-589) abolishes EMT in TNBC by suppressing ZEB1/2 (Rhodes et al., 2014). There are also additional HDACis of the hydroxamic acid type, including resminostat, abexinostat, and pracinostat, even though they are less tested in BC. In this regard, more research is warranted to examine whether they can be applied in BC. As discovered by Qin et al., panobinostat inhibited TNBC and non-TNBC cell growth, invasion, and migration, while promoting their apoptosis. Likewise, panobinostat suppresses BC proliferation and invasion within mouse models (Qin et al., 2019b). Romidepsin (FK2280), one of the cyclic peptide HDACi, has been approved by the FDA, and it can synergistically inhibit cell proliferation while promoting cell apoptosis when used in combination with decitabine (an inhibitor of methyltransferase) (Cooper et al., 2012). In inflammatory BC, romidepsin exposure can destroy the lymphatic vascular structure and tumor emboli by repressing HIF-1a and VEGF within inflammatory BC. Moreover, romidepsin synergistically suppresses primary tumor proliferation and multiple metastases when used in combination with paclitaxel (Robertson et al., 2013).

Valproic acid (VPA), the HDACi of the aliphatic fatty acid type, has been extensively studied, and it suppresses BC occurrence by upregulating apoptosis pathways and inducing cell cycle arrest (Fortunati et al., 2008; Travaglini et al., 2009). In addition, VPA promotes EMT by increasing ZEB1 and SNAIL levels HDAC2-dependently, but the HDAC2-related mechanism is still unknown (Zhang et al., 2019b). Additionally, VPA synergistically suppresses BC development when applied in combination with anti-tumor agents such as tamoxifen, epirubicin, cisplatin, camptothecin, and capecitabine (Marchion et al., 2005; Fortunati et al., 2008; Arakawa et al., 2009; Terranova-Barberio et al., 2016).

Entinostat (Ent, MS-275), the synthetic benzamide derivative of HDACi, exhibits potent immunomodulation on BC (McCaw et al., 2019; Connolly et al., 2021). In addition, Ent exposure can suppress EMT and the tumor-promoting cell phenotype, thereby inhibiting tumor occurrence and metastasis (Shah et al., 2014). Ent can induce the expression of retinoid acid to improve differentiation mediated by retinoic acid when it is applied together with doxorubicin and all-trans retinoic acid (ATRA); moreover, such a combination further increases doxorubicin-induced cytotoxicity. Moreover, Ent together with ATRA can manage the resistance to the aromatase inhibitor (AI) by decreasing the quantity of tumor-initiating cells (Merino et al., 2016). Additional new multifunctional inhibitors achieve favorable antitumor efficacy.

Sirtuin inhibitors suppress BC development through diverse structures, targets, and activities. In addition, they can deal with

the problem of multidrug resistance through combined use with chemotherapeutics. For instance, amurensin G suppresses SIRT1 and later inhibits MDR1 and FoxO1 levels within the doxorubicin-resistant BC cells, thus potentiating doxorubicin absorption into cells and suppressing oncogenic development (Oh et al., 2010). Splitomicin can decrease cell motility while potentiating paclitaxel's effect on resisting cell motility. Such an effect is intensified by the addition of trichostatin A (TSA), the HDAC6 inhibitor (Bonezzi et al., 2012). Some studies are conducted to evaluate SIRT1/2 inhibitors, including salermide, sirtinol, cambinol, splitomicin, nicotinamide, tenovin, suramin, indole derivatives, and analogs with similar structures. The aforementioned molecules can upregulate p53 acetylation or induce pro-apoptotic, SIRT1-epigenetically silenced gene expression to suppress BC cell growth and trigger p53-dependent apoptosis (Peck et al., 2010). Consequently, different SIRT inhibitors show synergistic effects with conventional antitumor agents in the treatment of BC. There are different pathways related to the drug resistance escape mechanism of SIRTs, indicating that more SIRT inhibitors may be prepared according to the known inhibitors for balancing efficacy and specificity. Phase-I and phase-II clinical studies have been conducted to evaluate the effects of HDAC and DNMT inhibitors on treating BC (Falahi et al., 2014). Epi-drugs exhibit poor anticancer effects on BC, and epi-drug monotherapy can just achieve an effective rate of 10% in BC cases, indicating that monotherapy may not be appropriate for treating BC. Nonetheless, according to the aforementioned clinical trials, when epi-drugs are used in combination with targeted or cytotoxic therapies, such as ER-targeted therapy, the OS and PFS are improved (Falahi et al., 2014). Consequently, the existing clinical trials mostly apply epi-drugs in combination with traditional treatments.

Epi-drugs in clinical practice

These encouraging preclinical findings have laid the sound basis to translate epi-drugs to clinical trials for treating BC. Table 4 summarizes epi-drug-related clinical trials for the treatment of BC (mostly from https://www.clinicaltrials.gov/). Many accomplished (NCT00262834, NCT00777049, and NCT01171924) along with ongoing (NCT00416130, NCT01638533, and NCT04676516) clinical trials have been conducted to predict the safety, pharmacodynamics, and pharmacokinetics of epi-drugs for determining the best doses and monotherapy schemes. It is to be noted that the tolerance of 300/400 mg oral SAHA (twice/day for a 14-day period, separated by a 7-day rest) has been verified (Vansteenkiste et al., 2008). However, the present work just enrolled two BC cases, making it impossible to accurately determine the response rate. More clinical trials are being conducted to predict the best SAHA dose. Apart from monotherapy, epi-drugs have been frequently utilized in combination with other drugs in clinics. For instance, one phase-II trial applied SAHA plus tamoxifen in treating BC resistant to hormone therapy, and the objective response rate (ORR) and clinical benefit rate were determined to be 19% and 40%, respectively (Munster et al., 2011). Moreover, the combined application of Ent and exemestane increased the OS from 19. 8 months (as obtained after exemestane monotherapy) to 28. 1 months (Yardley et al., 2013). One recent phase-II clinical trial applied SAHA in combination with tamoxifen and pembrolizumab in improving the response to immunotherapy among ER+ BC cases. The treatment strategy achieved an ORR and clinical benefit rate of 4% and 19%, respectively (Terranova-Barberio et al., 2020). Typically, as revealed by an ongoing phase-II trial (NCT04190056), the aforementioned combination strategy can trigger an immune response for treating ER+ BC, while reducing the dose and adverse reactions. BETis and SAHA synergistically treat BC with olaparib in preclinical trials (Min et al., 2015; Yang et al., 2017). Additionally, there are two ongoing trials (NCT03901469 and NCT03742245) analyzing whether epi-drugs plus PARPis are effective and safe by suppressing DNA damage repair. Taken together, these clinical trials further verify the effectiveness of epi-drugs in treating BC, which should be further investigated.

Limitation and prospects of epi-drugs in breast cancer

In this study, we reviewed histone modifications and their functions and potential cellular interactions, which might result in the development of potential efficient therapies with KATi and HDACi. Several synthetic compounds currently in pre-clinical studies have exhibited potent KATi activities against breast malignant cells. Moreover, they can effectively augment the anti-cancer activities of standard chemotherapeutic agents such as paclitaxel, doxorubicin, and cisplatin and sensitize drug-resistant cells to radiation therapy. Nevertheless, as shown, KATi and HDACi seem to be a promising group of anti-cancer drugs, particularly in combination with other anti-cancer drugs and/or radiotherapy. More large-scale promising evidence needs to be obtained from multi-center clinical trials. Meanwhile, their use in combination with other drugs and the schedule of such drug combinations need to be further investigated in both preclinical and clinical studies.

Currently, selectivity is one of the biggest challenges in developing drugs targeting epigenetic modifiers. Most currently developed drugs do not show selectivity to certain enzymes; instead, they target molecules that have certain common functions and structures. But epigenetic agents are most advantageous in their good tolerance and low severe adverse reaction rate, even though there are certain concerns about the safety of certain medicines (Cheng et al., 2019). Additionally, more reports indicate that the response rates are poor after short-term treatment, and resistance is developed in the end, which can be attributed to the transcriptional plasticity driven by epigenetics responding to environmental stress (Dawson, 2017). More multicenter and randomized phase-III studies should be

conducted to realize the full potential and specificity of HDACis therapy in various subtypes of breast cancer. Further clinical studies should include the most promising novel HDACi and isozyme-specific inhibitors.

Conclusion

The present work focuses on summarizing relevant studies on HMs related to BC and the possible mechanisms associated with abnormal HMs. Additionally, we also aim to analyze existing therapeutic agents together with those drugs approved and tested through pre-clinical and clinical trials, to assess their roles in HMs. Moreover, epi-drugs that targeted HMT inhibitors and HDAC inhibitors should be tested in preclinical and clinical studies for the treatment of BC. Epi-drugs that target histone methylation (HMT inhibitors) and histone deacetylation (HDAC inhibitors) are now under clinical trials or approved by the US Food and Drug Administration (FDA). Therefore, the review covers the difficulties in applying HM-targeting treatments in clinical applications and proposes feasible approaches for overcoming such difficulties and promoting their use in treating BC cases. Indeed, the full clinical therapeutic scope and commercial value of such agents in the field of oncology are only just emerging.

Author contributions

JF researched data for the manuscript, wrote the manuscript, contributed to the discussion of the content, and reviewed the manuscript before the submission. XM retrieved and organized documents, modified the manuscript, and contributed to subject planning. All authors read and approved the final version of the manuscript.

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

Asangani, I. A., Dommeti, V. L., Wang, X., Malik, R., Cieslik, M., Yang, R., et al. (2014). Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer. *Nature* 510 (7504), 278–282. doi:10.1038/nature13229

Ahmad, A., Sarkar, S. H., Bitar, B., Ali, S., Aboukameel, A., Sethi, S., et al. (2012). Garcinol regulates EMT and Wnt signaling pathways in vitro and in vivo, leading to anticancer activity against breast cancer cells. *Mol. Cancer Ther.* 11 (10), 2193–2201. doi:10.1158/1535-7163.MCT-12-0232-T

Al Sarakbi, W., Sasi, W., Jiang, W. G., Roberts, T., Newbold, R. F., and Mokbel, K. (2009). The mRNA expression of SETD2 in human breast cancer: Correlation with clinico-pathological parameters. *BMC Cancer* 9, 290. doi:10.1186/1471-2407-9-290

Alsamri, H., Hasasna, H. E., Baby, B., Alneyadi, A., Dhaheri, Y. A., Ayoub, M. A., et al. (2021). Carnosol is a novel inhibitor of p300 acetyltransferase in breast cancer. *Front. Oncol.* 11, 664403. doi:10.3389/fonc.2021.664403

Altaf, M., Utley, R. T., Lacoste, N., Tan, S., Briggs, S. D., and Cote, J. (2007). Interplay of chromatin modifiers on a short basic patch of histone H4 tail defines the boundary of telomeric heterochromatin. *Mol. Cell* 28 (6), 1002–1014. doi:10.1016/j.molcel.2007.12.002

Arakawa, Y., Saito, S., Yamada, H., and Aiba, K. (2009). Simultaneous treatment with camptothecin and valproic acid suppresses induction of Bcl-X(L) and promotes apoptosis of MCF-7 breast cancer cells. *Apoptosis* 14 (9), 1076–1085. doi:10.1007/s10495-009-0384-0

Arif, M., Pradhan, S. K., Thanuja, G. R., Vedamurthy, B. M., Agrawal, S., Dasgupta, D., et al. (2009). Mechanism of p300 specific histone acetyltransferase inhibition by small molecules. *J. Med. Chem.* 52 (2), 267–277. doi:10.1021/jm800657z

Atanassov, B. S., Mohan, R. D., Lan, X., Kuang, X., Lu, Y., Lin, K., et al. (2016). ATXN7L3 and ENY2 Coordinate activity of multiple H2B Deubiquitinases important for cellular proliferation and tumor growth. *Mol. Cell* 62 (4), 558–571. doi:10.1016/j.molcel.2016.03.030

Balasubramanyam, K., Altaf, M., Varier, R. A., Swaminathan, V., Ravindran, A., Sadhale, P. P., et al. (2004). Polyisoprenylated benzophenone, garcinol, a natural histone acetyltransferase inhibitor, represses chromatin transcription and alters global gene expression. *J. Biol. Chem.* 279 (32), 33716–33726. doi:10.1074/jbc.M402839200

Balasubramanyam, K., Swaminathan, V., Ranganathan, A., and Kundu, T. K. (2003). Small molecule modulators of histone acetyltransferase p300. *J. Biol. Chem.* 278 (21), 19134–19140. doi:10.1074/jbc.M301580200

Bali, P., Pranpat, M., Swaby, R., Fiskus, W., Yamaguchi, H., Balasis, M., et al. (2005). Activity of suberoylanilide hydroxamic Acid against human breast cancer cells with amplification of her-2. *Clin. Cancer Res.* 11 (17), 6382–6389. doi:10.1158/1078-0432.CCR-05-0344

Bandyopadhyay, K., Baneres, J. L., Martin, A., Blonski, C., Parello, J., and Gjerset, R. A. (2009). Spermidinyl-CoA-based HAT inhibitors block DNA repair and provide cancer-specific chemo- and radiosensitization. *Cell Cycle* 8 (17), 2779–2788. doi:10.4161/cc.8.17.9416

Bannister, A. J., Zegerman, P., Partridge, J. F., Miska, E. A., Thomas, J. O., Allshire, R. C., et al. (2001). Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* 410 (6824), 120–124. doi:10.1038/35065138

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

Beckers, T., Burkhardt, C., Wieland, H., Gimmnich, P., Ciossek, T., Maier, T., et al. (2007). Distinct pharmacological properties of second generation HDAC inhibitors with the benzamide or hydroxamate head group. *Int. J. Cancer* 121 (5), 1138–1148. doi:10.1002/ijc.22751

Bellarosa, D., Bressan, A., Bigioni, M., Parlani, M., Maggi, C. A., and Binaschi, M. (2012). SAHA/Vorinostat induces the expression of the CD137 receptor/ligand system and enhances apoptosis mediated by soluble CD137 receptor in a human breast cancer cell line. *Int. J. Oncol.* 41 (4), 1486–1494. doi:10.3892/ijo.2012.1551

Berger, S. L. (2007). The complex language of chromatin regulation during transcription. *Nature* 447 (7143), 407–412. doi:10.1038/nature05915

Boccuni, P., MacGrogan, D., Scandura, J. M., and Nimer, S. D. (2003). The human L(3)MBT polycomb group protein is a transcriptional repressor and interacts physically and functionally with TEL (ETV6). *J. Biol. Chem.* 278 (17), 15412–15420. doi:10.1074/jbc.M300592200

Bonezzi, K., Belotti, D., North, B. J., Ghilardi, C., Borsotti, P., Resovi, A., et al. (2012). Inhibition of SIRT2 potentiates the anti-motility activity of taxanes: Implications for antineoplastic combination therapies. *Neoplasia* 14 (9), 846–854. doi:10.1593/neo.12728

Borkin, D., He, S., Miao, H., Kempinska, K., Pollock, J., Chase, J., et al. (2015). Pharmacologic inhibition of the Menin-MLL interaction blocks progression of MLL leukemia in vivo. *Cancer Cell* 27 (4), 589–602. doi:10.1016/j.ccell.2015.02.016

Bowers, E. M., Yan, G., Mukherjee, C., Orry, A., Wang, L., Holbert, M. A., et al. (2010). Virtual ligand screening of the p300/CBP histone acetyltransferase: Identification of a selective small molecule inhibitor. *Chem. Biol.* 17 (5), 471–482. doi:10.1016/j.chembiol.2010.03.006

Brown, J. A., Bourke, E., Eriksson, L. A., and Kerin, M. J. (2016). Targeting cancer using KAT inhibitors to mimic lethal knockouts. *Biochem. Soc. Trans.* 44 (4), 979–986. doi:10.1042/BST20160081

Byler, S., Goldgar, S., Heerboth, S., Leary, M., Housman, G., Moulton, K., et al. (2014). Genetic and epigenetic aspects of breast cancer progression and therapy. *Anticancer Res.* 34 (3), 1071–1077.

Cao, L., Zhu, L., Yang, J., Su, J., Ni, J., Du, Y., et al. (2014). Correlation of low expression of hMOF with clinicopathological features of colorectal carcinoma, gastric cancer and renal cell carcinoma. *Int. J. Oncol.* 44 (4), 1207–1214. doi:10.3892/ijo.2014.2266

Cao, Q., Yu, J., Dhanasekaran, S. M., Kim, J. H., Mani, R. S., Tomlins, S. A., et al. (2008). Repression of E-cadherin by the polycomb group protein EZH2 in cancer. *Oncogene* 27 (58), 7274–7284. doi:10.1038/onc.2008.333

Caplan, L. (2014). Delay in breast cancer: Implications for stage at diagnosis and survival. *Front. Public Health* 2, 87. doi:10.3389/fpubh.2014.00087

Carlisi, D., Lauricella, M., D'Anneo, A., Buttitta, G., Emanuele, S., di Fiore, R., et al. (2015). The synergistic effect of SAHA and parthenolide in MDA-MB231 breast cancer cells. *J. Cell. Physiol.* 230 (6), 1276–1289. doi:10.1002/jcp.24863

Casciello, F., Al-Ejeh, F., Kelly, G., Brennan, D. J., Ngiow, S. F., Young, A., et al. (2017). G9a drives hypoxia-mediated gene repression for breast cancer cell survival and tumorigenesis. *Proc. Natl. Acad. Sci. U. S. A.* 114 (27), 7077–7082. doi:10.1073/pnas.1618706114

Casciello, F., Al-Ejeh, F., Miranda, M., Kelly, G., Baxter, E., Windloch, K., et al. (2020). G9a-mediated repression of CDH10 in hypoxia enhances breast tumour cell motility and associates with poor survival outcome. *Theranostics* 10 (10), 4515–4529. doi:10.7150/thno.41453

Cebrat, M., Kim, C. M., Thompson, P. R., Daugherty, M., and Cole, P. A. (2003). Synthesis and analysis of potential prodrugs of coenzyme A analogues for the inhibition of the histone acetyltransferase p300. *Bioorg. Med. Chem.* 11 (15), 3307–3313. doi:10.1016/s0968-0896(03)00265-7

Chang, C. C., Wu, M. J., Yang, J. Y., Camarillo, I. G., and Chang, C. J. (2015). Leptin-STAT3-G9a signaling promotes Obesity-mediated breast cancer progression. *Cancer Res.* 75 (11), 2375–2386. doi:10.1158/0008-5472.CAN-14-3076

Chang, C. J., Yang, J. Y., Xia, W., Chen, C. T., Xie, X., Chao, C. H., et al. (2011). EZH2 promotes expansion of breast tumor initiating cells through activation of RAF1-beta-catenin signaling. *Cancer Cell* 19 (1), 86–100. doi:10.1016/j.ccr.2010.10.035

Cheng, Y., He, C., Wang, M., Ma, X., Mo, F., Yang, S., et al. (2019). Targeting epigenetic regulators for cancer therapy: Mechanisms and advances in clinical trials. *Signal Transduct. Target. Ther.* 4, 62. doi:10.1038/s41392-019-0095-0

Chern, T. R., Liu, L., Petrunak, E., Stuckey, J. A., Wang, M., Bernard, D., et al. (2020). Discovery of potent small-molecule inhibitors of MLL methyltransferase. *ACS Med. Chem. Lett.* 11 (6), 1348–1352. doi:10.1021/acsmedchemlett.0c00229

Chien, Y. C., Liu, L. C., Ye, H. Y., Wu, J. Y., and Yu, Y. L. (2018). EZH2 promotes migration and invasion of triple-negative breast cancer cells via regulating TIMP2-MMP-2/-9 pathway. *Am. J. Cancer Res.* 8 (3), 422–434.

Chlebowski, R. T., and Anderson, G. L. (2012). Changing concepts: Menopausal hormone therapy and breast cancer. *J. Natl. Cancer Inst.* 104 (7), 517–527. doi:10.1093/jnci/djs014

Cho, M. H., Park, J. H., Choi, H. J., Park, M. K., Won, H. Y., Park, Y. J., et al. (2015). DOT1L cooperates with the c-Myc-p300 complex to epigenetically derepress CDH1 transcription factors in breast cancer progression. *Nat. Commun.* 6, 7821. doi:10.1038/ncomms8821

Cloos, P. A., Christensen, J., Agger, K., Maiolica, A., Rappsilber, J., Antal, T., et al. (2006). The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. *Nature* 442 (7100), 307–311. doi:10.1038/nature04837

Cole, A. J., Clifton-Bligh, R., and Marsh, D. J. (2015). Histone H2B monoubiquitination: Roles to play in human malignancy. *Endocr. Relat. Cancer* 22 (1), T19–T33. doi:10.1530/ERC-14-0185

Cole, P. A. (2008). Chemical probes for histone-modifying enzymes. *Nat. Chem. Biol.* 4 (10), 590–597. doi:10.1038/nchembio.111

Connolly, R. M., Zhao, F., Miller, K. D., Lee, M. J., Piekarz, R. L., Smith, K. L., et al. (2021). E2112: Randomized phase III trial of endocrine therapy plus entinostat or placebo in hormone receptor-positive advanced breast cancer. A trial of the ECOG-ACRIN cancer research group. *J. Clin. Oncol.* 39 (28), 3171–3181. doi:10.1200/JCO.21.00944

Cooper, S. J., von Roemeling, C. A., Kang, K. H., Marlow, L. A., Grebe, S. K., Menefee, M. E., et al. (2012). Reexpression of tumor suppressor, sFRP1, leads to antitumor synergy of combined HDAC and methyltransferase inhibitors in chemoresistant cancers. *Mol. Cancer Ther.* 11 (10), 2105–2115. doi:10.1158/1535-7163.MCT-11-0873

Cowan, L. A., Talwar, S., and Yang, A. S. (2010). Will DNA methylation inhibitors work in solid tumors? A review of the clinical experience with azacitidine and decitabine in solid tumors. *Epigenomics* 2 (1), 71–86. doi:10.2217/epi.09.44

Coward, W. R., Brand, O. J., Pasini, A., Jenkins, G., Knox, A. J., and Pang, L. (2018). Interplay between EZH2 and G9a regulates CXCL10 gene repression in Idiopathic Pulmonary Fibrosis. *Am. J. Respir. Cell Mol. Biol.* 58 (4), 449–460. doi:10.1165/rcmb.2017-0286OC

Crawford, N. T., McIntyre, A. J., McCormick, A., D'Costa, Z. C., Buckley, N. E., and Mullan, P. B. (2019). TBX2 interacts with heterochromatin protein 1 to recruit a novel repression complex to EGR1-targeted promoters to drive the proliferation of breast cancer cells. *Oncogene* 38 (31), 5971–5986. doi:10.1038/s41388-019-0853-z

Creyghton, M. P., Cheng, A. W., Welstead, G. G., Kooistra, T., Carey, B. W., Steine, E. J., et al. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc. Natl. Acad. Sci. U. S. A.* 107 (50), 21931–21936. doi:10.1073/pnas.1016071107

Cullis, P. M., Wolfenden, R., Cousens, L. S., and Alberts, B. M. (1982). Inhibition of histone acetylation by N-[2-(S-coenzyme A)acetyl] spermidine amide, a multisubstrate analog. *J. Biol. Chem.* 257 (20), 12165–12169. doi:10.1016/s0021-9258(18)33695-0

Dawson, M. A., and Kouzarides, T. (2012). Cancer epigenetics: From mechanism to therapy. *Cell* 150 (1), 12–27. doi:10.1016/j.cell.2012.06.013

Dawson, M. A., Prinjha, R. K., Dittmann, A., Giotopoulos, G., Bantscheff, M., Chan, W. I., et al. (2011). Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature* 478 (7370), 529–533. doi:10.1038/nature10509

Dawson, M. A. (2017). The cancer epigenome: Concepts, challenges, and therapeutic opportunities. *Science* 355 (6330), 1147–1152. doi:10.1126/science.aam7304

Deb, P., Bhan, A., Hussain, I., Ansari, K. I., Bobzean, S. A., Pandita, T. K., et al. (2016). Endocrine disrupting chemical, bisphenol-A, induces breast cancer associated gene HOXB9 expression in vitro and in vivo. *Gene* 590 (2), 234–243. doi:10.1016/j.gene.2016.05.009

Delmore, J. E., Issa, G. C., Lemieux, M. E., Rahl, P. B., Shi, J., Jacobs, H. M., et al. (2011). BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 146 (6), 904–917. doi:10.1016/j.cell.2011.08.017

Dhayalan, A., Rajavelu, A., Rathert, P., Tamas, R., Jurkowska, R. Z., Ragozin, S., et al. (2010). The Dnmt3a PWWP domain reads histone 3 lysine 36 trimethylation and guides DNA methylation. *J. Biol. Chem.* 285 (34), 26114–26120. doi:10.1074/jbc.M109.089433

Di Costanzo, A., Del Gaudio, N., Migliaccio, A., and Altucci, L. (2014). Epigenetic drugs against cancer: An evolving landscape. *Arch. Toxicol.* 88 (9), 1651–1668. doi:10.1007/s00204-014-1315-6

Dillon, S. C., Zhang, X., Trievel, R. C., and Cheng, X. (2005). The SET-domain protein superfamily: Protein lysine methyltransferases. *Genome Biol.* 6 (8), 227. doi:10.1186/gb-2005-6-8-227

Dong, C., Wu, Y., Wang, Y., Wang, C., Kang, T., Rychahou, P. G., et al. (2013). Interaction with Suv39H1 is critical for Snail-mediated E-cadherin repression in breast cancer. *Oncogene* 32 (11), 1351–1362. doi:10.1038/onc.2012.169

Dong, C., Yuan, T., Wu, Y., Wang, Y., Fan, T. W., Miriyala, S., et al. (2013). Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. *Cancer Cell* 23 (3), 316–331. doi:10.1016/j.ccr.2013.01.022

Dong, H. T., Liu, Q., Zhao, T., Yao, F., Xu, Y., Chen, B., et al. (2020). Long non-coding RNA LOXL1-AS1 drives breast cancer invasion and metastasis by Antagonizing miR-708-5p expression and activity. *Mol. Ther. Nucleic Acids* 19, 696–705. doi:10.1016/j.omtn.2019.12.016

Dou, D., Ge, X., Wang, X., Xu, X., Zhang, Z., Seng, J., et al. (2019). EZH2 contributes to cisplatin resistance in breast cancer by epigenetically suppressing miR-381 expression. *Onco. Targets. Ther.* 12, 9627–9637. doi:10.2147/OTT.S214104

Du, D., Katsuno, Y., Meyer, D., Budi, E. H., Chen, S. H., Koeppen, H., et al. (2018). Smad3-mediated recruitment of the methyltransferase SETDB1/ESET controls Snail1 expression and epithelial-mesenchymal transition. *EMBO Rep.* 19 (1), 135–155. doi:10.15252/embr.201744250

Du, J., Li, L., Ou, Z., Kong, C., Zhang, Y., Dong, Z., et al. (2012). FOXC1, a target of polycomb, inhibits metastasis of breast cancer cells. *Breast Cancer Res. Treat.* 131 (1), 65–73. doi:10.1007/s10549-011-1396-3

Duong, M. T., Akli, S., Macalou, S., Biernacka, A., Debeb, B. G., Yi, M., et al. (2013). Hbo1 is a cyclin E/CDK2 substrate that enriches breast cancer stem-like cells. *Cancer Res.* 73 (17), 5556–5568. doi:10.1158/0008-5472.CAN-13-0013

Eliseeva, E. D., Valkov, V., Jung, M., and Jung, M. O. (2007). Characterization of novel inhibitors of histone acetyltransferases. *Mol. Cancer Ther.* 6 (9), 2391–2398. doi:10.1158/1535-7163.MCT-07-0159

Eram, M. S., Bustos, S. P., Lima-Fernandes, E., Siarheyeva, A., Senisterra, G., Hajian, T., et al. (2014). Trimethylation of histone H3 lysine 36 by human methyltransferase PRDM9 protein. *J. Biol. Chem.* 289 (17), 12177–12188. doi:10.1074/jbc.M113.523183

Falahi, F., van Kruchten, M., Martinet, N., Hospers, G. A., and Rots, M. G. (2014). Current and upcoming approaches to exploit the reversibility of epigenetic mutations in breast cancer. *Breast Cancer Res.* 16 (4), 412. doi:10.1186/s13058-014-0412-z

Fan, L., Strasser-Weippl, K., Li, J. J., St Louis, J., Finkelstein, D. M., Yu, K. D., et al. (2014). Breast cancer in China. *Lancet. Oncol.* 15 (7), e279–89. doi:10.1016/S1470-2045(13)70567-9

Fang, R., Barbera, A. J., Xu, Y., Rutenberg, M., Leonor, T., Bi, Q., et al. (2010). Human LSD2/KDM1b/AOF1 regulates gene transcription by modulating intragenic H3K4me2 methylation. *Mol. Cell* 39 (2), 222–233. doi:10.1016/j.molcel.2010.07.008

Fang, Y., Liao, G., and Yu, B. (2019). LSD1/KDM1A inhibitors in clinical trials: Advances and prospects. *J. Hematol. Oncol.* 12 (1), 129. doi:10.1186/s13045-019-0811-9

Fenizia, C., Bottino, C., Corbetta, S., Fittipaldi, R., Floris, P., Gaudenzi, G., et al. (2019). SMYD3 promotes the epithelial-mesenchymal transition in breast cancer. *Nucleic Acids Res.* 47 (3), 1278–1293. doi:10.1093/nar/gky1221

Filippakopoulos, P., Qi, J., Picaud, S., Shen, Y., Smith, W. B., Fedorov, O., et al. (2010). Selective inhibition of BET bromodomains. *Nature* 468 (7327), 1067–1073. doi:10.1038/nature09504

Fiorentino, F., Mai, A., and Rotili, D. (2018). Lysine acetyltransferase inhibitors: Structure-activity relationships and potential therapeutic implications. *Future Med. Chem.* 10 (9), 1067–1091. doi:10.4155/fmc-2017-0244

Fortunati, N., Bertino, S., Costantino, L., Bosco, O., Vercellinatto, I., Catalano, M. G., et al. (2008). Valproic acid is a selective antiproliferative agent in estrogen-sensitive breast cancer cells. *Cancer Lett.* 259 (2), 156–164. doi:10.1016/j.canlet.2007.10.006

Fujii, S., Ito, K., Ito, Y., and Ochiai, A. (2008). Enhancer of zeste homologue 2 (EZH2) down-regulates RUNX3 by increasing histone H3 methylation. *J. Biol. Chem.* 283 (25), 17324–17332. doi:10.1074/jbc.M800224200

Gala, K., Li, Q., Sinha, A., Razavi, P., Dorso, M., Sanchez-Vega, F., et al. (2018). KMT2C mediates the estrogen dependence of breast cancer through regulation of ERα enhancer function. *Oncogene* 37 (34), 4692–4710. doi:10.1038/s41388-018-0273-5

Garcia, P. L., Miller, A. L., Kreitzburg, K. M., Council, L. N., Gamblin, T. L., Christein, J. D., et al. (2016). The BET bromodomain inhibitor JQ1 suppresses growth of pancreatic ductal adenocarcinoma in patient-derived xenograft models. *Oncogene* 35 (7), 833–845. doi:10.1038/onc.2015.126

Ghizzoni, M., Wu, J., Gao, T., Haisma, H. J., Dekker, F. J., and George Zheng, Y. (2012). 6-alkylsalicylates are selective Tip60 inhibitors and target the acetyl-CoA binding site. *Eur. J. Med. Chem.* 47 (1), 337–344. doi:10.1016/j.ejmech.2011.11.001

Gong, C., Yao, S., Gomes, A. R., Man, E. P., Lee, H. J., Gong, G., et al. (2016). BRCA1 positively regulates FOXO3 expression by restricting FOXO3 gene methylation and epigenetic silencing through targeting EZH2 in breast cancer. *Oncogenesis* 5, e214. doi:10.1038/oncsis.2016.23

Gupta, S. C., Patchva, S., and Aggarwal, B. B. (2013). Therapeutic roles of curcumin: Lessons learned from clinical trials. *AAPS J.* 15 (1), 195–218. doi:10.1208/s12248-012-9432-8

Hamamoto, R., Silva, F. P., Tsuge, M., Nishidate, T., Katagiri, T., Nakamura, Y., et al. (2006). Enhanced SMYD3 expression is essential for the growth of breast cancer cells. *Cancer Sci.* 97 (2), 113–118. doi:10.1111/j.1349-7006.2006.00146.x

Han, Y., Tanios, F., Reeps, C., Zhang, J., Schwamborn, K., Eckstein, H. H., et al. (2016). Histone acetylation and histone acetyltransferases show significant alterations in human abdominal aortic aneurysm. *Clin. Epigenetics* 8, 3. doi:10.1186/s13148-016-0169-6

Healey, M. A., Hu, R., Beck, A. H., Collins, L. C., Schnitt, S. J., Tamimi, R. M., et al. (2014). Association of H3K9me3 and H3K27me3 repressive histone marks with breast cancer subtypes in the Nurses' Health Study. *Breast Cancer Res. Treat.* 147 (3), 639–651. doi:10.1007/s10549-014-3089-1

Heintzman, N. D., Stuart, R. K., Hon, G., Fu, Y., Ching, C. W., Hawkins, R. D., et al. (2007). Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat. Genet.* 39 (3), 311–318. doi:10.1038/ng1966

Hemshekhar, M., Sebastin Santhosh, M., Kemparaju, K., and Girish, K. S. (2012). Emerging roles of anacardic acid and its derivatives: A pharmacological overview. *Basic Clin. Pharmacol. Toxicol.* 110 (2), 122–132. doi:10.1111/j.1742-7843.2011.00833.x

Hiatt, R. A., Sibley, A., Venkatesh, B., Cheng, J., Dixit, N., Fox, R., et al. (2022). From cancer Epidemiology to policy and practice: The role of a comprehensive cancer center. *Curr. Epidemiol. Rep.* 9, 10–21. doi:10.1007/s40471-021-00280-7

Holm, K., Grabau, D., Lovgren, K., Aradottir, S., Gruvberger-Saal, S., Howlin, J., et al. (2012). Global H3K27 trimethylation and EZH2 abundance in breast tumor subtypes. *Mol. Oncol.* 6 (5), 494–506. doi:10.1016/j.molonc.2012.06.002

Hu, C., Li, M., Guo, T., Wang, S., Huang, W., Yang, K., et al. (2019). Anti-metastasis activity of curcumin against breast cancer via the inhibition of stem cell-like properties and EMT. *Phytomedicine* 58, 152740. doi:10.1016/j.phymed.2018.11.001

Hu, X., Xiang, D., Xie, Y., Tao, L., Zhang, Y., Jin, Y., et al. (2019). LSD1 suppresses invasion, migration and metastasis of luminal breast cancer cells via activation of GATA3 and repression of TRIM37 expression. *Oncogene* 38 (44), 7017–7034. doi:10.1038/s41388-019-0923-2

Huang, M., Huang, J., Zheng, Y., and Sun, Q. (2019). Histone acetyltransferase inhibitors: An overview in synthesis, structure-activity relationship and molecular mechanism. *Eur. J. Med. Chem.* 178, 259–286. doi:10.1016/j.ejmech.2019.05.078

Huyen, Y., Zgheib, O., Ditullio, R. A., Jr., Gorgoulis, V. G., Zacharatos, P., Petty, T. J., et al. (2004). Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. *Nature* 432 (7015), 406–411. doi:10.1038/nature03114

Iizuka, M., Matsui, T., Takisawa, H., and Smith, M. M. (2006). Regulation of replication licensing by acetyltransferase Hbo1. *Mol. Cell. Biol.* 26 (3), 1098–1108. doi:10.1128/MCB.26.3.1098-1108.2006

Iizuka, M., Sarmento, O. F., Sekiya, T., Scrable, H., Allis, C. D., and Smith, M. M. (2008). Hbo1 Links p53-dependent stress signaling to DNA replication licensing. *Mol. Cell. Biol.* 28 (1), 140–153. doi:10.1128/MCB.00662-07

Iizuka, M., Takahashi, Y., Mizzen, C. A., Cook, R. G., Fujita, M., Allis, C. D., et al. (2009). Histone acetyltransferase Hbo1: Catalytic activity, cellular abundance, and links to primary cancers. *Gene* 436 (1-2), 108–114. doi:10.1016/j.gene.2009.01.020

Jenuwein, T., and Allis, C. D. (2001). Translating the histone code. *Science* 293 (5532), 1074–1080. doi:10.1126/science.1063127

Jeong, G. Y., Park, M. K., Choi, H. J., An, H. W., Park, Y. U., Choi, H. J., et al. (2021). NSD3-Induced methylation of H3K36 activates NOTCH signaling to drive breast tumor initiation and metastatic progression. *Cancer Res.* 81 (1), 77–90. doi:10.1158/0008-5472.CAN-20-0360

Jeong, K. W., Andreu-Vieyra, C., You, J. S., Jones, P. A., and Stallcup, M. R. (2014). Establishment of active chromatin structure at enhancer elements by mixed-lineage leukemia 1 to initiate estrogen-dependent gene expression. *Nucleic Acids Res.* 42 (4), 2245–2256. doi:10.1093/nar/gkt1236

Jeong, K. W., Kim, K., Situ, A. J., Ulmer, T. S., An, W., and Stallcup, M. R. (2011). Recognition of enhancer element-specific histone methylation by TIP60 in transcriptional activation. *Nat. Struct. Mol. Biol.* 18 (12), 1358–1365. doi:10.1038/nsmb.2153

Jin, M. L., Kim, Y. W., Jin, H. L., Kang, H., Lee, E. K., Stallcup, M. R., et al. (2018). Aberrant expression of SETD1A promotes survival and migration of estrogen receptor alpha-positive breast cancer cells. *Int. J. Cancer* 143 (11), 2871–2883. doi:10.1002/ijc.31853

Jin, Y., Park, S., Park, S. Y., Lee, C. Y., Eum, D. Y., Shim, J. W., et al. (2022). G9a knockdown suppresses cancer aggressiveness by facilitating Smad protein phosphorylation through increasing BMP5 expression in luminal A type breast cancer. *Int. J. Mol. Sci.* 23 (2), 589. doi:10.3390/ijms23020589

Jones, B., Su, H., Bhat, A., Lei, H., Bajko, J., Hevi, S., et al. (2008). The histone H3K79 methyltransferase Dot1L is essential for mammalian development and heterochromatin structure. *PLoS Genet.* 4 (9), e1000190. doi:10.1371/journal.pgen.1000190

Jorgensen, S., Schotta, G., and Sorensen, C. S. (2013). Histone H4 lysine 20 methylation: Key player in epigenetic regulation of genomic integrity. *Nucleic Acids Res.* 41 (5), 2797–2806. doi:10.1093/nar/gkt012

Jozwik, K. M., Chernukhin, I., Serandour, A. A., Nagarajan, S., and Carroll, J. S. (2016). FOXA1 directs H3K4 monomethylation at enhancers via recruitment of the methyltransferase MLL3. *Cell Rep.* 17 (10), 2715–2723. doi:10.1016/j.celrep.2016.11.028

Karthik, I. P., Desai, P., Sukumar, S., Dimitrijevic, A., Rajalingam, K., and Mahalingam, S. (2018). E4BP4/NFIL3 modulates the epigenetically repressed

RAS effector RASSF8 function through histone methyltransferases. *J. Biol. Chem.* 293 (15), 5624–5635. doi:10.1074/jbc.RA117.000623

Kato, I., Kasukabe, T., and Kumakura, S. (2020). MeninMLL inhibitors induce ferroptosis and enhance the antiproliferative activity of auranofin in several types of cancer cells. *Int. J. Oncol.* 57 (4), 1057–1071. doi:10.3892/ijo.2020.5116

Kaypee, S., Sudarshan, D., Shanmugam, M. K., Mukherjee, D., Sethi, G., and Kundu, T. K. (2016). Aberrant lysine acetylation in tumorigenesis: Implications in the development of therapeutics. *Pharmacol. Ther.* 162, 98–119. doi:10.1016/j.pharmthera.2016.01.011

Kim, J., Shin, Y., Lee, S., Kim, M., Punj, V., Lu, J. F., et al. (2018). Regulation of breast cancer-induced Osteoclastogenesis by MacroH2A1.2 involving EZH2-mediated H3K27me3. *Cell Rep.* 24 (1), 224–237. doi:10.1016/j.celrep.2018.06.020

Kim, J. H., Sharma, A., Dhar, S. S., Lee, S. H., Gu, B., Chan, C. H., et al. (2014). UTX and MLL4 coordinately regulate transcriptional programs for cell proliferation and invasiveness in breast cancer cells. *Cancer Res.* 74 (6), 1705–1717. doi:10.1158/0008-5472.CAN-13-1896

Kim, M. Y., Woo, E. M., Chong, Y. T., Homenko, D. R., and Kraus, W. L. (2006). Acetylation of estrogen receptor alpha by p300 at lysines 266 and 268 enhances the deoxyribonucleic acid binding and transactivation activities of the receptor. *Mol. Endocrinol.* 20 (7), 1479–1493. doi:10.1210/me.2005-0531

Kim, S. S., Lee, M. H., and Lee, M. O. (2020). Histone methyltransferases regulate the transcriptional expression of ERα and the proliferation of tamoxifen-resistant breast cancer cells. *Breast Cancer Res. Treat.* 180 (1), 45–54. doi:10.1007/s10549-019-05517-0

Kim, T., and Buratowski, S. (2009). Dimethylation of H3K4 by Set1 recruits the Set3 histone deacetylase complex to 5' transcribed regions. *Cell* 137 (2), 259–272. doi:10.1016/j.cell.2009.02.045

Kim, Y., Kim, Y. S., Kim, D. E., Lee, J. S., Song, J. H., Kim, H. G., et al. (2013). BIX-01294 induces autophagy-associated cell death via EHMT2/G9a dysfunction and intracellular reactive oxygen species production. *Autophagy* 9 (12), 2126–2139. doi:10.4161/auto.26308

Kim, Y. J., Greer, C. B., Cecchini, K. R., Harris, L. N., Tuck, D. P., and Kim, T. H. (2013). HDAC inhibitors induce transcriptional repression of high copy number genes in breast cancer through elongation blockade. *Oncogene* 32 (23), 2828–2835. doi:10.1038/onc.2013.32

Klose, R. J., Kallin, E. M., and Zhang, Y. (2006). JmjC-domain-containing proteins and histone demethylation. *Nat. Rev. Genet.* 7 (9), 715–727. doi:10.1038/nrg1945

Krishna, S., Kumar, S., Singh, D. K., Lakra, A. D., Banerjee, D., and Siddiqi, M. I. (2018). Multiple Machine Learning based-Chemoinformatics models for identification of histone acetyl Transferase inhibitors. *Mol. Inf.* 37 (8), e1700150. doi:10.1002/minf.201700150

Kuo, A. J., Cheung, P., Chen, K., Zee, B. M., Kioi, M., Lauring, J., et al. (2011). NSD2 links dimethylation of histone H3 at lysine 36 to oncogenic programming. *Mol. Cell* 44 (4), 609–620. doi:10.1016/j.molcel.2011.08.042

Kuo, A. J., Song, J., Cheung, P., Ishibe-Murakami, S., Yamazoe, S., Chen, J. K., et al. (2012). The BAH domain of ORC1 links H4K20me2 to DNA replication licensing and Meier-Gorlin syndrome. *Nature* 484 (7392), 115–119. doi:10.1038/nature10956

Kwie, F. H., Briet, M., Soupaya, D., Hoffmann, P., Maturano, M., Rodriguez, F., et al. (2011). New potent bisubstrate inhibitors of histone acetyltransferase p300: Design, synthesis and biological evaluation. *Chem. Biol. Drug Des.* 77 (1), 86–92. doi:10.1111/j.1747-0285.2010.01056.x

Lau, O. D., Kundu, T. K., Soccio, R. E., Ait-Si-Ali, S., Khalil, E. M., Vassilev, A., et al. (2000). HATs off: Selective synthetic inhibitors of the histone acetyltransferases p300 and PCAF. *Mol. Cell* 5 (3), 589–595. doi:10.1016/s1097-2765(00)80452-9

Lee, J. Y., Kuo, C. W., Tsai, S. L., Cheng, S. M., Chen, S. H., Chan, H. H., et al. (2016). Inhibition of HDAC3- and HDAC6-promoted Survivin expression plays an important role in SAHA-induced autophagy and Viability reduction in breast cancer cells. *Front. Pharmacol.* 7, 81. doi:10.3389/fphar.2016.00081

Lee, Y. J., Won, A. J., Lee, J., Jung, J. H., Yoon, S., Lee, B. M., et al. (2012). Molecular mechanism of SAHA on regulation of autophagic cell death in tamoxifen-resistant MCF-7 breast cancer cells. *Int. J. Med. Sci.* 9 (10), 881–893. doi:10.7150/ijms.5011

Li, L. X., Zhou, J. X., Calvet, J. P., Godwin, A. K., Jensen, R. A., and Li, X. (2018). Lysine methyltransferase SMYD2 promotes triple negative breast cancer progression. *Cell Death Dis.* 9 (3), 326. doi:10.1038/s41419-018-0347-x

Li, W., Wu, H., Sui, S., Wang, Q., Xu, S., and Pang, D. (2021). Targeting histone modifications in breast cancer: A Precise Weapon on the Way. *Front. Cell Dev. Biol.* 9, 736935. doi:10.3389/fcell.2021.736935

Li, Z., Yu, D., Li, H., Lv, Y., and Li, S. (2019). Long noncoding RNA UCA1 confers tamoxifen resistance in breast cancer endocrinotherapy through regulation of the EZH2/p21 axis and the PI3K/AKT signaling pathway. *Int. J. Oncol.* 54 (3), 1033–1042. doi:10.3892/ijo.2019.4679

Liang, Y., Hu, J., Li, J., Liu, Y., Yu, J., Zhuang, X., et al. (2015). Epigenetic activation of TWIST1 by MTDH promotes cancer stem-like cell Traits in breast cancer. *Cancer Res.* 75 (17), 3672–3680. doi:10.1158/0008-5472.CAN-15-0930

Liu, C., Ho, P. C., Wong, F. C., Sethi, G., Wang, L. Z., and Goh, B. C. (2015). Garcinol: Current status of its anti-oxidative, anti-inflammatory and anti-cancer effects. *Cancer Lett.* 362 (1), 8–14. doi:10.1016/j.canlet.2015.03.019

Liu, L., Kimball, S., Liu, H., Holowatyj, A., and Yang, Z. Q. (2015). Genetic alterations of histone lysine methyltransferases and their significance in breast cancer. *Oncotarget* 6 (4), 2466–2482. doi:10.18632/oncotarget.2967

Liu, R., Zhang, Z., Yang, H., Zhou, K., Geng, M., Zhou, W., et al. (2019). Design, synthesis, and biological evaluation of a new class of histone acetyltransferase p300 inhibitors. *Eur. J. Med. Chem.* 180, 171–190. doi:10.1016/j.ejmech.2019.07.026

Liu, S. S., Wu, F., Jin, Y. M., Chang, W. Q., and Xu, T. M. (2020). HDAC11: A rising star in epigenetics. *Biomed. Pharmacother.* 131, 110607. doi:10.1016/j.biopha.2020.110607

Liu, X., Li, C., Zhang, R., Xiao, W., Niu, X., Ye, X., et al. (2018). The EZH2-H3K27me3-DNMT1 complex orchestrates epigenetic silencing of the wwc1 gene, a Hippo/YAP pathway upstream effector, in breast cancer epithelial cells. *Cell. Signal.* 51, 243–256. doi:10.1016/j.cellsig.2018.08.011

Liu, Y. N., Lee, W. W., Wang, C. Y., Chao, T. H., Chen, Y., and Chen, J. H. (2005). Regulatory mechanisms controlling human E-cadherin gene expression. *Oncogene* 24 (56), 8277–8290. doi:10.1038/sj.onc.1208991

Liu, Z., Chen, P., Gao, H., Gu, Y., Yang, J., Peng, H., et al. (2014). Ubiquitylation of autophagy receptor Optineurin by HACE1 activates selective autophagy for tumor suppression. *Cancer Cell* 26 (1), 106–120. doi:10.1016/j.ccr.2014.05.015

Lu, P., Gu, Y., Li, L., Wang, F., Yang, X., and Yang, Y. (2019). Belinostat suppresses cell proliferation by inactivating Wnt/β-catenin pathway and promotes apoptosis through regulating PKC pathway in breast cancer. *Artif. Cells Nanomed. Biotechnol.* 47 (1), 3955–3960. doi:10.1080/21691401.2019.1671855

Lu, W., and Katzenellenbogen, B. S. (2017). Estrogen receptor-β modulation of the ERα-p53 loop regulating gene expression, proliferation, and apoptosis in breast cancer. *Horm. Cancer* 8 (4), 230–242. doi:10.1007/s12672-017-0298-1

Luan, Q. X., Zhang, B. G., Li, X. J., and Guo, M. Y. (2016). MiR-129-5p is downregulated in breast cancer cells partly due to promoter H3K27m3 modification and regulates epithelial-mesenchymal transition and multi-drug resistance. *Eur. Rev. Med. Pharmacol. Sci.* 20 (20), 4257–4265.

Luo, X. G., Zhang, C. L., Zhao, W. W., Liu, Z. P., Liu, L., Mu, A., et al. (2014). Histone methyltransferase SMYD3 promotes MRTF-A-mediated transactivation of MYL9 and migration of MCF-7 breast cancer cells. *Cancer Lett.* 344 (1), 129–137. doi:10.1016/j.canlet.2013.10.026

Lynch, H., Wen, H., Kim, Y. C., Snyder, C., Kinarsky, Y., Chen, P. X., et al. (2013). Can unknown predisposition in familial breast cancer be family-specific? *Breast J.* 19 (5), 520–528. doi:10.1111/tbj.12145

Mu, X., Chen, M., Xiao, B., Yang, B., Singh, S., and Zhang, B. (2019). EZH2 confers sensitivity of breast cancer cells to taxol by Attenuating p21 expression epigenetically. *DNA Cell Biol.* 38 (7), 651–659. doi:10.1089/dna.2019.4699

Manzo, F., Tambaro, F. P., Mai, A., and Altucci, L. (2009). Histone acetyltransferase inhibitors and preclinical studies. *Expert Opin. Ther. Pat.* 19 (6), 761–774. doi:10.1517/13543770902895727

Marchion, D. C., Bicaku, E., Daud, A. I., Richon, V., Sullivan, D. M., and Munster, P. N. (2004). Sequence-specific potentiation of topoisomerase II inhibitors by the histone deacetylase inhibitor suberoylanilide hydroxamic acid. *J. Cell. Biochem.* 92 (2), 223–237. doi:10.1002/jcb.20045

Marchion, D. C., Bicaku, E., Daud, A. I., Sullivan, D. M., and Munster, P. N. (2005). In vivo synergy between topoisomerase II and histone deacetylase inhibitors: Predictive correlates. *Mol. Cancer Ther.* 4 (12), 1993–2000. doi:10.1158/1535-7163.MCT-05-0194

Margueron, R., and Reinberg, D. (2011). The Polycomb complex PRC2 and its mark in life. *Nature* 469 (7330), 343–349. doi:10.1038/nature09784

Matkar, S., Sharma, P., Gao, S., Gurung, B., Katona, B. W., Liao, J., et al. (2015). An epigenetic pathway regulates sensitivity of breast cancer cells to HER2 inhibition via FOXO/c-Myc Axis. *Cancer Cell* 28 (4), 472–485. doi:10.1016/j.ccell.2015.09.005

Mbese, Z., Khwaza, V., and Aderibigbe, B. A. (2019). Curcumin and its derivatives as potential therapeutic agents in prostate, colon and breast cancers. *Molecules* 24 (23), E4386. doi:10.3390/molecules24234386

McCaw, T. R., Li, M., Starenki, D., Liu, M., Cooper, S. J., Arend, R. C., et al. (2019). Histone deacetylase inhibition promotes intratumoral CD8(+) T-cell responses, sensitizing murine breast tumors to anti-PD1. *Cancer Immunol. Immunother.* 68 (12), 2081–2094. doi:10.1007/s00262-019-02430-9

McGinty, R. K., Kim, J., Chatterjee, C., Roeder, R. G., and Muir, T. W. (2008). Chemically ubiquitylated histone H2B stimulates hDot1L-mediated intranucleosomal methylation. *Nature* 453 (7196), 812–816. doi:10.1038/nature06906

Merarchi, M., Sethi, G., Shanmugam, M. K., Fan, L., Arfuso, F., and Ahn, K. S. (2019). Role of natural products in modulating histone deacetylases in cancer. *Molecules* 24 (6), E1047. doi:10.3390/molecules24061047

Merino, V. F., Nguyen, N., Jin, K., Sadik, H., Cho, S., Korangath, P., et al. (2016). Combined treatment with epigenetic, differentiating, and chemotherapeutic agents Cooperatively targets tumor-initiating cells in triple-negative breast cancer. *Cancer Res.* 76 (7), 2013–2024. doi:10.1158/0008-5472.CAN-15-1619

Mertz, J. A., Conery, A. R., Bryant, B. M., Sandy, P., Balasubramanian, S., Mele, D. A., et al. (2011). Targeting MYC dependence in cancer by inhibiting BET bromodomains. *Proc. Natl. Acad. Sci. U. S. A.* 108 (40), 16669–16674. doi:10.1073/pnas.1108190108

Milite, C., Feoli, A., Sasaki, K., La Pietra, V., Balzano, A. L., Marinelli, L., et al. (2015). A novel cell-permeable, selective, and noncompetitive inhibitor of KAT3 histone acetyltransferases from a combined molecular pruning/classical isosterism approach. *J. Med. Chem.* 58 (6), 2779–2798. doi:10.1021/jm5019687

Miller, T., Krogan, N. J., Dover, J., Erdjument-Bromage, H., Tempst, P., Johnston, M., et al. (2001). Compass: A complex of proteins associated with a trithorax-related SET domain protein. *Proc. Natl. Acad. Sci. U. S. A.* 98 (23), 12902–12907. doi:10.1073/pnas.231473398

Min, A., Im, S. A., Kim, D. K., Song, S. H., Kim, H. J., Lee, K. H., et al. (2015). Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), enhances anti-tumor effects of the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib in triple-negative breast cancer cells. *Breast Cancer Res.* 17, 33. doi:10.1186/s13058-015-0534-y

Miranda, T. B., Cortez, C. C., Yoo, C. B., Liang, G., Abe, M., Kelly, T. K., et al. (2009). DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. *Mol. Cancer Ther.* 8 (6), 1579–1588. doi:10.1158/1535-7163.MCT-09-0013

Mo, R., Rao, S. M., and Zhu, Y. J. (2006). Identification of the MLL2 complex as a coactivator for estrogen receptor alpha. *J. Biol. Chem.* 281 (23), 15714–15720. doi:10.1074/jbc.M513245200

Montenegro, M. F., Sanchez-Del-Campo, L., Gonzalez-Guerrero, R., Martinez-Barba, E., Pinero-Madrona, A., Cabezas-Herrera, J., et al. (2016). Tumor suppressor SET9 guides the epigenetic plasticity of breast cancer cells and serves as an early-stage biomarker for predicting metastasis. *Oncogene* 35 (47), 6143–6152. doi:10.1038/onc.2016.154

Munster, P. N., Thurn, K. T., Thomas, S., Raha, P., Lacevic, M., Miller, A., et al. (2011). A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. *Br. J. Cancer* 104 (12), 1828–1835. doi:10.1038/bjc.2011.156

Nakagawa, M., Oda, Y., Eguchi, T., Aishima, S., Yao, T., Hosoi, F., et al. (2007). Expression profile of class I histone deacetylases in human cancer tissues. *Oncol. Rep.* 18 (4), 769–774. doi:10.3892/or.18.4.769

Natarajan, T. G., Kallakury, B. V., Sheehan, C. E., Bartlett, M. B., Ganesan, N., Preet, A., et al. (2010). Epigenetic regulator MLL2 shows altered expression in cancer cell lines and tumors from human breast and colon. *Cancer Cell Int.* 10, 13. doi:10.1186/1475-2867-10-13

Nayak, S. R., Harrington, E., Boone, D., Hartmaier, R., Chen, J., Pathiraja, T. N., et al. (2015). A role for histone H2B variants in endocrine-resistant breast cancer. *Horm. Cancer* 6 (5-6), 214–224. doi:10.1007/s12672-015-0230-5

Ng, H. H., Xu, R. M., Zhang, Y., and Struhl, K. (2002). Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. *J. Biol. Chem.* 277 (38), 34655–34657. doi:10.1074/jbc.C200433200

Ngo, L., Brown, T., and Zheng, Y. G. (2019). Bisubstrate inhibitors to target histone acetyltransferase 1. *Chem. Biol. Drug Des.* 93 (5), 865–873. doi:10.1111/cbdd.13476

Nguyen, A. T., and Zhang, Y. (2011). The diverse functions of Dot1 and H3K79 methylation. *Genes Dev.* 25 (13), 1345–1358. doi:10.1101/gad.2057811

Nie, L., Wei, Y., Zhang, F., Hsu, Y. H., Chan, L. C., Xia, W., et al. (2019). CDK2-mediated site-specific phosphorylation of EZH2 drives and maintains triple-negative breast cancer. *Nat. Commun.* 10 (1), 5114. doi:10.1038/s41467-019-13105-5

Nieto-Jimenez, C., Alcaraz-Sanabria, A., Perez-Pena, J., Corrales-Sanchez, V., Serrano-Heras, G., Galan-Moya, E. M., et al. (2017). Targeting basal-like breast tumors with bromodomain and extraterminal domain (BET) and polo-like kinase inhibitors. *Oncotarget* 8 (12), 19478–19490. doi:10.18632/oncotarget.14465

Ocana, A., Nieto-Jimenez, C., and Pandiella, A. (2017). BET inhibitors as novel therapeutic agents in breast cancer. *Oncotarget* 8 (41), 71285–71291. doi:10.18632/oncotarget.19744

Oh, W. K., Cho, K. B., Hien, T. T., Kim, T. H., Kim, H. S., Dao, T. T., et al. (2010). Amurensin G, a potent natural SIRT1 inhibitor, rescues doxorubicin responsiveness via down-regulation of multidrug resistance 1. *Mol. Pharmacol.* 78 (5), 855–864. doi:10.1124/mol.110.065961

Oktyabri, D., Ishimura, A., Tange, S., Terashima, M., and Suzuki, T. (2016). DOT1L histone methyltransferase regulates the expression of BCAT1 and is involved in sphere formation and cell migration of breast cancer cell lines. *Biochimie* 123, 20–31. doi:10.1016/j.biochi.2016.01.005

Olsen, E. A., Kim, Y. H., Kuzel, T. M., Pacheco, T. R., Foss, F. M., Parker, S., et al. (2007). Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J. Clin. Oncol.* 25 (21), 3109–3115. doi:10.1200/JCO.2006.10.2434

Ozdag, H., Teschendorff, A. E., Ahmed, A. A., Hyland, S. J., Blenkiron, C., Bobrow, L., et al. (2006). Differential expression of selected histone modifier genes in human solid cancers. *BMC Genomics* 7, 90. doi:10.1186/1471-2164-7-90

Park, S. E., Yi, H. J., Suh, N., Park, Y. Y., Koh, J. Y., Jeong, S. Y., et al. (2016). Inhibition of EHMT2/G9a epigenetically increases the transcription of Beclin-1 via an increase in ROS and activation of NF-κB. *Oncotarget* 7 (26), 39796–39808. doi:10.18632/oncotarget.9290

Park, S. H., Fong, K. W., Mong, E., Martin, M. C., Schiltz, G. E., and Yu, J. (2021). Going beyond polycomb: EZH2 functions in prostate cancer. *Oncogene* 40 (39), 5788–5798. doi:10.1038/s41388-021-01982-4

Park, S. H., Ozden, O., Liu, G., Song, H. Y., Zhu, Y., Yan, Y., et al. (2016). SIRT2-Mediated deacetylation and Tetramerization of pyruvate kinase directs Glycolysis and tumor growth. *Cancer Res.* 76 (13), 3802–3812. doi:10.1158/0008-5472.CAN-15-2498

Park, U. H., Kang, M. R., Kim, E. J., Kwon, Y. S., Hur, W., Yoon, S. K., et al. (2016). ASXL2 promotes proliferation of breast cancer cells by linking ERα to histone methylation. *Oncogene* 35 (28), 3742–3752. doi:10.1038/onc.2015.443

Pasculli, B., Barbano, R., and Parrella, P. (2018). Epigenetics of breast cancer: Biology and clinical implication in the era of precision medicine. *Semin. Cancer Biol.* 51, 22–35. doi:10.1016/j.semcancer.2018.01.007

Patel, A., Vought, V. E., Dharmarajan, V., and Cosgrove, M. S. (2008). A conserved arginine-containing motif crucial for the assembly and enzymatic activity of the mixed lineage leukemia protein-1 core complex. *J. Biol. Chem.* 283 (47), 32162–32175. doi:10.1074/jbc.M806317200

Peck, B., Chen, C. Y., Ho, K. K., Di Fruscia, P., Myatt, S. S., Coombes, R. C., et al. (2010). SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2. *Mol. Cancer Ther.* 9 (4), 844–855. doi:10.1158/1535-7163.MCT-09-0971

Pena-Llopis, S., Wan, Y., and Martinez, E. D. (2016). Unique epigenetic gene profiles define human breast cancers with poor prognosis. *Oncotarget* 7 (52), 85819–85831. doi:10.18632/oncotarget.13334

Piao, L., Suzuki, T., Dohmae, N., Nakamura, Y., and Hamamoto, R. (2015). SUV39H2 methylates and stabilizes LSD1 by inhibiting polyubiquitination in human cancer cells. *Oncotarget* 6 (19), 16939–16950. doi:10.18632/oncotarget.4760

Piekarz, R. L., Frye, R., Turner, M., Wright, J. J., Allen, S. L., Kirschbaum, M. H., et al. (2009). Phase II multi-institutional trial of the histone deacetylase inhibitor romidepsin as monotherapy for patients with cutaneous T-cell lymphoma. *J. Clin. Oncol.* 27 (32), 5410–5417. doi:10.1200/JCO.2008.21.6150

Pullikuth, A. K., Routh, E. D., Zimmerman, K. D., Chifman, J., Chou, J. W., Soike, M. H., et al. (2021). Bulk and single-cell profiling of breast tumors Identifies TREM-1 as a dominant immune suppressive marker associated with poor outcomes. *Front. Oncol.* 11, 734959. doi:10.3389/fonc.2021.734959

Punzi, S., Balestrieri, C., D'Alesio, C., Bossi, D., Dellino, G. I., Gatti, E., et al. (2019). WDR5 inhibition halts metastasis dissemination by repressing the mesenchymal phenotype of breast cancer cells. *Breast Cancer Res.* 21 (1), 123. doi:10.1186/s13058-019-1216-y

Puppe, J., Drost, R., Liu, X., Joosse, S. A., Evers, B., Cornelissen-Steijger, P., et al. (2009). BRCA1-deficient mammary tumor cells are dependent on EZH2 expression and sensitive to Polycomb Repressive Complex 2-inhibitor 3-deazaneplanocin A. *Breast Cancer Res.* 11 (4), R63. doi:10.1186/bcr2354

Qin, G., Li, Y., Xu, X., Wang, X., Zhang, K., Tang, Y., et al. (2019). Panobinostat (LBH589) inhibits Wnt/β-catenin signaling pathway via upregulating APCL expression in breast cancer. *Cell. Signal.* 59, 62–75. doi:10.1016/j.cellsig.2019.03.014

Qin, Y., Vasilatos, S. N., Chen, L., Wu, H., Cao, Z., Fu, Y., et al. (2019). Inhibition of histone lysine-specific demethylase 1 elicits breast tumor immunity and enhances antitumor efficacy of immune checkpoint blockade. *Oncogene* 38 (3), 390–405. doi:10.1038/s41388-018-0451-5

Quintayo, M. A., Munro, A. F., Thomas, J., Kunkler, I. H., Jack, W., Kerr, G. R., et al. (2012). GSK3β and cyclin D1 expression predicts outcome in early breast cancer patients. *Breast Cancer Res. Treat.* 136 (1), 161–168. doi:10.1007/s10549-012-2229-8

Rabello Ddo, A., de Moura, C. A., de Andrade, R. V., Motoyama, A. B., and Silva, F. P. (2013). Altered expression of MLL methyltransferase family genes in breast cancer. *Int. J. Oncol.* 43 (2), 653–660. doi:10.3892/ijo.2013.1981

Rada-Iglesias, A., Bajpai, R., Swigut, T., Brugmann, S. A., Flynn, R. A., and Wysocka, J. (2011). A unique chromatin signature uncovers early developmental enhancers in humans. *Nature* 470 (7333), 279–283. doi:10.1038/nature09692

Rangasamy, D. (2010). Histone variant H2A.Z can serve as a new target for breast cancer therapy. *Curr. Med. Chem.* 17 (28), 3155–3161. doi:10.2174/092986710792231941

Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B. D., Sun, Z. W., Schmid, M., et al. (2000). Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406 (6796), 593–599. doi:10.1038/35020506

Ren, G., Baritaki, S., Marathe, H., Feng, J., Park, S., Beach, S., et al. (2012). Polycomb protein EZH2 regulates tumor invasion via the transcriptional repression of the metastasis suppressor RKIP in breast and prostate cancer. *Cancer Res.* 72 (12), 3091–3104. doi:10.1158/0008-5472.CAN-11-3546

Rhodes, L. V., Tate, C. R., Segar, H. C., Burks, H. E., Phamduy, T. B., Hoang, V., et al. (2014). Suppression of triple-negative breast cancer metastasis by pan-DAC inhibitor panobinostat via inhibition of ZEB family of EMT master regulators. *Breast Cancer Res. Treat.* 145 (3), 593–604. doi:10.1007/s10549-014-2979-6

Ring, A., Nguyen, C., Smbatyan, G., Tripathy, D., Yu, M., Press, M., et al. (2018). CBP/β-Catenin/FOXM1 is a novel therapeutic target in triple negative breast cancer. *Cancers (Basel)* 10 (12), E525. doi:10.3390/cancers10120525

Rivera, C., Saavedra, F., Alvarez, F., Diaz-Celis, C., Ugalde, V., Li, J., et al. (2015). Methylation of histone H3 lysine 9 occurs during translation. *Nucleic Acids Res.* 43 (19), 9097–9106. doi:10.1093/nar/gkv929

Robert, N. J., Dieras, V., Glaspy, J., Brufsky, A. M., Bondarenko, I., Lipatov, O. N., et al. (2011). RIBBON-1: Randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab for first-line treatment of human epidermal growth factor receptor 2-negative, locally recurrent or metastatic breast cancer. *J. Clin. Oncol.* 29 (10), 1252–1260. doi:10.1200/JCO.2010.28.0982

Robertson, F. M., Chu, K., Boley, K. M., Ye, Z., Liu, H., Wright, M. C., et al. (2013). The class I HDAC inhibitor Romidepsin targets inflammatory breast cancer tumor emboli and synergizes with paclitaxel to inhibit metastasis. *J. Exp. Ther. Oncol.* 10 (3), 219–233.

Roth, S. Y., Denu, J. M., and Allis, C. D. (2001). Histone acetyltransferases. *Annu. Rev. Biochem.* 70, 81–120. doi:10.1146/annurev.biochem.70.1.81

Saji, S., Kawakami, M., Hayashi, S., Yoshida, N., Hirose, M., Horiguchi, S., et al. (2005). Significance of HDAC6 regulation via estrogen signaling for cell motility and prognosis in estrogen receptor-positive breast cancer. *Oncogene* 24 (28), 4531–4539. doi:10.1038/sj.onc.1208646

Salz, T., Deng, C., Pampo, C., Siemann, D., Qiu, Y., Brown, K., et al. (2015). Histone methyltransferase hSETD1A is a novel regulator of metastasis in breast cancer. *Mol. Cancer Res.* 13 (3), 461–469. doi:10.1158/1541-7786.MCR-14-0389

Santos-Rosa, H., Schneider, R., Bannister, A. J., Sherriff, J., Bernstein, B. E., Emre, N. C., et al. (2002). Active genes are tri-methylated at K4 of histone H3. *Nature* 419 (6905), 407–411. doi:10.1038/nature01080

Sato, Y., Kujirai, T., Arai, R., Asakawa, H., Ohtsuki, C., Horikoshi, N., et al. (2016). A Genetically Encoded Probe for Live-cell Imaging of H4K20 monomethylation. *J. Mol. Biol.* 428 (20), 3885–3902. doi:10.1016/j.jmb.2016.08.010

Seidel, C., Florean, C., Schnekenburger, M., Dicato, M., and Diederich, M. (2012). Chromatin-modifying agents in anti-cancer therapy. *Biochimie* 94 (11), 2264–2279. doi:10.1016/j.biochi.2012.05.012

Shah, P., Gau, Y., and Sabnis, G. (2014). Histone deacetylase inhibitor entinostat reverses epithelial to mesenchymal transition of breast cancer cells by reversing the repression of E-cadherin. *Breast Cancer Res. Treat.* 143 (1), 99–111. doi:10.1007/s10549-013-2784-7

Shanmugam, M. K., Lee, J. H., Chai, E. Z., Kanchi, M. M., Kar, S., Arfuso, F., et al. (2016). Cancer prevention and therapy through the modulation of transcription factors by bioactive natural compounds. *Semin. Cancer Biol.* 40-41, 35–47. doi:10.1016/j.semcancer.2016.03.005

Shi, P., Zhou, M., and Yang, Y. (2020). Upregulated tumor sirtuin 2 expression correlates with reduced TNM stage and better overall survival in surgical breast cancer patients. *Ir. J. Med. Sci.* 189 (1), 83–89. doi:10.1007/s11845-019-02071-y

Shi, Y. K., Li, Z. H., Han, X. Q., Yi, J. H., Wang, Z. H., Hou, J. L., et al. (2010). The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces growth inhibition and enhances taxol-induced cell death in breast cancer. *Cancer Chemother. Pharmacol.* 66 (6), 1131–1140. doi:10.1007/s00280-010-1455-1

Shilatifard, A. (2008). Molecular implementation and physiological roles for histone H3 lysine 4 (H3K4) methylation. *Curr. Opin. Cell Biol.* 20 (3), 341–348. doi:10.1016/j.ceb.2008.03.019

Si, X., Zang, R., Zhang, E., Liu, Y., Shi, X., Zhang, E., et al. (2016). LncRNA H19 confers chemoresistance in ERα-positive breast cancer through epigenetic silencing of the pro-apoptotic gene BIK. *Oncotarget* 7 (49), 81452–81462. doi:10.18632/oncotarget.13263

Simon, R. P., Robaa, D., Alhalabi, Z., Sippl, W., and Jung, M. (2016). KATching-up on small molecule modulators of lysine acetyltransferases. *J. Med. Chem.* 59 (4), 1249–1270. doi:10.1021/acs.jmedchem.5b01502

Siouda, M., Dujardin, A. D., Barbollat-Boutrand, L., Mendoza-Parra, M. A., Gibert, B., Ouzounova, M., et al. (2020). CDYL2 epigenetically regulates MIR124 to control NF-κB/STAT3-Dependent breast cancer cell plasticity. *iScience* 23 (6), 101141. doi:10.1016/j.isci.2020.101141

Song, Z., Zhang, X., Lin, Y., Wei, Y., Liang, S., and Dong, C. (2019). LINC01133 inhibits breast cancer invasion and metastasis by negatively regulating SOX4 expression through EZH2. *J. Cell. Mol. Med.* 23 (11), 7554–7565. doi:10.1111/jcmm.14625

Souto, J. A., Conte, M., Alvarez, R., Nebbioso, A., Carafa, V., Altucci, L., et al. (2008). Synthesis of benzamides related to anacardic acid and their histone acetyltransferase (HAT) inhibitory activities. *ChemMedChem* 3 (9), 1435–1442. doi:10.1002/cmdc.200800096

Steed, K. L., Jordan, H. R., and Tollefsbol, T. O. (2020). SAHA and EGCG promote apoptosis in triple-negative breast cancer cells, possibly through the modulation of cIAP2. *Anticancer Res.* 40 (1), 9–26. doi:10.21873/anticanres.13922

Su, C. H., Lin, I. H., Tzeng, T. Y., Hsieh, W. T., and Hsu, M. T. (2016). Regulation of IL-20 expression by Estradiol through KMT2B-mediated epigenetic modification. *PLoS One* 11 (11), e0166090. doi:10.1371/journal.pone.0166090

Su, Y., Hopfinger, N. R., Nguyen, T. D., Pogash, T. J., Santucci-Pereira, J., and Russo, J. (2018). Epigenetic reprogramming of epithelial mesenchymal transition in triple negative breast cancer cells with DNA methyltransferase and histone deacetylase inhibitors. *J. Exp. Clin. Cancer Res.* 37 (1), 314. doi:10.1186/s13046-018-0988-8

Subramanian, K., Jia, D., Kapoor-Vazirani, P., Powell, D. R., Collins, R. E., Sharma, D., et al. (2008). Regulation of estrogen receptor alpha by the SET7 lysine methyltransferase. *Mol. Cell* 30 (3), 336–347. doi:10.1016/j.molcel.2008.03.022

Sulaiman, A., McGarry, S., Li, L., Jia, D., Ooi, S., Addison, C., et al. (2018). Dual inhibition of Wnt and Yes-associated protein signaling retards the growth of triple-negative breast cancer in both mesenchymal and epithelial states. *Mol. Oncol.* 12 (4), 423–440. doi:10.1002/1878-0261.12167

Sun, Y., Jiang, X., Chen, S., and Price, B. D. (2006). Inhibition of histone acetyltransferase activity by anacardic acid sensitizes tumor cells to ionizing radiation. *FEBS Lett.* 580 (18), 4353–4356. doi:10.1016/j.febslet.2006.06.092

Tachibana, M., Sugimoto, K., Nozaki, M., Ueda, J., Ohta, T., Ohki, M., et al. (2002). G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev.* 16 (14), 1779–1791. doi:10.1101/gad.989402

Tajbakhsh, A., Hasanzadeh, M., Rezaee, M., Khedri, M., Khazaei, M., ShahidSales, S., et al. (2018). Therapeutic potential of novel formulated forms of curcumin in the treatment of breast cancer by the targeting of cellular and physiological dysregulated pathways. *J. Cell. Physiol.* 233 (3), 2183–2192. doi:10.1002/jcp.25961

Tajima, K., Matsuda, S., Yae, T., Drapkin, B. J., Morris, R., Boukhali, M., et al. (2019). SETD1A protects from senescence through regulation of the mitotic gene expression program. *Nat. Commun.* 10 (1), 2854. doi:10.1038/s41467-019-10786-w

Takemoto, Y., Ito, A., Niwa, H., Okamura, M., Fujiwara, T., Hirano, T., et al. (2016). Identification of Cyproheptadine as an inhibitor of SET domain containing lysine methyltransferase 7/9 (Set7/9) that regulates estrogen-dependent transcription. *J. Med. Chem.* 59 (8), 3650–3660. doi:10.1021/acs.jmedchem.5b01732

Talbert, P. B., and Henikoff, S. (2021). Histone variants at a glance. *J. Cell Sci.* 134 (6), jcs244749. doi:10.1242/jcs.244749

Tan, J., Yang, X., Zhuang, L., Jiang, X., Chen, W., Lee, P. L., et al. (2007). Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. *Genes Dev.* 21 (9), 1050–1063. doi:10.1101/gad.1524107

Taniguchi, H., Jacinto, F. V., Villanueva, A., Fernandez, A. F., Yamamoto, H., Carmona, F. J., et al. (2012). Silencing of Kruppel-like factor 2 by the histone methyltransferase EZH2 in human cancer. *Oncogene* 31 (15), 1988–1994. doi:10.1038/onc.2011.387

Tardat, M., Brustel, J., Kirsh, O., Lefevbre, C., Callanan, M., Sardet, C., et al. (2010). The histone H4 Lys 20 methyltransferase PR-Set7 regulates replication origins in mammalian cells. *Nat. Cell Biol.* 12 (11), 1086–1093. doi:10.1038/ncb2113

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 (D1), D941–D947. doi:10.1093/nar/gky1015

Terranova-Barberio, M., Pawlowska, N., Dhawan, M., Moasser, M., Chien, A. J., Melisko, M. E., et al. (2020). Exhausted T cell signature predicts immunotherapy response in ER-positive breast cancer. *Nat. Commun.* 11 (1), 3584. doi:10.1038/s41467-020-17414-y

Terranova-Barberio, M., Roca, M. S., Zotti, A. I., Leone, A., Bruzzese, F., Vitagliano, C., et al. (2016). Valproic acid potentiates the anticancer activity of capecitabine in vitro and in vivo in breast cancer models via induction of thymidine phosphorylase expression. *Oncotarget* 7 (7), 7715–7731. doi:10.18632/oncotarget.6802

Tillinghast, G. W., Partee, J., Albert, P., Kelley, J. M., Burtow, K. H., and Kelly, K. (2003). Analysis of genetic stability at the EP300 and CREBBP loci in a panel of cancer cell lines. *Genes Chromosom. Cancer* 37 (2), 121–131. doi:10.1002/gcc.10195

Towbin, B. D., Gonzalez-Aguilera, C., Sack, R., Gaidatzis, D., Kalck, V., Meister, P., et al. (2012). Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery. *Cell* 150 (5), 934–947. doi:10.1016/j.cell.2012.06.051

Trabert, B., Sherman, M. E., Kannan, N., and Stanczyk, F. Z. (2020). Progesterone and breast cancer. *Endocr. Rev.* 41 (2), bnz001. doi:10.1210/endrev/bnz001

Travaglini, L., Vian, L., Billi, M., Grignani, F., and Nervi, C. (2009). Epigenetic reprogramming of breast cancer cells by valproic acid occurs regardless of estrogen receptor status. *Int. J. Biochem. Cell Biol.* 41 (1), 225–234. doi:10.1016/j.biocel.2008.08.019

Trisciuoglio, D., Di Martile, M., and Del Bufalo, D. (2018). Emerging role of histone acetyltransferase in stem cells and cancer. *Stem Cells Int.* 2018, 8908751. doi:10.1155/2018/8908751

Truax, A. D., Thakkar, M., and Greer, S. F. (2012). Dysregulated recruitment of the histone methyltransferase EZH2 to the class II transactivator (CIITA) promoter IV in breast cancer cells. *PLoS One* 7 (4), e36013. doi:10.1371/journal.pone.0036013

Tsang, J. Y., Lai, S. T., Ni, Y. B., Shao, Y., Poon, I. K., Kwan, J. S., et al. (2021). SETD2 alterations and histone H3K36 trimethylation in phyllodes tumor of breast. *Breast Cancer Res. Treat.* 187 (2), 339–347. doi:10.1007/s10549-021-06181-z

Tu, W. B., Shiah, Y. J., Lourenco, C., Mullen, P. J., Dingar, D., Redel, C., et al. (2018). MYC interacts with the G9a histone methyltransferase to drive transcriptional repression and tumorigenesis. *Cancer Cell* 34 (4), 579–595. doi:10.1016/j.ccell.2018.09.001

Unnikrishnan, A., Gafken, P. R., and Tsukiyama, T. (2010). Dynamic changes in histone acetylation regulate origins of DNA replication. *Nat. Struct. Mol. Biol.* 17 (4), 430–437. doi:10.1038/nsmb.1780

Vansteenkiste, J., Van Cutsem, E., Dumez, H., Chen, C., Ricker, J. L., Randolph, S. S., et al. (2008). Early phase II trial of oral vorinostat in relapsed or refractory breast, colorectal, or non-small cell lung cancer. *Invest. New Drugs* 26 (5), 483–488. doi:10.1007/s10637-008-9131-6

Vavouri, T., and Lehner, B. (2012). Human genes with CpG island promoters have a distinct transcription-associated chromatin organization. *Genome Biol.* 13 (11), R110. doi:10.1186/gb-2012-13-11-r110

Verma, S. K., Tian, X., LaFrance, L. V., Duquenne, C., Suarez, D. P., Newlander, K. A., et al. (2012). Identification of potent, selective, cell-active inhibitors of the histone lysine methyltransferase EZH2. *ACS Med. Chem. Lett.* 3 (12), 1091–1096. doi:10.1021/ml3003346

Vo, N., and Goodman, R. H. (2001). CREB-binding protein and p300 in transcriptional regulation. *J. Biol. Chem.* 276 (17), 13505–13508. doi:10.1074/jbc.R000025200

Voss, A. K., and Thomas, T. (2009). MYST family histone acetyltransferases take center stage in stem cells and development. *Bioessays* 31 (10), 1050–1061. doi:10.1002/bies.200900051

Vougiouklakis, T., Saloura, V., Park, J. H., Takamatsu, N., Miyamoto, T., Nakamura, Y., et al. (2018). Development of novel SUV39H2 inhibitors that exhibit growth suppressive effects in mouse xenograft models and regulate the phosphorylation of H2AX. *Oncotarget* 9 (61), 31820–31831. doi:10.18632/oncotarget.25806

Wadia, J. S., and Dowdy, S. F. (2005). Transmembrane delivery of protein and peptide drugs by TAT-mediated transduction in the treatment of cancer. *Adv. Drug Deliv. Rev.* 57 (4), 579–596. doi:10.1016/j.addr.2004.10.005

Wang, C., Fu, M., Angeletti, R. H., Siconolfi-Baez, L., Reutens, A. T., Albanese, C., et al. (2001). Direct acetylation of the estrogen receptor alpha hinge region by p300 regulates transactivation and hormone sensitivity. *J. Biol. Chem.* 276 (21), 18375–18383. doi:10.1074/jbc.M100800200

Wang, J., Duan, Z., Nugent, Z., Zou, J. X., Borowsky, A. D., Zhang, Y., et al. (2016). Reprogramming metabolism by histone methyltransferase NSD2 drives endocrine resistance via coordinated activation of pentose phosphate pathway enzymes. *Cancer Lett.* 378 (2), 69–79. doi:10.1016/j.canlet.2016.05.004

Wang, J. J., Zou, J. X., Wang, H., Duan, Z. J., Wang, H. B., Chen, P., et al. (2019). Histone methyltransferase NSD2 mediates the survival and invasion of triple-negative breast cancer cells via stimulating ADAM9-EGFR-AKT signaling. *Acta Pharmacol. Sin.* 40 (8), 1067–1075. doi:10.1038/s41401-018-0199-z

Wang, L., Collings, C. K., Zhao, Z., Cozzolino, K. A., Ma, Q., Liang, K., et al. (2017). A cytoplasmic COMPASS is necessary for cell survival and triple-negative breast cancer pathogenesis by regulating metabolism. *Genes Dev.* 31 (20), 2056–2066. doi:10.1101/gad.306092.117

Wang, L., Xu, M. L., Wang, C., Dong, Q. Q., Miao, Z., Chen, X. Y., et al. (2020). SET and MYND domain-containing protein 3 inhibits tumor cell sensitivity to cisplatin. *Oncol. Lett.* 19 (5), 3469–3476. doi:10.3892/ol.2020.11465

Wang, T., Mao, B., Cheng, C., Zou, Z., Gao, J., Yang, Y., et al. (2018). YAP promotes breast cancer metastasis by repressing growth differentiation factor-15. *Biochim. Biophys. Acta. Mol. Basis Dis.* 1864 (5), 1744–1753. doi:10.1016/j.bbadis.2018.02.020

Wang, Y., Han, Y., Fan, E., and Zhang, K. (2015). Analytical strategies used to identify the readers of histone modifications: A review. *Anal. Chim. Acta* 891, 32–42. doi:10.1016/j.aca.2015.06.049

Wang, Y. F., Zhang, J., Su, Y., Shen, Y. Y., Jiang, D. X., Hou, Y. Y., et al. (2017). G9a regulates breast cancer growth by modulating iron homeostasis through the repression of ferroxidase hephaestin. *Nat. Commun.* 8 (1), 274. doi:10.1038/s41467-017-00350-9

Wawruszak, A., Borkiewicz, L., Okon, E., Kukula-Koch, W., Afshan, S., and Halasa, M. (2021). Vorinostat (SAHA) and breast cancer: An overview. *Cancers (Basel)* 13 (18), 4700. doi:10.3390/cancers13184700

Wawruszak, A., Gumbarewicz, E., Okon, E., Jeleniewicz, W., Czapinski, J., Halasa, M., et al. (2019). Histone deacetylase inhibitors reinforce the phenotypical markers of breast epithelial or mesenchymal cancer cells but inhibit their migratory properties. *Cancer Manag. Res.* 11, 8345–8358. doi:10.2147/CMAR.S210029

Wawruszak, A., Luszczki, J. J., Grabarska, A., Gumbarewicz, E., Dmoszynska-Graniczka, M., Polberg, K., et al. (2015). Assessment of interactions between cisplatin and two histone deacetylase inhibitors in MCF7, T47D and MDA-MB-231 human breast cancer cell lines - an Isobolographic analysis. *PLoS One* 10 (11), e0143013. doi:10.1371/journal.pone.0143013

Winters, S., Martin, C., Murphy, D., and Shokar, N. K. (2017). Breast cancer Epidemiology, prevention, and screening. *Prog. Mol. Biol. Transl. Sci.* 151, 1–32. doi:10.1016/bs.pmbts.2017.07.002

Wu, H., Min, J., Lunin, V. V., Antoshenko, T., Dombrovski, L., Zeng, H., et al. (2010). Structural biology of human H3K9 methyltransferases. *PLoS One* 5 (1), e8570. doi:10.1371/journal.pone.0008570

Wu, H. T., Liu, Y. E., Hsu, K. W., Wang, Y. F., Chan, Y. C., Chen, Y., et al. (2020). MLL3 induced by Luteolin causes apoptosis in tamoxifen-resistant breast cancer cells through H3K4 monomethylation and suppression of the PI3K/AKT/mTOR pathway. *Am. J. Chin. Med.* 48 (5), 1221–1241. doi:10.1142/S0192415X20500603

Wu, J., Wang, J., Li, M., Yang, Y., Wang, B., and Zheng, Y. G. (2011). Small molecule inhibitors of histone acetyltransferase Tip60. *Bioorg. Chem.* 39 (1), 53–58. doi:10.1016/j.bioorg.2010.11.003

Wu, S., Luo, Z., Yu, P. J., Xie, H., and He, Y. W. (2016). Suberoylanilide hydroxamic acid (SAHA) promotes the epithelial mesenchymal transition of triple negative breast cancer cells via HDAC8/FOXA1 signals. *Biol. Chem.* 397 (1), 75–83. doi:10.1515/hsz-2015-0215

Xiao, X. S., Cai, M. Y., Chen, J. W., Guan, X. Y., Kung, H. F., Zeng, Y. X., et al. (2011). High expression of p300 in human breast cancer correlates with tumor Recurrence and predicts adverse prognosis. *Chin. J. Cancer Res.* 23 (3), 201–207. doi:10.1007/s11670-011-0201-5

Xun, J., Wang, D., Shen, L., Gong, J., Gao, R., Du, L., et al. (2017). JMJD3 suppresses stem cell-like characteristics in breast cancer cells by downregulation of Oct4 independently of its demethylase activity. *Oncotarget* 8 (13), 21918–21929. doi:10.18632/oncotarget.15747

Yi, C., Li, G., Wang, W., Sun, Y., Zhang, Y., Zhong, C., et al. (2021). Disruption of YY1-EZH2 interaction using synthetic peptides inhibits breast cancer development. *Cancers* 13 (10), 2402. doi:10.3390/cancers13102402

Yamaguchi, H., Du, Y., Nakai, K., Ding, M., Chang, S. S., Hsu, J. L., et al. (2018). EZH2 contributes to the response to PARP inhibitors through its PARP-mediated poly-ADP ribosylation in breast cancer. *Oncogene* 37 (2), 208–217. doi:10.1038/onc.2017.311

Yang, F., Sun, L., Li, Q., Han, X., Lei, L., Zhang, H., et al. (2012). SET8 promotes epithelial-mesenchymal transition and confers TWIST dual transcriptional activities. *EMBO J.* 31 (1), 110–123. doi:10.1038/emboj.2011.364

Yang, G., Yuan, Y., Yuan, H., Wang, J., Yun, H., Geng, Y., et al. (2021). Histone acetyltransferase 1 is a succinyltransferase for histones and non-histones and promotes tumorigenesis. *EMBO Rep.* 22 (2), e50967. doi:10.15252/embr.202050967

Yang, H., Kwon, C. S., Choi, Y., and Lee, D. (2016). Both H4K20 mono-methylation and H3K56 acetylation mark transcription-dependent histone

turnover in fission yeast. *Biochem. Biophys. Res. Commun.* 476 (4), 515–521. doi:10.1016/j.bbrc.2016.05.155

Yang, L., Zhang, Y., Shan, W., Hu, Z., Yuan, J., Pi, J., et al. (2017). Repression of BET activity sensitizes homologous recombination-proficient cancers to PARP inhibition. *Sci. Transl. Med.* 9 (400), eaal1645. doi:10.1126/scitranslmed.aal1645

Yang, T., Wang, P., Yin, X., Zhang, J., Huo, M., Gao, J., et al. (2021). The histone deacetylase inhibitor PCI-24781 impairs calcium influx and inhibits proliferation and metastasis in breast cancer. *Theranostics* 11 (5), 2058–2076. doi:10.7150/thno.48314

Yang, X. J., and Seto, E. (2007). HATs and HDACs: From structure, function and regulation to novel strategies for therapy and prevention. *Oncogene* 26 (37), 5310–5318. doi:10.1038/sj.onc.1210599

Yardley, D. A., Ismail-Khan, R. R., Melichar, B., Lichinitser, M., Munster, P. N., Klein, P. M., et al. (2013). Randomized phase II, double-blind, placebo-controlled study of exemestane with or without entinostat in postmenopausal women with locally recurrent or metastatic estrogen receptor-positive breast cancer progressing on treatment with a nonsteroidal aromatase inhibitor. *J. Clin. Oncol.* 31 (17), 2128–2135. doi:10.1200/JCO.2012.43.7251

Yomtoubian, S., Lee, S. B., Verma, A., Izzo, F., Markowitz, G., Choi, H., et al. (2020). Inhibition of EZH2 catalytic activity selectively targets a metastatic Subpopulation in triple-negative breast cancer. *Cell Rep.* 30 (3), 755–770. doi:10.1016/j.celrep.2019.12.056

Yu, C. W., Cheng, K. C., Chen, L. C., Lin, M. X., Chang, Y. C., and Hwang-Verslues, W. W. (2018). Pro-inflammatory cytokines IL-6 and CCL2 suppress expression of circadian gene Period2 in mammary epithelial cells. *Biochim. Biophys. Acta. Gene Regul. Mech.* 1861 (11), 1007–1017. doi:10.1016/j.bbagrm.2018.09.003

Yu, Y., Qi, J., Xiong, J., Jiang, L., Cui, D., He, J., et al. (2019). Epigenetic Co-Deregulation of EZH2/TET1 is a senescence-Countering, actionable Vulnerability in triple-negative breast cancer. *Theranostics* 9 (3), 761–777. doi:10.7150/thno.29520

Yuan, W., Xu, M., Huang, C., Liu, N., Chen, S., and Zhu, B. (2011). H3K36 methylation antagonizes PRC2-mediated H3K27 methylation. *J. Biol. Chem.* 286 (10), 7983–7989. doi:10.1074/jbc.M110.194027

Yuan, Z., Syed, M. A., Panchal, D., Rogers, D., Joo, M., and Sadikot, R. T. (2012). Curcumin mediated epigenetic modulation inhibits TREM-1 expression in response to lipopolysaccharide. *Int. J. Biochem. Cell Biol.* 44 (11), 2032–2043. doi:10.1016/j.biocel.2012.08.001

Yun, J. M., Jialal, I., and Devaraj, S. (2011). Epigenetic regulation of high glucose-induced proinflammatory cytokine production in monocytes by curcumin. *J. Nutr. Biochem.* 22 (5), 450–458. doi:10.1016/j.jnutbio.2010.03.014

Zeng, Y., Qiu, R., Yang, Y., Gao, T., Zheng, Y., Huang, W., et al. (2019). Regulation of EZH2 by SMYD2-mediated lysine methylation is implicated in tumorigenesis. *Cell Rep.* 29 (6), 1482–1498. doi:10.1016/j.celrep.2019.10.004

Zhao, L., Pang, A., and Li, Y. (2018). Function of GCN5 in the TGF-β1-induced epithelial-to-mesenchymal transition in breast cancer. *Oncol. Lett.* 16 (3), 3955–3963. doi:10.3892/ol.2018.9134

Zhang, J., Sui, S., Wu, H., Zhang, J., Zhang, X., Xu, S., et al. (2019). The transcriptional landscape of lncRNAs reveals the oncogenic function of LINC00511 in ER-negative breast cancer. *Cell Death Dis.* 10 (8), 599. doi:10.1038/s41419-019-1835-3

Zhang, H., Zhang, Y., Chen, C., Zhu, X., Zhang, C., Xia, Y., et al. (2018). A double-negative feedback loop between DEAD-box protein DDX21 and Snail regulates epithelial-mesenchymal transition and metastasis in breast cancer. *Cancer Lett.* 437, 67–78. doi:10.1016/j.canlet.2018.08.021

Zhang, J., Yao, D., Jiang, Y., Huang, J., Yang, S., and Wang, J. (2017). Synthesis and biological evaluation of benzimidazole derivatives as the G9a Histone Methyltransferase inhibitors that induce autophagy and apoptosis of breast cancer cells. *Bioorg. Chem.* 72, 168–181. doi:10.1016/j.bioorg.2017.04.005

Zhang, K. J., Tan, X. L., and Guo, L. (2020). The long non-coding RNA DANCR regulates the inflammatory phenotype of breast cancer cells and promotes breast cancer progression via EZH2-dependent suppression of SOCS3 transcription. *Mol. Oncol.* 14 (2), 309–328. doi:10.1002/1878-0261.12622

Zhang, R., Li, X., Liu, Z., Wang, Y., Zhang, H., and Xu, H. (2020). EZH2 inhibitors-mediated epigenetic reactivation of FOSB inhibits triple-negative breast cancer progress. *Cancer Cell Int.* 20, 175. doi:10.1186/s12935-020-01260-5

Zhang, R., Wang, J., Zhao, L., Liu, S., Du, D., Ding, H., et al. (2018). Identification of novel inhibitors of histone acetyltransferase hMOF through high throughput screening. *Eur. J. Med. Chem.* 157, 867–876. doi:10.1016/j.ejmech.2018.08.026

Zhang, S., Tang, Z., Qing, B., Tang, R., Duan, Q., Ding, S., et al. (2019). Valproic acid promotes the epithelial-to-mesenchymal transition of breast cancer cells through stabilization of Snail and transcriptional upregulation of Zeb1. *Eur. J. Pharmacol.* 865, 172745. doi:10.1016/j.ejphar.2019.172745

Zhang, Y., Liu, J., Lin, J., Zhou, L., Song, Y., Wei, B., et al. (2016). The transcription factor GATA1 and the histone methyltransferase SET7 interact to promote VEGF-mediated angiogenesis and tumor growth and predict clinical outcome of breast cancer. *Oncotarget* 7 (9), 9859–9875. doi:10.18632/oncotarget.7126

Zhou, W., Feng, X., Han, H., Guo, S., and Wang, G. (2016). Synergistic effects of combined treatment with histone deacetylase inhibitor suberoylanilide hydroxamic acid and TRAIL on human breast cancer cells. *Sci. Rep.* 6, 28004. doi:10.1038/srep28004

Zuo, Y., Xu, H., Chen, Z., Xiong, F., Zhang, B., Chen, K., et al. (2020). 17AAG synergizes with Belinostat to exhibit a negative effect on the proliferation and invasion of MDAMB231 breast cancer cells. *Oncol. Rep.* 43 (6), 1928–1944. doi:10.3892/or.2020.7563

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 03 October 2022
DOI 10.3389/fphar.2022.965244

OPEN ACCESS

EDITED BY
Peixin Dong,
Hokkaido University, Japan

REVIEWED BY
Zhongdong Hu,
Beijing University of Chinese Medicine, China
Karen Maegley,
Pfizer, United States
Wan Yan Jun,
Peking University, China

*CORRESPONDENCE
Lixiang Xue,
lixiangxue@hsc.pku.edu.cn
Hongyan Guo,
bysyghy@163.com

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 09 June 2022
ACCEPTED 14 September 2022
PUBLISHED 03 October 2022

CITATION
Zhang X, Huo X, Guo H and Xue L (2022), Combined inhibition of PARP and EZH2 for cancer treatment: Current status, opportunities, and challenges. *Front. Pharmacol.* 13:965244. doi: 10.3389/fphar.2022.965244

Combined inhibition of PARP and EZH2 for cancer treatment: Current status, opportunities, and challenges

Xi Zhang[1], Xiao Huo[2,3], Hongyan Guo[1]* and Lixiang Xue[2,3]*

[1]Department of Obstetrics and Gynecology, Peking University Third Hospital, Haidian, China, [2]Center of Basic Medical Research, Institute of Medical Innovation and Research, Peking University Third Hospital, Haidian, China, [3]Biobank, Peking University Third Hospital, Haidian, China

Tumors with BRCA1/2 mutations or homologous recombination repair defects are sensitive to PARP inhibitors through the mechanism of synthetic lethality. Several PARP inhibitors are currently approved for ovarian, breast and pancreatic cancer in clinical practice. However, more than 40% of patients with BRCA1/2 mutations are insensitive to PARP inhibitors, which has aroused attention to the mechanism of PARP resistance and sensitization schemes. PARP inhibitor resistance is related to homologous recombination repair, stability of DNA replication forks, PARylation and epigenetic modification. Studies on epigenetics have become the hotspots of research on PARP inhibitor resistance. As an important epigenetic regulator of transcription mediated by histone methylation, EZH2 interacts with PARP through DNA homologous recombination, DNA replication, posttranslational modification, tumor immunity and other aspects. EZH2 inhibitors have been just shifting from the bench to the bedside, but the combination scheme in cancer therapy has not been fully explored yet. Recently, a revolutionary drug design combining PARP inhibitors and EZH2 inhibitors based on PROTAC techniques has shed light on the resolution of PARP inhibitor resistance. This review summarizes the interactions between EZH2 and PARP, suggests the potential PARP inhibitor sensitization effect of EZH2 inhibitors, and further discusses the potential populations that benefit from the combination of EZH2 inhibitors and PARP inhibitors.

KEYWORDS

PARP, EZH2, DNA damage repair, tumor immune microenvironment, tumor metabolism, PROTAC

Abbreviations: SSB, Single strand break; DSB, Double strand break; HRD, Homologous repair defect; HR, Homologous repair; NHEJ, Non-homologous end joining.

1 Introduction

DNA damage repair (DDR) is a guard to maintain genome stability. DDR pathways are initiated when DNA damage occurs in cells by homologous recombination repair (HR), nonhomologous end joining (NHEJ) and DNA single-strand break repair (SSBR) (Davis and Chen, 2013; Wright et al., 2018). Poly ADP-ribose polymerase (PARP) plays a key role in DNA single-strand repair; hence, the use of PARP inhibitors in tumors with DNA double-strand repair defects blocks both DNA double-strand and single-strand repair, resulting in synthetic lethal effects and antitumor effects (Sonnenblick et al., 2015).

Clinical trials of PARP inhibitors have made continuous progress in solid tumors such as breast cancer, ovarian cancer and pancreatic cancer, but the problem of drug resistance of PARP inhibitors has gradually emerged, and one of the solutions is drug combination (Lee and Matulonis, 2020; Li et al., 2020). Preclinical studies and clinical trials focused on the combination of target drugs with PARP inhibitors include cell cycle-regulating drugs, such as the inhibitors of ATR, ATM, SHK1, SHK2, and WEE1 (Li et al., 2020), antiangiogenic drugs, such as anti-VEGF. (Bizzaro et al., 2021); immune checkpoint inhibitors, such as anti-PD-1 and anti-PD-L1, some of which have entered clinical trials but have not yet achieved advanced clinical decision. How to design a drug combination program and how to determine the best indication of the combination scheme are the propositions worth considering.

Epigenetic dysregulation has long been considered a key factor affecting tumor cell fate. EZH2 (Enhancer of Zeste homolog 2) is one of the most important epigenetic factors involved in the regulation of tumorigenesis, development and metastasis. The canonical pathway of EZH2 (Enhancer of Zeste homolog 2) catalyzes the trimethylation of histone H3K27 to silence the transcription of target genes. The noncanonical pathway includes nonhistone methylation and even transcriptional activation as well as interaction with other transcription factors (Wang and Wang, 2020). Various tumors express high levels of EZH2, which is related to advanced stage and poor prognosis (Chase and Cross, 2011; Kim and Roberts, 2016). Several EZH2 inhibitors have entered clinical trials, such as tazemetostat, GSK126, CPI-1205, PF-06821497, and SHR2554. Tazemetostat is the first EZH2 inhibitor approved by the FDA for the treatment of locally advanced or metastatic epithelial sarcoma, and relapsed or refractory follicular lymphoma (Gounder et al., 2020; Morschhauser et al., 2020). However, EZH2 inhibitors still have limited efficacy in some tumors with high expression of EZH2, such as ovarian cancer, which calls for deeper exploration of new drug combination schemes (Li et al., 2021).

Although EZH2 and PARP have distinct mechanisms and functions, respectively, both EZH2 and PARP share some common features in regulating cell fate through the cell cycle, DNA damage response, programmed cell death and other biological processes (Scott et al., 2015; Alemasova and Lavrik, 2019; Laugesen et al., 2019), which may have complex interactions. Various lines of evidence indicate that PARP and EZH2 have close crosstalk; hence, PARP inhibitors and EZH2 inhibitors may have synergistic antitumor or antagonistic effects. This review summarizes the progress on the interaction of PARP and EZH2, focusing on the aspects of DNA damage repair and the direct modification that PARP adds to EZH2, and analyzes the possible relationship between PARP and EZH2 in the tumor immune and metabolic microenvironment. In addition, advanced techniques for drug design that boost the combination of PARPi and EZH2i are also discussed.

2 Seesaw effect: The promotion of DNA repair by EZH2 is released by Poly ADP-ribose polymerase inhibitors

2.1 EZH2 is involved in the DNA damage response of tumor cells

EZH2 is one of the key factors in the response of tumor cells to DNA damage and determines their subsequent cell fate. Cells recognize DNA damage sites through two cell cycle checkpoints, namely, G1/S and G2/M, and induce cell cycle arrest, allowing cells to stay in G1 or G2 phase for DNA damage repair (Wu et al., 2011). In tumor cells with DNA double-strand damage induced by adriamycin (ADR) and etoposide (ETO), knockdown of EZH2 can mediate the deactivation of both G1/S and G2/M cell cycle checkpoints and induce apoptosis by a mechanism that depends on the presence of p53 mutations in tumor cells. Tumor cells with wild-type p53 respond to DNA damage and promote DNA damage repair through the p53-p21 pathway. FBXO32 is a target of EZH2 (Ciarapica et al., 2014; Wang et al., 2018) and is involved in mediating the degradation of the proteasome pathway of p53 downstream molecule p21; hence, the knockdown of EZH2 upregulates FBXO32 and further blocks DNA damage repair. In p53 mutant tumor cells, phosphorylation of the cell cycle checkpoint kinase ChK1 is involved in mediating the G2/M cell cycle block in response to DNA damage. EZH2 inhibitors downregulate the level of ChK1 phosphorylation through an unknown mechanism, thereby inhibiting the DNA damage response (Wu et al., 2011), and ChK1 inhibitors are more sensitive in EZH2-deficient tumor cells, more apparently inducing cell apoptosis (Leon et al., 2020).

2.2 EZH2 impacts DNA damage repair in tumor cells

EZH2 inhibitors harm homologous recombination repair in ovarian cancer cell lines by downregulating the expression

of nonhomologous recombination repair-associated genes and thus inhibiting homologous recombination repair by treating them with EZH2 inhibitors. This mechanism of EZH2 involvement in regulating the DNA damage repair modality is CARM1-dependent (Karakashev et al., 2020). CARM1 is an arginine methyltransferase that transcriptionally represses the subunit BAF155 of the SWI/SNF complex (SWI/SNF complex), which is involved in the regulation of chromosome remodeling and is antagonistic to the PRC2 complex with EZH2 as the catalytic subunit (Kadoch et al., 2016); thus, high expression of CARM1 upregulates EZH2. In ovarian cancer cell lines with high CARM1 expression, EZH2 levels are upregulated, allowing activation of homologous recombination repair by exerting transcriptional repression on nonhomologous recombination repair-related genes, such as MAD2L2 (mitotic arrest deficient 2 like 2, MAD2L2) (Karakashev et al., 2020).

SLFN11 (schlafen family member 11) is recruited to DNA damage sites and inhibits homologous repair (Mu et al., 2016), which could sensitize the effect of DNA-damaging agents, such as PARP inhibitors and cisplatin (Stewart et al., 2017). The inactivation of SLFN11 is related to resistance to PARP inhibitors (Coussy et al., 2020), and reactivating SLFN11 by epigenetic agents could alleviate the resistance of PARP inhibitors (Murai et al., 2016; Tang et al., 2018). SLFN11 is a target gene of EZH2, and DNA damage-induced EZH2 activation suppresses the expression of SLFN11(Gardner et al., 2017). In small cell lung cancer, EZH2 inhibitors could release the expression of SLFN11, which may sensitize PARP inhibitors (Sabari et al., 2017).

In summary, EZH2 inhibitors assist PARP inhibitors in mimicking “drug-induced synthetic lethality".

2.3 Orchestration of EZH2 and Poly ADP-ribose polymerase in DNA damage repair in tumor cells

EZH2 affects PARP-associated DNA damage repair through the canonical pathway in an H3K27me3-dependent manner. Traditionally, EZH2 acts as a histone methyltransferase catalyzing H3K27me3, which transcriptionally inactivates target genes. During the DNA damage response, EZH2 localizes to DNA damage sites in the nucleus, and this process is accompanied by the upregulation of global H3K27me3 levels (Yamamoto et al., 2019). As previously described, EZH2 affects the DNA damage repair response in tumor cells, and experiments in ovarian cancer cell lines, mouse models, and PDX models of ovarian cancer patients have verified that EZH2 promotes homologous recombination repair through H3K27me3 modification, while EZH2 inhibitor treatment prevents homologous recombination repair, thereby enhancing the antitumor effects of PARP inhibitors (Karakashev et al., 2020), which is described as a pharmacological synthetic lethal condition (e.g., Figure 1). Additionally, EZH2 also affects PARP expression levels by regulating PARP degradation. Fas-associated death domain (FADD) is a member of the tumor necrosis factor receptor superfamily that activates the downstream caspase cascade response to mediate programmed cell death (Gurung et al., 2014). EZH2 downregulates the transcriptional level of FADD, decreasing the degradation of PARP mediated by FADD and thus upregulating PARP expression (Han et al., 2020). Therefore, EZH2 inhibitors probably have the capacity to synchronize both the single-strand DNA damage-repair function and the expression of PARP simultaneously.

EZH2 may also be involved in PARP-associated DNA damage responses in tumor cells *via* a nonhistone methyltransferase catalytic pathway. In a human osteosarcoma cell line with DNA damage induced by ionizing radiation, EZH2 was recruited to the DNA double-strand damage site marked by γ-H2AX, and Suz12 and EED were also recruited to DNA damage sites as other components of the PRC2 complex; however, with PARP inhibitor treatment, EZH2 was unable to localize at the DNA double-strand damage site, suggesting that this process is PARPdependent. However, immunofluorescence revealed that these DNA damage sites did not overlap with elevated H3K27me3 markers, suggesting that EZH2 did not catalyze H3K27me3 during this process (Campbell et al., 2013). Another study showed only transient elevation of H3K27me3 at the DNA damage site, followed by rapid disappearance (Chou et al., 2010), suggesting that EZH2 may sequentially act through a noncanonical pathway in a canonical manner, but the mechanism remains to be elucidated.

Additionally, the expression of EZH2 is also affected by PARP inhibition. In a lymphoblastoid B-cell line, the inhibition of PARP reduces the expression of EZH2, followed by an elevation of global H3K27me3 (Martin et al., 2015). PARylation modified by PARP affects both the expression and activity of EZH2, which is discussed in section 3.1.

The application of PARP inhibitors in tumors with BRCA mutations blocks both DNA single-strand and double-strand damage repair, the classic synthetic lethal mechanism, whereas in BRCA wild-type tumors, even though PARP inhibitors prevent the repair of DNA single-strand breaks and further DNA double-strand breaks occur, tumor cells can still repair DNA damage due to homologous recombination repair. EZH2 inhibitors promote nonhomologous recombination repair and inhibit homologous recombination repair by deregulating histone trimethylation-mediated transcriptional repression of target genes, thus mimicking “drug-induced synthetic lethality” in concert with PARP inhibitors.

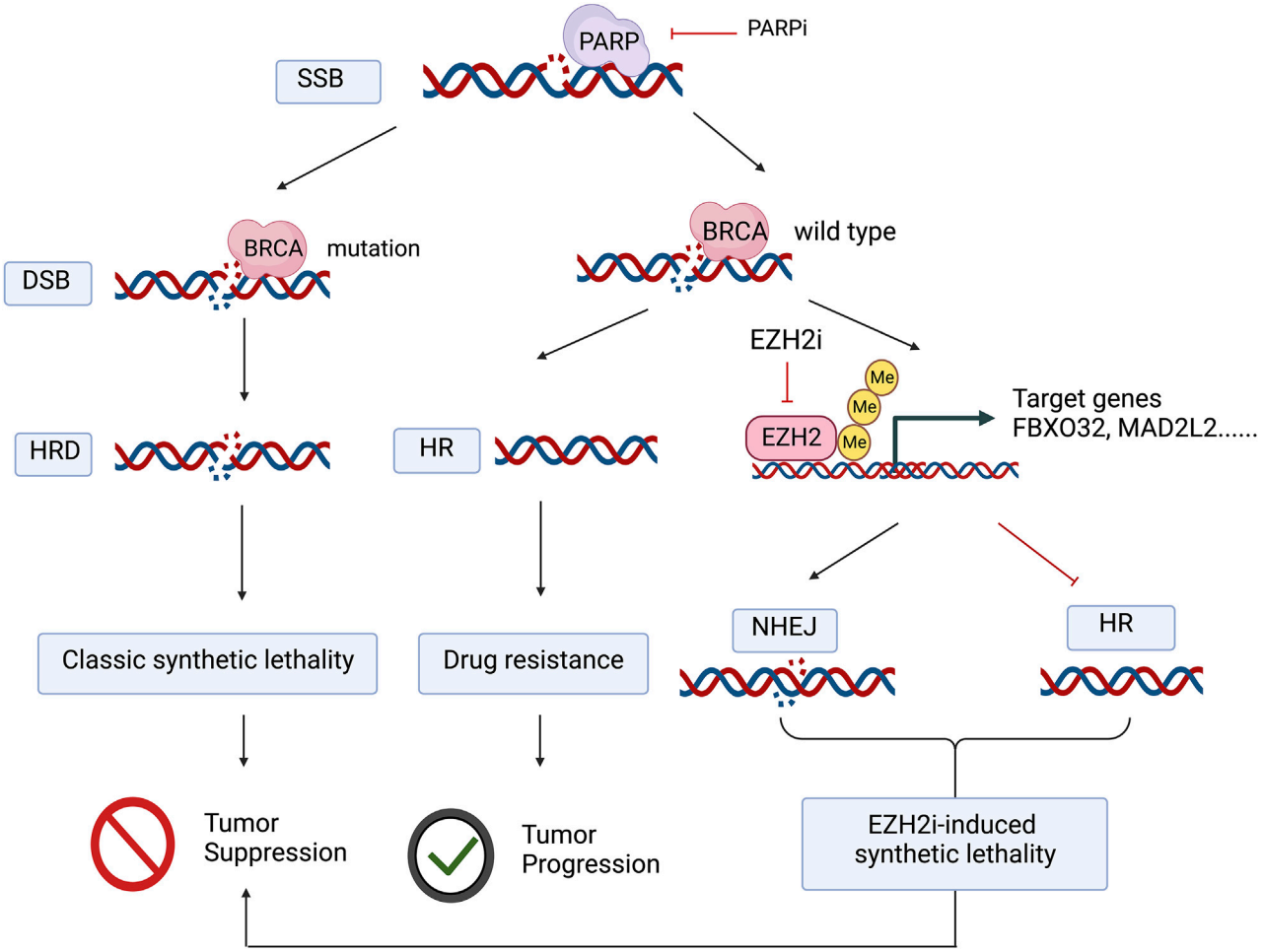


FIGURE 1
The application of PARP inhibitors in tumors with BRCA mutations blocks both DNA single-strand and double-strand damage repair, the classic synthetic lethal mechanism, whereas in BRCA wild-type tumors, even though PARP inhibitors prevent the repair of DNA single-strand breaks and further DNA double-strand breaks occur, tumor cells can still repair DNA damage due to homologous recombination repair. EZH2 inhibitors promote nonhomologous recombination repair and inhibit homologous recombination repair by deregulating histone trimethylation-mediated transcriptional repression of target genes, thus mimicking "drug-induced synthetic lethality" in concert with PARP inhibitors. Figure 1 was created with BioRender.com with a publication license.

3 Association between EZH2 and Poly ADP-ribose polymerase in the tumor microenvironment

3.1 PARylation modification of EZH2 directly by Poly ADP-ribose polymerase

PARP consists of catalytic and regulatory subunits and acts as a ribosylase in posttranslational modification, using NAD^+ as a substrate for ADP-ribose. Among the members of the PARP family (PARP1-16), PARP3, PARP6-12 and PARP14-16 catalyze mono-ADP-ribosylation of proteins, while PARP1, PARP2, PARP4, and PARP5a/b catalyze poly-ADP-ribosylation (PARylation) (Min and Im, 2020; Sanderson and Cohen, 2020). The subcellular localization determines whether PARP is involved in intranuclear events, such as epigenetic modification of DNA and histones (Ciccarone et al., 2017), DNA damage repair (Min and Im, 2020), and RNA metabolism (Ke et al., 2019), resulting in divergent cell fates, such as survival or apoptosis (Virag et al., 2013).

PARP inhibitors, including olaparib, rucaparib and niraparib, target PARP1 and PARP2, which are localized in the nucleus and are able to directly modify the PARylation of EZH2 under specific conditions. With DNA damage induced by alkylating agents in tumor cells, PARP1 reduces the affinity of EZH2 for H3K27 sites and inhibits its enzymatic activity through PARylation, while poly ADP-ribose glycohydrolase (PARG) can

reverse this effect (Caruso et al., 2018). This conclusion has been confirmed by studies based on breast and ovarian cancer cell lines, where PARP1 increased the PARylation modification of EZH2 during alkylating agent-induced DNA damage or hydrogen peroxide-induced oxidative stress, further inducing the breakdown of the PRC2 complex and degradation of EZH2 (Yamaguchi et al., 2018). Thus, direct modification of EZH2 by PARP1 may be involved in regulating the response of tumor cells to DNA damage and oxidative stress, but it remains unclear which downstream signaling pathways may be altered as a result of such modifications.

3.2 Association between EZH2 and Poly ADP-ribose polymerase in the tumor immune microenvironment

Although studies have shown the promising combination of EZH2 inhibitors and PARP inhibitors in tumor therapy, the combination scheme of EZH2 inhibitors and PARP inhibitors does not always show antitumor effects, which may be related to the tumor immune microenvironment. Immune cells in the tumor microenvironment include $CD4^+$ T cells, $CD8^+$ T cells, and dendritic cells, which mainly play antitumor roles, and regulatory T cells (Tregs), as well as MDSCs with immunosuppressive effects. Additionally, tumor-associated macrophages (TAMs) play a bidirectional role in tumor immunity through the M1 and M2 polarization directions (Pantelidou et al., 2019; Ding et al., 2020; Ghonim et al., 2021; Wu et al., 2021). Immune cell infiltration in the tumor microenvironment as well as the interaction between immune cells, tumor cells and stromal cells may influence the effect of antitumor drugs, and cytokines and chemokines are involved in mediating such effects (Wu and Dai, 2017). For example, experiments in tumor-bearing mice revealed that IL-17 secreted by helper T cells (T helper 17, Th17) in the tumor microenvironment promotes upregulation of granulocyte colony stimulating factor (G-CSF) levels through the NF-κB and ERK signaling pathways, and myeloid-derived suppressive cell (Myeloid-derived suppressor cells, MDSC) infiltration increases and induces tumor resistance to anti-VEGF-targeted drugs (Chung et al., 2013).

EZH2 may affect the antitumor effects of PARP inhibitors by remodeling the tumor microenvironment. Evidence has shown that the PARP inhibition effect is dependent on the infiltration degree of T cells (Pantelidou et al., 2019; Sen et al., 2019); however, EZH2 downregulates the degree of T-cell infiltration in the tumor microenvironment (Peng et al., 2015), which hurdles the effect of PARP inhibitors. EZH2 inhibitors increase the infiltration of T cells in the tumor microenvironment (Zingg et al., 2017; Goswami et al., 2018). *In vitro* cellular assays have demonstrated that the PARP inhibitor olaparib activates the cGAS/STING pathway in triple-negative breast cancer cells, and *in vivo* experiments have further revealed that olaparib also induces the activation of the STING/TBK1/IRF3 pathway, which assists DCs in recognizing tumor antigens and further recruits and activates $CD8^+$ T cells. Consistent with this, the sensitivity of olaparib was decreased in tumor-bearing mice with knockdown of $CD8^+$ T cells (Pantelidou et al., 2019). Not coincidentally, EZH2 inhibitors were also able to promote STING pathway-mediated T-cell infiltration and antitumor effects (Morel et al., 2021; Xu et al., 2021), suggesting that these two inhibitors may play synergistic roles in modulating the tumor immune microenvironment.

However, the combination of EZH2 inhibitors with PARP inhibitors can also negatively affect immune cells in the tumor microenvironment, such as macrophages. Macrophages in the tumor microenvironment are divided into M1-type macrophages, which exert antitumor effects, and M2-type macrophages, which exert protumor effects, and different members of the colony-stimulating factor family are able to modulate the polarization of macrophages toward M1 or M2, respectively (Wang et al., 2014). In human breast cancer cell line MB-231 tumor-bearing mice, the combination of PARP inhibitor and EZH2 inhibitor or the simultaneous knockdown of PARP and EZH2 promoted the polarization of tumor-associated macrophages (TAMs) toward M2 and the generation of neovascularization in tumors (Yang et al., 2020).

In summary, EZH2 inhibitors upregulate the infiltration of immune cells in the tumor microenvironment and induce reprogramming of immunosuppressive cells, which enhances the antitumor efficacy of PARP inhibitors. On the other hand, the combination of these two drugs also disturbs the tumor immune microenvironment; therefore, the combination of the two drugs needs to be carefully discussed in various tumors.

3.3 Potential coordination between EZH2 inhibitors and Poly ADP-ribose polymerase inhibitors in tumor metabolism

Tumor cells compete with other cells in the microenvironment for metabolic materials to create a favorable microenvironment. The altered metabolic pattern of tumor cells involves glucose metabolism (Lin et al., 2020), lipid metabolism (Snaebjornsson et al., 2020), amino acid metabolism (Bott et al., 2019), etc., and drug resistance can be also induced by altered tumor metabolism under the intervention of antitumor drugs, while reprogramming of metabolism may exist as a potential new drug target (Li and Zhang, 2016).

The hub between PARP and EZH2 in metabolism may lie in NAD+/NADH and NADP+/NADPH. PARP posttranslationally modifies proteins in an NAD^+-dependent manner, converting NAD^+ to nicotinamide (NAM), which is synthesized by the

remedial synthesis pathway to restore NAD^+ levels. NAD^+ is generated as $NADP^+$ by the action of kinase, and the reduction products are NADH and NADPH, respectively. NAD^+/NADH and $NADP^+$/NADPH are involved in glycolysis, nucleotide synthesis and fatty acid synthesis as important cofactors (Bian et al., 2019; Navas and Carnero, 2021). EZH2 downregulates aldehyde oxidase 1 (AOX1) expression levels in a H3K27me3-dependent manner, which activates the tryptophan-kynurenine pathway and further increases the synthesis of NADP (Vantaku et al., 2020), which is the same downstream product of PARP catalysis.

Another intertwined point between PARP and EZH2 could be concluded in the aspect of lipid metabolism. Firstly, PARP and lipid metabolism are closely linked, and lipid metabolism affects PARP expression (Zhang et al., 2014; Qin et al., 2018) and enzymatic activity (Lin et al., 2008). PARP is associated with fatty acid synthesis (Szántó et al., 2021), lipid peroxidation (Bai et al., 2011; Huang et al., 2017), adipocyte differentiation (Szántó and Bai, 2020), and other lipid metabolic processes. Note worthily, these processes can affect the expression and activity of EZH2 or are regulated by EZH2 (Zhang et al., 2022). RNAseq and proteomic data suggest that the PARP inhibitor olaparib causes cells to undergo altered lipid metabolism, highlighted by processes such as fatty acid biosynthesis and fatty acid *ß*-oxidation (Mehta et al., 2021). The expression of sterol regulatory element-binding protein 1 (SREBP1), a transcription factor that promotes lipid synthesis, especially cholesterol synthesis is downregulated by posttranslational modification of PARP, and knockdown of PARP or treatment with PARP inhibitors both promote hepatic lipid accumulation (Szanto et al., 2014; Pang et al., 2018).

Secondly, PARP is able to alter the ratio of polyunsaturated fatty acid (PUFA) composition in skin tissues. For example, DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) levels can be upregulated and further leads to the formation of a proinflammatory microenvironment (Kiss et al., 2015). At this point, it was also found that applying a certain type of EZH2 inhibitor or shRNA-EZH2 knockdown approach, PUFA was upregulated in multiple solid tumor cell lines and animal models (Zhang et al., 2022). Moreover, in breast cancer cells, ω-3 polyunsaturated fatty acids such as DHA and EPA induce the degradation of EZH2 by the proteasome pathway, thereby downregulating EZH2 protein levels and alleviating the transcriptional repression of EZH2 target genes such as E-cadherin and IGFBP3(Dimri et al., 2010).

Thirdly, as a key regulator family in lipid metabolism, peroxisome proliferator-activated receptor (PPAR) is a member of the intranuclear receptor transcription factor superfamily, which mainly includes PPARα, PPARβ and PPARγ(Wang et al., 2020). PPARα is mainly expressed in hepatocytes, cardiomyocytes and brown adipocytes. PPARα target genes are key enzymes for fatty acid oxidation, so PPARα has an important role in regulating fatty acid oxidation (Kersten, 2014). The EZH2-PPARγ axis has been confirmed to promote cancer proliferation (Hu et al., 2021). The posttranslational modification of PPARα by PARP and SIRT1 (Sirtuin) has a competitive effect, and PARP prevents the binding of PPARα to the promoter region of fatty acid oxidation-related genes through PARylation modification, while SIRT1 catalyzes deacetylation to promote the localization of PPARα in the promoter region of target genes, thereby inhibiting fatty acid oxidation (Huang et al., 2017). It has been suggested that the deacetylation of EZH2 by SIRT1 inhibits the binding of EZH2 to its target genes and weakens the pro-carcinogenic effect of EZH2 (Wan et al., 2015); on the other hand, knockdown or inhibition of EZH2 can promote the expression of SIRT1. It can be speculated that there may be an intersection between EZH2 and PARP-induced downstream metabolism-related changes and vice versa.

At last point, both PARP and EZH2 are involved in the adipocytes differentiation and the formation of lipid droplet, respectively. Adipocytes eventually differentiate into brown, white and beige adipocytes. Adipocyte terminal differentiation-related genes are regulated by PPARγ. PPARγ is mainly expressed in adipose tissue (Cristancho and Lazar, 2011) and promotes adipocyte differentiation by forming a positive feedback loop with C/EBPα (Cebpb CCAAT/enhancer binding protein) (Farmer, 2006). During adipocyte differentiation, PARP was recruited to the promoter regions of PPARγ2 target genes such as CD36 and aP2 to promote the expression of both genes. This process was accompanied by the downregulation of H3K9me3 and upregulation of H3K4me3 in the promoter region of PPARγ2 (Erener et al., 2012), suggesting that PARP may be involved in regulating adipocyte differentiation by affecting epigenetic modifications. In line with this phenomenon, EZH2 coincides with the ability to catalyze histone methylation in the promoter region of PPARγ2, the result of which promotes processes such as liver fibrosis (Du et al., 2021) and pancreatic cancer cell proliferation (Hu et al., 2021). Interestingly, our previous research has also found EZH2 inhibitor GSK126 upregulates the expression level of fatty acid synthesis related genes and results in lipid droplet accumulation in liver (Zhang et al., 2022). Hence, although there is still a lack of direct evidence between PARP and EZH2 in regulating or being regulated in lipid metabolic processes, several hints have indicated the possibility of an interaction between them in the given circumstance.

3.4 The limitation of the combined strategy due to tumor-suppressor role of EZH2

Beyond the traditional oncogenic role, EZH2 also acts as a tumor-suppressor in certain condition, which possibly brings limitation to the combined strategy (Gan et al., 2018). In Kras-driven lung adenocarcinoma mouse model, loss of *Ezh2* release the insulin-like growth factor 1 (Igf1), further amplify the

TABLE 1 EZH2 inhibitors in combination with PARP inhibitors in pre-clinical researches.

PARPi + EZH2i	EZH2 target gene	Cancer	Genetic characters	Material	Methods	Results	Year
Olaparib + EZH2 siRNA	β-catenin	Ovarian cancer	BRCA1/2^{wild}	Ovarian cancer cell line HeyA8	CCK8	si-EZH2 increases the sensitivity of Olaparib by regulating *β*-catenin signal pathway	2021Sun et al., 2021
Olaparib + GSK126	MAD2L2	Ovarian cancer	CARM1^{high}BRCA1/2^{wild}	CARM1^{high} A1847 and CARM1^{KO} A1847 cell line; subcutaneous xenograft mice models	colony formation assays	Olaparib and GSK126 show synergistic effect in suppressing CARM1-high *in vitro* and *in vivo*	2020 Karakashev et al., 2020
				CARM1^{high}and CARM1^{low} patient-Derived Xenografts mice models	Xenograft Models	The mechanism is that EZH2 inhibition induces MAD2L2 expression and non-homologous end-joining	
Dual PARP and EZH2 inhibitor	—	Breast cancer	ER (-)PR (-)HER2(-)BRCA1/2^{wild}	TNBC cell lines MDA-MB-231 and MDA-MB-468	MTT assay	Dual target agent shows better inhibitory activity than single agent of Olaparib or EZH2, and their combined treatment	2021 Wang et al., 2021
Olaparib + GSK126, PARP^{KO} + EZH2^{KO}	RELA/B	Breast cancer	ER (-)PR (-)HER2(-)BRCA1/2^{wild}	TNBC cell lines MDA-MB-231	CellTiter-Glo Assay Kit, colony formation assays	PARP1-PRC2 double depletion, and combined administration of Olaparib and GSK126 promotes cancer growth	2020 Yang et al., 2020
Olaparib + GSK343	HOXA9, DAB2IP	Breast cancer	EZH2^{high}BRCA^{mut}	BRCA^{mut} cell lines SUM149, MDA-MD-436 and UWB1.289	Colony formation assay and soft agar assay	EZH2 inhibitor sensitizes PARP inhibitor in BRCA^{mut} cell lines	2018 Yamaguchi et al., 2018
Olaparib + GSK126	MUS81	Breast cancer	BRCA2$^{-/-}$	HeLa, VU423 (BRCA2$^{-/-}$), A2780, U2OS, HEK 293T cell lines; KB2P PARPi-naïve tumor-bearing mice model	Clonogenic survival assay; Xenograft Models	EZH2 inhibitor promotes PARP inhibitor resistance by stop recruiting MUS81 and cause fork stabilization	2017 Rondinelli et al., 2017
Olaparib + UNC1999	—	Acute myeloid leukemia	BRCA1$^{-/-}$	LCLs, HeLa and HEK293 cell lines	Bio Rad TC20 Automated Cell Counter	EZH2 inhibitor sensitizes PARP inhibitor in BRCA cells	2018 Caruso et al., 2018
				BRCA1-mutated and BRCA1-reconstituted MDA-MB-436 cell lines			

activation of Akt-ERK signaling and promote tumor formation (Wang et al., 2017). In pediatric high-grade gliomas, specifically diffuse midline gliomas (DMG) which is characterized by the signature K27M mutation in histone H3, EZH2 ablation promotes tumor cell proliferation, while EZH2 overexpression reverses this effect in H3WT DMG mouse models (Dhar et al., 2022). Therefore, the combination strategy should be cautiously evaluated in different type of tumors due to the double-edge effect of EZH2.

4 Preclinical studies of combination strategy of EZH2 inhibitors and Poly ADP-ribose polymerase inhibitors

The regimens of EZH2 inhibitors in combination with other drugs include those with chemotherapeutic agents, immunotherapy and targeted therapies. The combined effects of EZH2 inhibitors and PARP inhibitors are summarized in Table 1 and vary in different cancer types. Currently,

experiments and clinical trials raise a question worthy of consideration: which population benefits more from a combination of EZH2 inhibitors and PARP inhibitors? The BRCA mutation state is a distinguishing feature, and the effect of combination rules still relies on the state of BRCA deficiency (Wicha, 2009; Schlacher, 2017; Chen, 2021); however, studies in ovarian cancer suggest that EZH2 inhibitors sensitize PARP inhibitors in CARM1-high patients and that the CARM1-high population highly overlaps with wild-type BRCA, suggesting that EZH2 inhibitors are expected to be an effective drug combination regimen for PARP inhibitors in specific ovarian cancer patient groups, further expanding the applicability of PARP (Hatchi and Livingston, 2020; Karakashev and Zhang, 2020).

It is noteworthy that in certain genetically characterized populations, the combination of EZH2 inhibitors with PARP inhibitors shows negative effects. In BRCA2-deficient breast cancer cell lines, the combination of the EZH2 inhibitor GSK126 and the PARP inhibitor rucaparib diminishes the single-agent antitumor effect of the latter. A similar effect is confirmed in animal model, that EZH2 inhibitors induce PARP inhibitor resistance (Rondinelli et al., 2017). The EZH2 inhibitor interferes with the localization of MUS81 to replication forks, thereby enhancing DNA stability and further inducing PARP inhibitor resistance.

In addition, the drug structure may also affect the effect of the combination of the two drugs, and how to design the structure of the targeted drug is a proposition worthy of consideration. An ideal drug is to inhibit the enzyme activity along with the protein levels of both PARP and EZH2, and this concept is expected to be realized by proteolysis-targeting chimera (PROTAC). By anchoring the ubiquitin ligase E7 at the appropriate site of the EZH2 inhibitor GSK126 molecule by PROTAC technology, EZH2 enzyme activity was inhibited while inducing the degradation of its proteasome pathway, and the levels of other components of the PRC2 complex, such as SUZ12 and EED, were simultaneously downregulated (Liu et al., 2021). Adding a linker connected to the EZH2 inhibitor EPZ6438 to E3 ligase systems also shows a profound effect (Tu et al., 2021). Therefore, both the canonical and noncanonical oncogenic pathways of EZH2 are blocked by the PROTAC strategy (Wang et al., 2022). A novel PARP inhibitor anchored by ubiquitin ligase E3, designed based on PROTAC technology, has also been reported to have a significantly lower IC50 than the conventional PARP inhibitor Niraparib (Zhao et al., 2019). In addition, a new compound as dual-target inhibitor of PARP and EZH2, synthesized on the basis of olaparib and tazemetostat by linking the two drug molecules through hydrogen bonding, inhibited tumors 15–80 times more effectively than the PARP inhibitor alone in a BRCA wild-type triple-negative breast cancer cell line (Wang et al., 2021).

5 Perspectives

With the development of PARP clinical trials in ovarian cancer, studies related to drug resistance to PARP inhibitors are gradually receiving attention. Clinical studies of PARP in combination with other drugs have focused on kinase inhibitors, WEE inhibitors, immunotherapy, and other drugs, and epigenetic drugs may play an important role as potential drug combination solutions. However, the following aspects are noteworthy:

First, how to design the drug combination regimen. The combined dose and administration method of two or more drugs are prominent factors that may affect their effects. Second, how to determine the best population for the combination of drugs. Both PARP and EZH2 have a wide range of biological functions in addition to acting on tumor cells, such as being involved in various cells, including immune cells and adipocytes, and they also have a regulatory effect on tumor metabolism and immune function. EZH2 acts both tumor-promoter and tumor-suppressor roles in certain type of cancer. Therefore, it is necessary to comprehensively evaluate the molecular characteristics, immune typing, and metabolic typing of tumors to confirm the indications for combination therapy. Third, the design of new drugs based on existing drug combinations is promising. The most excited advance mentioned above has been reported lately that the compound with dual PARP and EZH2 inhibitors, showed good inhibitory activity against PARP-1 and EZH2 and good inhibitory effects on multiple type of triple-negative breast cancer (TNBC) cell lines with wild-type BRCA, with a slight harm on normal cells, suggesting possible safety of the combined strategy in clinical context (Wang et al., 2021). However, the evidences *in vivo* are still required. Besides, simultaneous inhibition of enzyme activity and protein expression will be considered in the future to improve the efficacy of tumor treatment. Fourth, targeting drug delivery and enrichment is a proposition that deserves further exploration. Both EZH2 and PARP1 are multitargets, so targeting tumor cells through novel drug loading and delivery systems is one of the solutions to improve the existing therapeutic efficacy in this case. The current nanodelivery, targeted loading, and delivery systems that target tumor cells, indicating markers, offer technical possibilities for this strategy.

Author contributions

LX and HG contributed to the concept development and outline arrangement. XZ and XH contributed to relevant references collecting and essay editing. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

Conflict of interest

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

The reviewer WJ declared a shared parent affiliation with the authors to the handling editor at the time of review.

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/fphar.2022.965244/full#supplementary-material

References

Alemasova, E. E., and Lavrik, O. I. (2019). Poly(ADP-ribosyl)ation by PARP1: Reaction mechanism and regulatory proteins. *Nucleic Acids Res.* 47, 3811–3827. doi:10.1093/nar/gkz120

Bai, P., Cantó, C., Oudart, H., Brunyánszki, A., Cen, Y., Thomas, C., et al. (2011). PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metab.* 13, 461–468. doi:10.1016/j.cmet.2011.03.004

Bian, C., Zhang, C., Luo, T., Vyas, A., Chen, S.-H., Liu, C., et al. (2019). NADP(+) is an endogenous PARP inhibitor in DNA damage response and tumor suppression. *Nat. Commun.* 10, 693. doi:10.1038/s41467-019-08530-5

Bizzaro, F., Fuso Nerini, I., Taylor, M. A., Anastasia, A., Russo, M., Damia, G., et al. (2021). VEGF pathway inhibition potentiates PARP inhibitor efficacy in ovarian cancer independent of BRCA status. *J. Hematol. Oncol.* 14, 186. doi:10.1186/s13045-021-01196-x

Bott, A. J., Maimouni, S., and Zong, W. X. (2019). The pleiotropic effects of glutamine metabolism in cancer. *Cancers* 11, E770. doi:10.3390/cancers11060770

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, 2675–2683. doi:10.4161/cc.25795

Caruso, L. B., Martin, K. A., Lauretti, E., Hulse, M., Siciliano, M., Lupey-Green, L. N., et al. (2018). Poly(ADP-ribose) Polymerase 1, PARP1, modifies EZH2 and inhibits EZH2 histone methyltransferase activity after DNA damage. *Oncotarget* 9, 10585–10605. doi:10.18632/oncotarget.24291

Chase, A., and Cross, N. C. P. (2011). Aberrations of EZH2 in cancer. *Clin. Cancer Res.* 17, 2613–2618. doi:10.1158/1078-0432.CCR-10-2156

Chen, M.-K. (2021). Efficacy of PARP inhibition combined with EZH2 inhibition depends on BRCA mutation status and microenvironment in breast cancer. *Febs J.* 288, 2884–2887. doi:10.1111/febs.15730

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, 18475–18480. doi:10.1073/pnas.1012946107

Chung, A. S., Wu, X. M., Zhuang, G. L., Ngu, H., Kasman, I., Zhang, J. H., et al. (2013). An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy. *Nat. Med.* 19, 1114–1123. doi:10.1038/nm.3291

Ciarapica, R., De Salvo, M., Carcarino, E., Bracaglia, G., Adesso, L., Leoncini, P. P., et al. (2014). The Polycomb group (PcG) protein EZH2 supports the survival of PAX3-FOXO1 alveolar rhabdomyosarcoma by repressing FBXO32 (Atrogin1/MAFbx). *Oncogene* 33, 4173–4184. doi:10.1038/onc.2013.471

Ciccarone, F., Zampieri, M., and Caiafa, P. (2017). PARP1 orchestrates epigenetic events setting up chromatin domains. *Semin. Cell Dev. Biol.* 63, 123–134. doi:10.1016/j.semcdb.2016.11.010

Coussy, F., El-Botty, R., Chateau-Joubert, S., Dahmani, A., Montaudon, E., Leboucher, S., et al. (2020). BRCAness, SLFN11, and RB1 loss predict response to topoisomerase I inhibitors in triple-negative breast cancers. *Sci. Transl. Med.* 12, eaax2625. doi:10.1126/scitranslmed.aax2625

Cristancho, A. G., and Lazar, M. A. (2011). Forming functional fat: A growing understanding of adipocyte differentiation. *Nat. Rev. Mol. Cell Biol.* 12, 722–734. doi:10.1038/nrm3198

Davis, A. J., and Chen, D. J. (2013). DNA double strand break repair via non-homologous end-joining. *Transl. Cancer Res.* 2, 130–143. doi:10.3978/j.issn.2218-676X.2013.04.02

Dhar, S., Gadd, S., Patel, P., Vaynshteyn, J., Raju, G. P., Hashizume, R., et al. (2022). A tumor suppressor role for EZH2 in diffuse midline glioma pathogenesis. *Acta Neuropathol. Commun.* 10, 47. doi:10.1186/s40478-022-01336-5

Dimri, M., Bommi, P. V., Sahasrabuddhe, A. A., Khandekar, J. D., and Dimri, G. P. (2010). Dietary omega-3 polyunsaturated fatty acids suppress expression of EZH2 in breast cancer cells. *Carcinogenesis* 31, 489–495. doi:10.1093/carcin/bgp305

Ding, L. Y., Wang, Q. W., Kearns, M., Jiang, T., Konstantinopoulos, P., Matulonis, U., et al. (2020). PARP inhibition modulates the tumor immune microenvironment in Brca1-deficient ovarian tumor. *Clin. Cancer Res.* 26, 23.

Du, Z. P., Liu, M., Wang, Z. H., Lin, Z. Y., Feng, Y. Y., Tian, D., et al. (2021). EZH2-mediated inhibition of KLF14 expression promotes HSCs activation and liver fibrosis by downregulating PPARγ. *Cell Prolif.* 54. doi:10.1111/cpr.13072

Erener, S., Hesse, M., Kostadinova, R., and Hottiger, M. O. (2012). Poly(ADP-ribose)polymerase-1 (PARP1) controls adipogenic gene expression and adipocyte function. *Mol. Endocrinol.* 26, 79–86. doi:10.1210/me.2011-1163

Farmer, S. R. (2006). Transcriptional control of adipocyte formation. *Cell Metab.* 4, 263–273. doi:10.1016/j.cmet.2006.07.001

Gan, L., Yang, Y., Li, Q., Feng, Y., Liu, T., and Guo, W. (2018). Epigenetic regulation of cancer progression by EZH2: From biological insights to therapeutic potential. *Biomark. Res.* 6, 10. doi:10.1186/s40364-018-0122-2

Gardner, E. E., Lok, B. H., Schneeberger, V. E., Desmeules, P., Miles, L. A., Arnold, P. K., et al. (2017). Chemosensitive relapse in small cell lung cancer proceeds through an EZH2-SLFN11 Axis. *Cancer Cell* 31, 286–299. doi:10.1016/j.ccell.2017.01.006

Ghonim, M. A., Ibba, S. V., Tarhuni, A. F., Errami, Y., Luu, H. H., Dean, M. J., et al. (2021). Targeting PARP-1 with metronomic therapy modulates MDSC suppressive function and enhances anti-PD-1 immunotherapy in colon cancer. *J. Immunother. Cancer* 9, e001643. doi:10.1136/jitc-2020-001643

Goswami, S., Apostolou, I., Zhang, J., Skepner, J., Anandhan, S., Zhang, X., et al. (2018). Modulation of EZH2 expression in T cells improves efficacy of anti-CTLA-4 therapy. *J. Clin. Invest.* 128, 3813–3818. doi:10.1172/JCI99760

Gounder, M., Schöffski, P., Jones, R. L., Agulnik, M., Cote, G. M., Villalobos, V. M., et al. (2020). Tazemetostat in advanced epithelioid sarcoma with loss of INI1/SMARCB1: An international, open-label, phase 2 basket study. *Lancet. Oncol.* 21, 1423–1432. doi:10.1016/S1470-2045(20)30451-4

Gurung, P., Anand, P. K., Malireddi, R. K. S., Walle, L. V., Van Opdenbosch, N., Dillon, C. P., et al. (2014). FADD and caspase-8 mediate priming and activation of the canonical and noncanonical Nlrp3 inflammasomes. *J. Immunol.* 192, 1835–1846. doi:10.4049/jimmunol.1302839

Han, B., Meng, X., Wu, P., Li, Z., Li, S., Zhang, Y., et al. (2020). ATRX/EZH2 complex epigenetically regulates FADD/PARP1 axis, contributing to TMZ resistance in glioma. *Theranostics* 10, 3351–3365. doi:10.7150/thno.41219

Hatchi, E., and Livingston, D. M. (2020). Opening a door to PARP inhibitor-induced lethality in HR-proficient human tumor cells. *Cancer Cell* 37, 139–140. doi:10.1016/j.ccell.2020.01.005

Hu, J. L., Zheng, Z. N., Lei, J., Cao, Y. X., Li, Q. Y., Zheng, Z., et al. (2021). Targeting the EZH2-PPAR Axis is a potential therapeutic pathway for pancreatic cancer. *Ppar Res.* 2021, 5589342. doi:10.1155/2021/5589342

Huang, K., Du, M., Tan, X., Yang, L., Li, X., Jiang, Y., et al. (2017). PARP1-mediated PPARα poly(ADP-ribosyl)ation suppresses fatty acid oxidation in non-alcoholic fatty liver disease. *J. Hepatol.* 66, 962–977. doi:10.1016/j.jhep.2016.11.020

Kadoch, C., Copeland, R. A., and Keilhack, H. (2016). PRC2 and SWI/SNF chromatin remodeling complexes in health and disease. *Biochemistry* 55, 1600–1614. doi:10.1021/acs.biochem.5b01191

Karakashev, S., Fukumoto, T., Zhao, B., Lin, J. H., Wu, S., Fatkhutdinov, N., et al. (2020). EZH2 inhibition sensitizes CARM1-high, homologous recombination proficient ovarian cancers to PARP inhibition. *Cancer Cell* 37, 157–167. doi:10.1016/j.ccell.2019.12.015

Karakashev, S., and Zhang, R. G. (2020). Targeting CARM1 in ovarian cancer with EZH2 and PARP inhibitors. *Mol. Cell. Oncol.* 7, 1760675. doi:10.1080/23723556.2020.1760675

Ke, Y. S., Zhang, J., Lv, X. P., Zeng, X. L., and Ba, X. Q. (2019). Novel insights into PARPs in gene expression: Regulation of RNA metabolism. *Cell. Mol. Life Sci.* 76, 3283–3299. doi:10.1007/s00018-019-03120-6

Kersten, S. (2014). Integrated physiology and systems biology of PPARα. *Mol. Metab.* 3, 354–371. doi:10.1016/j.molmet.2014.02.002

Kim, K. H., and Roberts, C. W. M. (2016). Targeting EZH2 in cancer. *Nat. Med.* 22, 128–134. doi:10.1038/nm.4036

Kiss, B., Szanto, M., Szklenar, M., Brunyanszki, A., Marosvolgyi, T., Sarosi, E., et al. (2015). Poly(ADP) ribose polymerase-1 ablation alters eicosanoid and docosanoid signaling and metabolism in a murine model of contact hypersensitivity. *Mol. Med. Rep.* 11, 2861–2867. doi:10.3892/mmr.2014.3044

Laugesen, A., Hojfeldt, J. W., and Helin, K. (2019). Molecular mechanisms directing PRC2 recruitment and H3K27 methylation. *Mol. Cell* 74, 8–18. doi:10.1016/j.molcel.2019.03.011

Lee, E. K., and Matulonis, U. A. (2020). PARP inhibitor resistance mechanisms and implications for post-progression combination therapies. *Cancers* 12, E2054. doi:10.3390/cancers12082054

Leon, T. E., Rapoz-D'silva, T., Bertoli, C., Rahman, S., Magnussen, M., Philip, B., et al. (2020). EZH2-Deficient T-cell acute lymphoblastic leukemia is sensitized to CHK1 inhibition through enhanced replication stress. *Cancer Discov.* 10, 998–1017. doi:10.1158/2159-8290.CD-19-0789

Li, C., Wang, Y., Gong, Y. Q., Zhang, T. R., Huang, J. Q., Tan, Z., et al. (2021). Finding an easy way to harmonize: A review of advances in clinical research and combination strategies of EZH2 inhibitors. *Clin. Epigenetics* 13, 62. doi:10.1186/s13148-021-01045-1

Li, H., Liu, Z. Y., Wu, N. Y. Y., Chen, Y. C., Cheng, Q., and Wang, J. (2020). PARP inhibitor resistance: The underlying mechanisms and clinical implications. *Mol. Cancer* 19, 107. doi:10.1186/s12943-020-01227-0

Li, Z. Y., and Zhang, H. F. (2016). Reprogramming of glucose, fatty acid and amino acid metabolism for cancer progression. *Cell. Mol. Life Sci.* 73, 377–392. doi:10.1007/s00018-015-2070-4

Lin, P.-H., Lin, C.-H., Huang, C.-C., Fang, J.-P., and Chuang, M.-C. (2008). 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin modulates the induction of DNA strand breaks and poly(ADP-ribose) polymerase-1 activation by 17beta-estradiol in human breast carcinoma cells through alteration of CYP1A1 and CYP1B1 expression. *Chem. Res. Toxicol.* 21, 1337–1347. doi:10.1021/tx700396d

Lin, X. P., Xiao, Z. Z., Chen, T., Liang, S. H., and Guo, H. Q. (2020). Glucose metabolism on tumor plasticity, diagnosis, and treatment. *Front. Oncol.* 10, 317. doi:10.3389/fonc.2020.00317

Liu, Z., Hu, X., Wang, Q., Wu, X., Zhang, Q., Wei, W., et al. (2021). Design and synthesis of EZH2-based PROTACs to degrade the PRC2 complex for targeting the noncatalytic activity of EZH2. *J. Med. Chem.* 64, 2829–2848. doi:10.1021/acs.jmedchem.0c02234

Martin, K. A., Cesaroni, M., Denny, M. F., Lupey, L. N., and Tempera, I. (2015). Global transcriptome analysis reveals that poly(ADP-Ribose)Polymerase 1 regulates gene expression through EZH2. *Mol. Cell. Biol.* 35, 3934–3944. doi:10.1128/MCB.00635-15

Mehta, A. K., Cheney, E. M., Hartl, C. A., Pantelidou, C., Oliwa, M., Castrillon, J. A., et al. (2021). Targeting immunosuppressive macrophages overcomes PARP inhibitor resistance in BRCA1-associated triple-negative breast cancer. *Nat. Cancer* 2, 66–82. doi:10.1038/s43018-020-00148-7

Min, A., and Im, S. A. (2020). PARP inhibitors as therapeutics: Beyond modulation of PARylation. *Cancers* 12, E394. doi:10.3390/cancers12020394

Morel, K. L., Sheahan, A. V., Burkhart, D. L., Baca, S. C., Boufaied, N., Liu, Y., et al. (2021). EZH2 inhibition activates a dsRNA-STING-interferon stress axis that potentiates response to PD-1 checkpoint blockade in prostate cancer. *Nat. Cancer* 2, 444–456. doi:10.1038/s43018-021-00185-w

Morschhauser, F., Tilly, H., Chaidos, A., Mckay, P., Phillips, T., Assouline, S., et al. (2020). Tazemetostat for patients with relapsed or refractory follicular lymphoma: An open-label, single-arm, multicentre, phase 2 trial. *Lancet. Oncol.* 21, 1433–1442. doi:10.1016/S1470-2045(20)30441-1

Mu, Y. H., Lou, J. M., Srivastava, M., Zhao, B., Feng, X. H., Liu, T., et al. (2016). SLFN11 inhibits checkpoint maintenance and homologous recombination repair. *EMBO Rep.* 17, 94–109. doi:10.15252/embr.201540964

Murai, J., Feng, Y., Yu, G. Y. K., Ru, Y. B., Tang, S. W., Shen, Y. Q., et al. (2016). Resistance to PARP inhibitors by SLFN11 inactivation can be overcome by ATR inhibition. *Oncotarget* 7, 76534–76550. doi:10.18632/oncotarget.12266

Navas, L. E., and Carnero, A. (2021).NAD(+) metabolism, stemness, the immune response, and cancer, *Signal Transduct. Target. Ther.*, 6.

Pang, J., Cui, J., Xi, C., Shen, T., Gong, H., Dou, L., et al. (2018). Inhibition of poly(ADP-ribose) polymerase increased lipid accumulation through SREBP1 modulation. *Cell. Physiol. biochem.* 49, 645–652. doi:10.1159/000493028

Pantelidou, C., Sonzogni, O., De Oliveria Taveira, M., Mehta, A. K., Kothari, A., Wang, D., et al. (2019). PARP inhibitor efficacy depends on CD8(+) T-cell recruitment via intratumoral STING pathway activation in BRCA-deficient models of triple-negative breast cancer. *Cancer Discov.* 9, 722–737. doi:10.1158/2159-8290.CD-18-1218

Peng, D., Kryczek, I., Nagarsheth, N., Zhao, L., Wei, S., Wang, W., et al. (2015). Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. *Nature* 527, 249–253. doi:10.1038/nature15520

Qin, X., Xie, G., Wu, X., Xu, X., Su, M., and Yang, B. (2018). Prenatal exposure to perfluorooctanoic acid induces nerve growth factor expression in cerebral cortex cells of mouse offspring. *Environ. Sci. Pollut. Res. Int.* 25, 18914–18920. doi:10.1007/s11356-018-2117-9

Rondinelli, B., Gogola, E., Yucel, H., Duarte, A. A., Van De Ven, M., Van Der Sluijs, R., et al. (2017). EZH2 promotes degradation of stalled replication forks by recruiting MUS81 through histone H3 trimethylation. *Nat. Cell Biol.* 19, 1371–1378. doi:10.1038/ncb3626

Sabari, J. K., Lok, B. H., Laird, J. H., Poirier, J. T., and Rudin, C. M. (2017). Unravelling the biology of SCLC: Implications for therapy. *Nat. Rev. Clin. Oncol.* 14, 549–561. doi:10.1038/nrclinonc.2017.71

Sanderson, D. J., and Cohen, M. S. (2020). Mechanisms governing PARP expression, localization, and activity in cells. *Crit. Rev. Biochem. Mol. Biol.* 55, 541–554. doi:10.1080/10409238.2020.1818686

Schlacher, K. (2017). PARPi focus the spotlight on replication fork protection in cancer. *Nat. Cell Biol.* 19, 1309–1310. doi:10.1038/ncb3638

Scott, C. L., Swisher, E. M., and Kaufmann, S. H. (2015). Poly (ADP-Ribose) polymerase inhibitors: Recent advances and future development. *J. Clin. Oncol.* 33, 1397–1406. -+. doi:10.1200/JCO.2014.58.8848

Sen, T., Rodriguez, B. L., Chen, L., Corte, C. M. D., Morikawa, N., Fujimoto, J., et al. (2019). Targeting DNA damage response promotes antitumor immunity through STING-mediated T-cell activation in small cell lung cancer. *Cancer Discov.* 9, 646–661. doi:10.1158/2159-8290.CD-18-1020

Snaebjornsson, M. T., Janaki-Raman, S., and Schulze, A. (2020). Greasing the wheels of the cancer machine: The role of lipid metabolism in cancer. *Cell Metab.* 31, 62–76. doi:10.1016/j.cmet.2019.11.010

Sonnenblick, A., De Azambuja, E., Azim, H. A., Jr., and Piccart, M. (2015). An update on PARP inhibitors-moving to the adjuvant setting. *Nat. Rev. Clin. Oncol.* 12, 27–41. doi:10.1038/nrclinonc.2014.163

Stewart, C. A., Tong, P., Cardnell, R. J., Sen, T., Li, L. R., Gay, C. M., et al. (2017). Dynamic variations in epithelial-to-mesenchymal transition (EMT), ATM, and SLFN11 govern response to PARP inhibitors and cisplatin in small cell lung cancer. *Oncotarget* 8, 28575–28587. doi:10.18632/oncotarget.15338

Sun, Y., Wu, J., Dong, X., Zhang, J., Meng, C., and Liu, G. (2021). MicroRNA-506-3p increases the response to PARP inhibitors and cisplatin by targeting EZH2/β-catenin in serous ovarian cancers. *Transl. Oncol.* 14, 100987. doi:10.1016/j.tranon.2020.100987

Szántó, M., and Bai, P. (2020). The role of ADP-ribose metabolism in metabolic regulation, adipose tissue differentiation, and metabolism. *Genes Dev.* 34, 321–340. doi:10.1101/gad.334284.119

Szanto, M., Brunyanszki, A., Marton, J., Vamosi, G., Nagy, L., Fodor, T., et al. (2014). Deletion of PARP-2 induces hepatic cholesterol accumulation and decrease in HDL levels. *Biochim. Biophys. Acta* 1842, 594–602. doi:10.1016/j.bbadis.2013.12.006

Szántó, M., Gupte, R., Kraus, W. L., Pacher, P., and Bai, P. (2021). PARPs in lipid metabolism and related diseases. *Prog. Lipid Res.* 84, 101117. doi:10.1016/j.plipres.2021.101117

Tang, S. W., Thomas, A., Murai, J., Trepel, J. B., Bates, S. E., Rajapakse, V. N., et al. (2018). Overcoming resistance to DNA-targeted agents by epigenetic activation of schlafen 11 (SLFN11) expression with class I histone deacetylase inhibitors. *Clin. Cancer Res.* 24, 1944–1953. doi:10.1158/1078-0432.CCR-17-0443

Tu, Y. L., Sun, Y. M., Qiao, S., Luo, Y., Liu, P. P., Jiang, Z. X., et al. (2021). Design, synthesis, and evaluation of VHL-based EZH2 degraders to enhance therapeutic activity against lymphoma. *J. Med. Chem.* 64, 10167–10184. doi:10.1021/acs.jmedchem.1c00460

Vantaku, V., Putluri, V., Bader, D. A., Maity, S., Ma, J., Arnold, J. M., et al. (2020). Epigenetic loss of AOX1 expression via EZH2 leads to metabolic deregulations and promotes bladder cancer progression. *Oncogene* 39, 6265–6285. doi:10.1038/s41388-019-0902-7

Virag, L., Robaszkiewicz, A., Rodriguez-Vargas, J. M., and Oliver, F. J. (2013). Poly(ADP-ribose) signaling in cell death. *Mol. Asp. Med.* 34, 1153–1167. doi:10.1016/j.mam.2013.01.007

Wan, J., Zhan, J., Li, S., Ma, J., Xu, W., Liu, C., et al. (2015). PCAF-primed EZH2 acetylation regulates its stability and promotes lung adenocarcinoma progression. *Nucleic Acids Res.* 43, 3591–3604. doi:10.1093/nar/gkv238

Wang, C., Li, X., Zhang, J., Ge, Z., Chen, H., and Hu, J. (2018). EZH2 contributes to 5-FU resistance in gastric cancer by epigenetically suppressing FBXO32 expression. *Onco. Targets. Ther.* 11, 7853–7864. doi:10.2147/OTT.S180131

Wang, C., Qu, L., Li, S., Yin, F., Ji, L., Peng, W., et al. (2021). Discovery of first-in-class dual PARP and EZH2 inhibitors for triple-negative breast cancer with wild-type BRCA. *J. Med. Chem.* 64, 12630–12650. doi:10.1021/acs.jmedchem.1c00567

Wang, J., and Wang, G. G. (2020). No easy way out for EZH2: Its pleiotropic, noncanonical effects on gene regulation and cellular function. *Int. J. Mol. Sci.* 21, E9501. doi:10.3390/ijms21249501

Wang, J., Yu, X. F., Gong, W. D., Liu, X. J., Park, K. S., Ma, A. Q., et al. (2022). EZH2 noncanonically binds cMyc and p300 through a cryptic transactivation domain to mediate gene activation and promote oncogenesis. *Nat. Cell Biol.* 24, 384–399. doi:10.1038/s41556-022-00850-x

Wang, N., Liang, H., and Zen, K. (2014). Molecular mechanisms that influence the macrophage M1-M2 polarization balance. *Front. Immunol.* 5, 614. doi:10.3389/fimmu.2014.00614

Wang, Y., Hou, N., Cheng, X., Zhang, J., Tan, X., Zhang, C., et al. (2017). Ezh2 acts as a tumor suppressor in kras-driven lung adenocarcinoma. *Int. J. Biol. Sci.* 13, 652–659. doi:10.7150/ijbs.19108

Wang, Y. P., Nakajima, T., Gonzalez, F. J., and Tanaka, N. (2020). PPARs as metabolic regulators in the liver: Lessons from liver-specific PPAR-null mice. *Int. J. Mol. Sci.* 21, E2061. doi:10.3390/ijms21062061

Wicha, M. S. (2009). Development of 'synthetic lethal' strategies to target BRCA1-deficient breast cancer. *Breast Cancer Res.* 11, 108. doi:10.1186/bcr2362

Wright, W. D., Shah, S. S., and Heyer, W. D. (2018). Homologous recombination and the repair of DNA double-strand breaks. *J. Biol. Chem.* 293, 10524–10535. doi:10.1074/jbc.TM118.000372

Wu, T., and Dai, Y. (2017). Tumor microenvironment and therapeutic response. *Cancer Lett.* 387, 61–68. doi:10.1016/j.canlet.2016.01.043

Wu, Z., Lee, S. T., Qiao, Y., Li, Z., Lee, P. L., Lee, Y. J., et al. (2011). Polycomb protein EZH2 regulates cancer cell fate decision in response to DNA damage. *Cell Death Differ.* 18, 1771–1779. doi:10.1038/cdd.2011.48

Wu, Z. Z., Cui, P. F., Tao, H. T., Zhang, S. J., Ma, J. X., Liu, Z. F., et al. (2021). The synergistic effect of PARP inhibitors and immune checkpoint inhibitors. *Clin. Med. Insights. Oncol.* 15, 1179554921996288. doi:10.1177/1179554921996288

Xu, T., Dai, J., Tang, L., Yang, L., Si, L., Sheng, X., et al. (2021). EZH2 inhibitor enhances the STING agonist–induced antitumor immunity in melanoma. *J. Invest. Dermatol.* 142, 1158–1170.e8. doi:10.1016/j.jid.2021.08.437

Yamaguchi, H., Du, Y., Nakai, K., Ding, M., Chang, S. S., Hsu, J. L., et al. (2018). EZH2 contributes to the response to PARP inhibitors through its PARP-mediated poly-ADP ribosylation in breast cancer. *Oncogene* 37, 208–217. doi:10.1038/onc.2017.311

Yamamoto, M., Jin, C., Hata, T., Yasumizu, Y., Zhang, Y., Hong, D., et al. (2019). MUC1-C integrates chromatin remodeling and PARP1 activity in the DNA damage response of triple-negative breast cancer cells. *Cancer Res.* 79, 2031–2041. doi:10.1158/0008-5472.CAN-18-3259

Yang, A. Y., Choi, E. B., Park, M. S., Kim, S. K., Park, M. S., and Kim, M. Y. (2020). PARP1 and PRC2 double deficiency promotes BRCA-proficient breast cancer growth by modification of the tumor microenvironment. *Febs J.* 23, 2888–2910. doi:10.1111/febs.15636

Zhang, L., Zou, J., Chai, E., Qi, Y., and Zhang, Y. (2014). Alpha-lipoic acid attenuates cardiac hypertrophy via downregulation of PARP-2 and subsequent activation of SIRT-1. *Eur. J. Pharmacol.* 744, 203–210. doi:10.1016/j.ejphar.2014.09.037

Zhang, T., Guo, Z., Huo, X., Gong, Y., Li, C., Huang, J., et al. (2022). Dysregulated lipid metabolism blunts the sensitivity of cancer cells to EZH2 inhibitor. *EBioMedicine* 77, 103872. doi:10.1016/j.ebiom.2022.103872

Zhao, Q. Y., Lan, T. L., Su, S., and Rao, Y. (2019). Induction of apoptosis in MDA-MB-231 breast cancer cells by a PARP1-targeting PROTAC small molecule. *Chem. Commun.* 55, 369–372. doi:10.1039/c8cc07813k

Zingg, D., Arenas-Ramirez, N., Sahin, D., Rosalia, R. A., Antunes, A. T., Haeusel, J., et al. (2017). The histone methyltransferase Ezh2 controls mechanisms of adaptive resistance to tumor immunotherapy. *Cell Rep.* 20, 854–867. doi:10.1016/j.celrep.2017.07.007

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 06 April 2023
DOI 10.3389/fphar.2023.928821

OPEN ACCESS

EDITED BY
Thomas Grewal,
The University of Sydney, Australia

REVIEWED BY
Nathan J. Bowen,
Clark Atlanta University, United States
Herryawan Ryadi Eziwar Dyari,
National University of Malaysia, Malaysia

*CORRESPONDENCE
Lixiang Xue,
lixiangxue@bjmu.edu.cn
Baoshan Cao,
caobaoshan0711@aliyun.com
Jiagui Song,
jiaguisong@bjmu.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 26 April 2022
ACCEPTED 28 March 2023
PUBLISHED 06 April 2023

CITATION
Xia W, Wang H, Zhou X, Wang Y, Xue L, Cao B and Song J (2023), The role of cholesterol metabolism in tumor therapy, from bench to bed.
Front. Pharmacol. 14:928821.
doi: 10.3389/fphar.2023.928821

The role of cholesterol metabolism in tumor therapy, from bench to bed

Wenhao Xia[1,2†], Hao Wang[1,3†], Xiaozhu Zhou[4], Yan Wang[1,5], Lixiang Xue[1,3,5*], Baoshan Cao[1,6*] and Jiagui Song[1,5,7*]

[1]Cancer Center of Peking University Third Hospital, Beijing, China, [2]School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China, [3]Department of Radiation Oncology, Peking University Third Hospital, Beijing, China, [4]Department of Clinical Pharmacy, School of Pharmacy, Capital Medical University, Beijing, China, [5]Third Hospital Institute of Medical Innovation and Research, Beijing, China, [6]Department of Medical Oncology and Radiation Sickness, Peking University Third Hospital, Beijing, China, [7]State Key Laboratory of Natural and Biomimetic Drugs, Peking University as the Third Responsibility Unit of Song Jiagui, Beijing, China

Cholesterol and its metabolites have important biological functions. Cholesterol is able to maintain the physical properties of cell membrane, play an important role in cellular signaling, and cellular cholesterol levels reflect the dynamic balance between biosynthesis, uptake, efflux and esterification. Cholesterol metabolism participates in bile acid production and steroid hormone biosynthesis. Increasing evidence suggests a strict link between cholesterol homeostasis and tumors. Cholesterol metabolism in tumor cells is reprogrammed to differ significantly from normal cells, and disturbances of cholesterol balance also induce tumorigenesis and progression. Preclinical and clinical studies have shown that controlling cholesterol metabolism suppresses tumor growth, suggesting that targeting cholesterol metabolism may provide new possibilities for tumor therapy. In this review, we summarized the metabolic pathways of cholesterol in normal and tumor cells and reviewed the pre-clinical and clinical progression of novel tumor therapeutic strategy with the drugs targeting different stages of cholesterol metabolism from bench to bedside.

KEYWORDS

cholesterol, cholesterol metabolism, tumor therapy, pharmacological targets, clinical trial

1 Introduction

Cholesterol is a ubiquitous sterol present in vertebrates with multiple biological functions. Cholesterol is an essential lipid component of the mammalian cell membrane that can maintain membrane integrity and mobility and form membrane microstructures (Cerqueira et al., 2016). In addition to serving as a membrane structural and functional component, cholesterol produces various oxysterol through enzymatic and non-enzymatic pathways. Cholesterol also represents a precursor of bile acid, and its oxidative effect allows for the biosynthesis of steroid hormones in the steroid-producing tissues (Luu et al., 2016). Cholesterol metabolism homeostasis is maintained by a complex network that regulates cholesterol biosynthesis, uptake, efflux, and storage (Giacomini et al., 2021). In addition, cholesterol also interacts with a variety of proteins, including receptors, channels and enzymes, which are thought to regulate protein stability, localization and activity (Hulce et al., 2013).

Tumor cells are highly proliferative and therefore rely on cholesterol to meet substantially increased nutrient needs for membrane synthesis and support their uncontrolled growth, thereby promoting tumorigenesis and progression (Riscal et al., 2019). Indeed, cholesterol, cholesterol derivatives and cholesterol synthesis intermediates can regulate tumor cell proliferation, motility, stemness and drug resistance (Kopecka et al., 2020a). Given these important functions of cholesterol metabolism in cancer, drugs targeting cholesterol metabolism and tumor treatment strategies have become a hot topic in the field of tumor research and have made significant progress in recent years. In this review, we introduce the metabolic pathways of cholesterol in normal and cancer cells, its role in the tumor therapy, and the latest progress in therapeutic drugs targeting different stages of cholesterol metabolism.

2 Overview of the cholesterol metabolism in normal cells

Cholesterol metabolism including biosynthesis, uptake, efflux and storage is a complex and important process under normal physiological conditions. In brief, cholesterol biosynthesis starts with acetyl-coA and involves synergy of more than 20 enzymes, most of them on the membrane of the endoplasmic reticulum (ER) (Luo et al., 2020). Several steps are tightly regulated throughout the process, and some intermediates produced during the process can be transferred and used as precursors for the biosynthesis of other compounds (Cerqueira et al., 2016; Luo et al., 2020). The biosynthesis cascade of cholesterol occurs in almost every mammalian cell, especially liver synthesis accounts for about 50% of the total cholesterol biosynthesis (Luo et al., 2020).

Cholesterol uptake consists of NPC1L1 (Niemann–Pick C1-like-1) protein-mediated absorption from the intestinal lumen and LDLR-mediated subsequent absorption from the blood (Luo et al., 2020). NPC1L1 is a glycosylated, multi-spanning membrane protein specifically expressed on the apical surface of enterocytes and the membrane of bile canaliculi of human hepatocytes (Altmann et al., 2004). It is a key mediator of cholesterol uptake and controls cholesterol uptake in enterocytes through clathrin-mediated endocytosis (Luo et al., 2020). The human *NPC1L1* gene is activated by SREBP2 and is upregulated by hepatocyte nuclear factor 4α (HNF4α) (Iwayanagi et al., 2008).

Although almost all mammalian cells can produce cholesterol, only hepatocytes, adrenal cells, and gonad cells are able to catabolize cholesterol. Thus, excess cholesterol of peripheral tissues is converted to cholesterol esters stored in lipid droplets or moved to the liver that can be converted to bile acids and excreted into the digestive system (Ouimet et al., 2019). Mechanistically, four members of the ATP binding cassette (ABC) transporter superfamily: ABC subfamily A member 1 (ABCA1), ABC subfamily G (ABCG) members 1, 5, and 8 regulate cholesterol efflux in a cell-type-specific manner. ABCA1 is widely expressed throughout the body and its main receptor mediating cholesterol efflux is lipid-free apolipoprotein A-I (apoA-I) (Rosenson et al., 2012) and produces HDL particles. ABCG1 is most abundant in macrophages, lower in hepatocytes, and absent in enterocytes (Kennedy et al., 2005). However, ABCG5 and ABCG8 are nearly exclusively expressed at the apical surface of enterocytes and hepatocytes, forming a heterodimer mediating the excretion of cholesterol into the bile and intestinal lumen (Graf et al., 2003).

As mentioned above, excess intracellular cholesterol is usually converted to cholesterol esters, which is an important means to prevent free cholesterol accumulation in cells. The formation of cholesterol esters is mediated by acyl coenzyme A cholesterol acetyltransferase (ACAT) (Petan et al., 2018). To date, two ACAT isoenzymes have been reported in mammals, including ACAT1 and ACAT2. ACAT1 is widely expressed throughout the body and is most abundant in macrophages, epithelial cells and steroid hormone-producing cells, indicating its involvement in maintaining cholesterol homeostasis, while ACAT2 is mainly expressed in enterocytes and also in hepatocytes, suggesting that it contributes to lipoprotein biosynthesis and assembly (Luo et al., 2020).

The molecular mechanism of cholesterol metabolism is strictly regulated to maintain cholesterol homeostasis, not only satisfy cell growth and proliferation with enough cholesterol, but also avoid excessive cholesterol accumulation. Cholesterol homeostasis is mainly regulated by 2 families of transcription factors: the sterol regulatory element binding proteins (SREBPs) and the liver X receptors (LXRs) (Luo et al., 2020). SREBP1 mainly regulates the genes involved in fatty acid (FA) synthesis, while SREBP2 controls the gene of the cholesterol biosynthesis pathway (Horton et al., 2003). When the cholesterol content is present in endoplasmic reticulum (ER) is low, SREBP2 activates the transcription and expression of the cholesterol biosynthetic enzymes HMGCR, increases the expression of the *NPC1L1* and *LDLR* genes (Luo et al., 2020) to increase the *de novo* cholesterol synthesis (Nohturfft and Zhang, 2009; Cai et al., 2019). When cholesterol content in endoplasmic reticulum (ER) is high, the activation of SREBP2 and cholesterol synthesis are blocked. Moreover, LXRs promotes activation of genes associated with bile acid generation (CYP7A1), cholesterol excretion (ABCG5, ABCG8), and reverse cholesterol transport (ABCA1, ABCG1) (Giacomini et al., 2021), ultimately promoting the elimination of the excess of cellular cholesterol.

Although cholesterol is essential for membrane fluidity and structural maintenance, signaling regulation, and energy storage, most mammalian cells cannot directly process cholesterol through the catalytic reaction, but may modify their steroid skeleton, which further generate oxysterols eventually and bile acid *via* cholesterol efflux ultimately upon the content of cholesterol is overload (Luu et al., 2016; Riscal et al., 2019). Oxysterols are oxidized forms of cholesterol, which present at extremely low concentrations in human (van Reyk et al., 2006). Oxysterols regulate cellular cholesterol homeostasis by inhibiting SREBP and activating LXR. Moreover, oxysterols are widely involved in post-transcriptional regulation of cholesterol homeostasis by changing enzyme stability and/or activity (e.g., promoting HMGCR degradation, affecting the activity of several cholesterol biosynthetic enzymes, *etc.*) (Luu et al., 2016).

3 Reprogrammed cholesterol metabolism in tumor cells

Cholesterol is generally beneficial for cancer growth and development, it promotes migration and invasion, inhibits apoptosis through activating oncogenic signaling pathways (Figure 1).

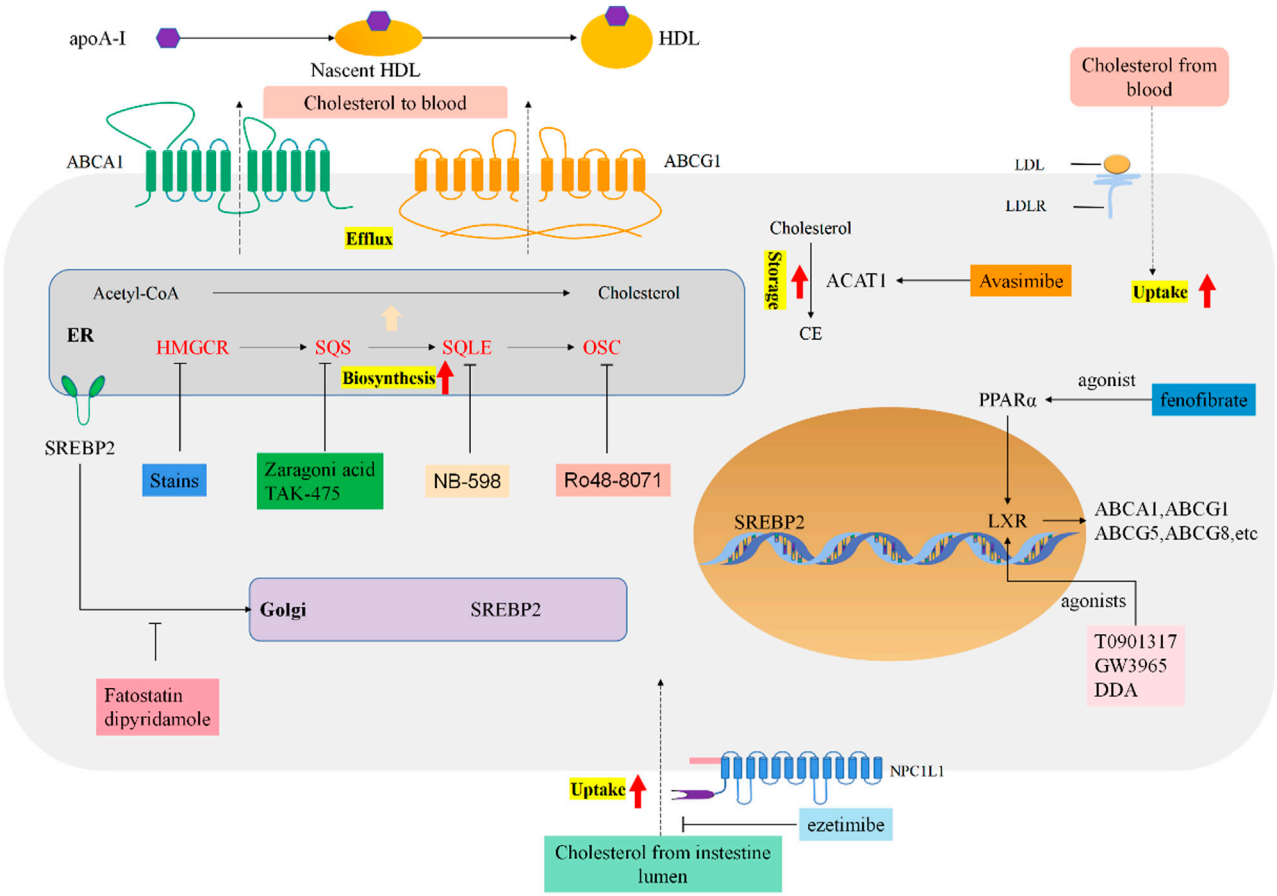


FIGURE 1
Reprogrammed cholesterol metabolism and indicated drug targets in tumor cells. Cholesterol metabolism and homeostasis regulation in cancer cells. Boxes of different colors indicate targeted therapeutic drugs and drug targets for each stage of cholesterol metabolism.

3.1 Cholesterol biosynthesis is enhanced in tumor cells

Tumor cells require excess cholesterol and intermediates of the cholesterol biosynthesis pathway to maintain cell proliferation, possibly related to the substantial cholesterol require for membrane synthesis (Cruz et al., 2013). Increased endogenous cholesterol synthesis and high cholesterol exposure both favor cancer progression (Kopecka et al., 2020b). Interestingly, intracellular cholesterol levels cause more cancer burden than systemic serum cholesterol, suggesting that abnormalities in cholesterol biosynthesis are strongly associated with tumorigenesis (Sorrentino et al., 2014; Kuzu et al., 2016).

Several enzymes such as SREBP2, HMGCR, SQS, OSC, and SQLE which are involved in cholesterol synthesis are significantly upregulated in liver cancer mouse model (Liang et al., 2018). SREBP2 and its downstream targets, including mevalonate-pathway enzymes, are significantly upregulated in glioblastoma (Lewis et al., 2015). HMGCR is overexpressed in prostate cancer, gastric cancer and colon cancer (Giacomini et al., 2021). Squalene synthase (SQS) is enhanced in lung cancer patients, induces cholesterol biosynthesis, which in turn maintains the enrichment of tumor necrosis factor receptor 1 (TNFR1) in lipid rafts to promote lung cancer metastasis (Yang et al., 2014). Inhibition of SQS reduces the levels of lipid raft-associated cholesterol, inhibits prostate cancer cell proliferation, and induces apoptotic (Brusselmans et al., 2007). The level of squalene cycloxidase (SQLE) is enhanced in breast cancer, lung cancer and colorectal cancer, and promotes cancer cell migration and invasion, which may be related to regulating the sterol components of lipid rafts as well (Giacomini et al., 2021). In metastatic mouse models of colorectal and pancreatic cancer, lanosterol synthase (LSS) promotes tumor neovascularization and metastasis (Maione et al., 2015). Oxide squalene cyclase (OSC) inhibitors hinder endothelial cell migration and promote apoptosis, which inhibits tumor angiogenesis and dissemination to the distance (Liang et al., 2014). In addition, enhanced expression of cholesterol synthesis genes is associated with poor survival in sarcoma, acute myeloid leukemia and melanoma patients, but in lower grade glioma it was associated with good survival (Kuzu et al., 2016). The latest research has revealed that activated cholesterol biosynthesis programs promotes triple-negative breast cancer progression (Cai et al., 2019) and increased cholesterol synthesis is associated with poor patient prognosis (Ehmsen et al., 2019).

Mechanistically, cholesterol biosynthesis has complex links with the signaling pathways and factors that regulate tumors. Several

oncogenic signals such as PI3K/AKT/mTOR, RTK/RAS, and TP53 have been shown to modulate cholesterol synthesis in cancer cells (Kuzu et al., 2016). For example, constitutive activation of PI3K/AKT signaling increases intracellular cholesterol levels through SREBP-1 activation, resulting in *de novo* cholesterol biosynthesis and LDL receptor (LDLR) expression, thereby enhancing exogenous cholesterol import in prostate cancer (Guo et al., 2011). On the other hand, cholesterol biosynthesis also has a critical role in maintaining cancer stem cells by activating signaling pathways of sonic hedgehog, Notch and receptor tyrosine kinases (Kim, 2019). Thus, targeting the cholesterol generation and mevalonate pathway represents a promising choice for tumor therapy.

3.2 Cholesterol uptake is enhanced in tumor cells

Increasing cholesterol uptake appears to be more efficient strategy compared to *de novo* cholesterol synthesis for cancer cells. It is reported that NPC1L1 promotes colon carcinogenesis by inducing cholesterol absorption and increasing plasma cholesterol levels (He et al., 2015). NPC1L1 knockdown reduces colitis-associated tumorigenesis, which may be associated with downregulation of β-catenin, p-c-Jun and p-ERK (He et al., 2015). One of the extracellular loops of NPC1L1 is the binding site of ezetimibe, thus providing support for targeted cholesterol uptake (Weinglass et al., 2008). Besides, it has been found that some anaplastic large cell lymphoma cells are completely dependent on cholesterol uptake to acquire cholesterol, due to the absence of SQLE. These cancer cells actively upregulate LDLR, which takes up exogenous cholesterol as an alternative strategy to support proliferation (Garcia-Bermudez et al., 2019). Indeed, LDLRs levels are increased in glioblastoma, leukemia, pancreatic and lung cancers (Huang et al., 2016; Gallagher et al., 2017) and LDLRs promotes epithelial-to-mesenchymal transition (EMT), increases the secretion of metalloproteinase MMP-9 and activates Wnt/β-catenin signaling pathway (Campion et al., 2020). However, the level of LDLR is decreased in human advanced prostate cancer. The roles of hypercholesterolemia in tumors are still controversial: elevated serum cholesterol level is positively correlated with the recurrence rate of prostate cancer (Allott et al., 2014). But it is also reported that high serum cholesterol levels increased the anti-tumor functions of natural killer cells and reduced the growth of liver tumors in mice (Pelton et al., 2014). Collectively, while cholesterol uptake is one of the sources for cancer cells to obtain cholesterol, how cancer cells coordinate the balance between cholesterol biosynthesis and uptake and whether it is altered with tumor progression remains to be further elucidated.

3.3 Cholesterol efflux is dysregulated in tumor cells

Deficiency of ABCA1, a main receptor mediating cholesterol efflux, increases mitochondrial cholesterol, inhibits release of mitochondrial cell death-promoting molecules, and thus facilitates cancer cell survival (Smith and Land, 2012; Kuzu et al., 2016). It has been demonstrated that ABCA1 can promote cell metastasis by regulating cholesterol levels, and patients with high ABCA1 expression had shorter times to metastasis in breast cancer (Aguirre-Portoles et al., 2018). PPARα and PPARγ activation promotes LXR-mediated ABCA1 expression, and PPARα blocks cholesterol biosynthesis by inhibiting sterol regulatory element binding protein 2 (SREBP-2) activity (Grabacka and Reiss, 2008). Thus, targeting PPARα appears to be an effective strategy to regulate cholesterol content. Indeed, the antitumor effect of fenofibrate (an agonist of PPARα) has been demonstrated (Giacomini et al., 2021).

3.4 Cholesterol esterification is enhanced in tumor cells

As mentioned above, cells are able to avoid excessive cholesterol accumulation through the cholesterol esterification pathway. Usually, cholesterol esterification reduces the amount of intracellular free cholesterol, protects tumor cells from their toxic effects, and reduces the amount of free cholesterol that can maintain SREBP-induced cholesterol biosynthesis and uptake (Chang et al., 2006). However, it is also reported that reducing cholesterol esterification was able to inhibit the growth and invasion of hepatoma carcinoma cells in a mouse xenograft model (Geng et al., 2016), suggesting that the function of cholesterol esterification depends on tumor types. Cholesteryl esters (CE), a common signature in cancer, is usually stored in lipid droplets that serve as a reservoir for neutral lipids such as triacylglycerols. The accumulation of CE can be converted by tumor cells into cholesterol utilization, as demonstrated by high expression of ACAT1 and cholesterol ester metabolizing enzyme lysosomal acid lipase (LAL) in tumor tissues. In fact, the accumulation of CE promotes proliferation and invasive capacity of breast cancer, and promotes the occurrence and metastatic potential of glioblastoma, prostate and pancreatic cancer (de Gonzalo-Calvo et al., 2015; Petan et al., 2018). CE accumulation is driven by loss of PTEN and consequent activation of PI3K/AKT/mTOR pathway that induces the expression of SREBP and LDLR, thereby promoting ACAT1-mediated cholesterol storage in lipid droplets (Yue et al., 2014). In glioblastomas, inhibition of ACAT1 inhibits adipogenesis and tumor growth (Geng et al., 2016). Consistently, ACAT1 overexpression was confirmed in many cancers, including hepatocellular carcinoma, castration-resistant prostate cancer, and pancreatic cancer (Giacomini et al., 2021). Therefore, targeted enhanced cholesterol esterification seems to be a promising therapeutic strategy. In fact, it has been shown that targeting ACAT1 has an anticancer potential (Yue et al., 2014).

3.5 Abnormal regulation of cholesterol homeostasis in tumor cells

As mentioned above, SREBP2 and LXR are essential for maintaining cholesterol homeostasis. SREBP promotes cancer cell growth, migration, and colony generation in esophageal squamous cell carcinoma (Zhong et al., 2019). SREBP and its downstream genes are significantly upregulated and promote cell survival and tumor growth in the hypoxic and nutrient-restricted tumor

microenvironment (Lewis et al., 2015). SREBP2 has also been shown to bind to mutant p53 and activate the expression of the mevalonate pathway in breast cancer cells (Freed-Pastor et al., 2012). Moreover, it is proved that RORγ (a nuclear receptor) promotes the recruitment of SREBP2, and activates the cholesterol biosynthesis (Cai et al., 2019). Thus, the SREBP and RORγ can serve as good targets for tumor therapy. In addition to SREBP, LXR is also an important driver of carcinogenesis. LXR inverse agonists and LXR agonists were shown to inhibit the proliferation and colony formation, and induce apoptosis in clear cell renal cell carcinoma (ccRCC) cells, but had no cytotoxic effect on normal renal tubular epithelial cells. Therefore, LXR may be a safe therapeutic target for ccRCC (Wu et al., 2019).

3.6 Oxysterols have multifunctional role in cancer cells

Oxysterols are involved in various cancers (Kuzu et al., 2016). Side-chain oxidation of cholesterol generates 22-hydrocholesterol (22-HC), 24-hydroxycholesterol (24-HC), 25-hydroxycholesterol (25-HC) and 27-hydroxycholesterol (27-HC), and oxidation occurring on the backbone generates 7α/β-hydroxycholesterol (7α-HC/7β-HC), 7-ketocholesterol (7-KC) and 5, 6α/β-epoxycholesterol (5, 6α -EC/5, 6β-EC). 22-HC is a high-affinity LXR ligand that induces ABCA1 expression, leading to cellular cholesterol efflux. 25-HC is a side-chain oxysterol that inhibits cholesterol biosynthesis by inhibiting SREBP (Riscal et al., 2019). Certain oxysterols have anticancer effects. In Jurkat T-cell lymphoma cells, 24-HC induces apoptosis through a mechanism involving 24-HC esters and lipid droplet accumulation (Yamanaka et al., 2014). 22-HC, 24-HC, 7α-HC/7β-HC and 5, 6α -EC/5, and 6β-EC all act as agonists of LXR to inhibit proliferation in breast cancer, ovarian cancer and prostate cancer through inducing G1 cell cycle arrest or apoptosis (Lin et al., 2013; Riscal et al., 2019; de Medina et al., 2021). Thus, oxysterols with cytotoxic activity may be potential therapeutic agents for cancer. However, 27-HC acts as an estrogen receptor (ER) agonist in breast cancer, which stimulates tumor growth and metastasis in multiple breast cancer models (McDonnell et al., 2014). A recent study demonstrated that chronic exposure of cancer cells to 27-HC, which likely models the situation in patients with hypercholesterolemia/dyslipidemia, resulted in the emergence of cells exhibiting increased tumorigenic and metastatic capacity (Liu et al., 2021). Intriguingly, the metabolites of 5, 6-epoxycholesterol (5, 6-EC) have opposing properties in breast cancer oncogenesis. In normal breast tissue, the metabolite dendrogenin A (DDA) displays tumour-suppressive properties. Yet in breast cancer, 5, 6-EC is metabolized to oncosterone (6-oxo-cholestan-3, 6-diol, cholestan-3, 6-diol-6-one, and OCDO), acting as an oncometabolite and tumor promoter in breast cancer. Therefore, blocking oncosterone biosynthesis or neutralizing oncosterone receptors may be a new pharmacological target for the treatment of breast cancer (de Medina et al., 2021).

Besides the tumor cells, oxysterols can also influence the tumor microenvironment. Immune cells expressing "generic" oxysterol receptors, such as LXR, and specific receptors in immune cells, such as G protein-coupled receptor 183 (GPR183), can recognize different oxysterols (Willinger, 2019). Baek et al. (2017) demonstrated that 27-HC increases the number and activity of polymorphonuclear neutrophils (PMN) and γδT cells, and reduces the cytotoxic $CD8^+$ T cell population. In addition, oxysterol promotes tumor growth by inhibiting dendritic cell (DC) migration to lymphoid and by promoting the recruitment of protumor neutrophils in the tumor microenvironment (Raccosta et al., 2013).

4 Targeting cholesterol in tumor therapy

4.1 Targeting cholesterol biosynthesis

4.1.1 Targeting HMGCR

As cholesterol metabolism has important functions in cancer progression, targeting cholesterol metabolism has been shown to be a viable antitumor strategy (Table 1). As previously described, HMGCR is one of the rate-limiting enzymes for the cholesterol-producing mevalonate pathway, so targeting HMGCR may be a good strategy for tumor therapy (Nielsen et al., 2012; Gu et al., 2019; Di Bello et al., 2020). Statins are the most common pharmacological inhibitors of HMGCR. Numerous epidemiological analyses suggest statins can reduce the incidence of certain tumors, but these conclusions are not consistent (Kuzu et al., 2016). One study suggests an association between statin and a slight reduction in cancer-related mortality for 13 different cancer types (Nielsen et al., 2012). However, there are also many epidemiological studies suggest no association between statin and cancer (Kuzu et al., 2016). Statins can enhance the effects of chemotherapeutic agents such as cisplatin, anthracyclines, paclitaxel, 5-fluorouracil, etoposide and malfaran (Osmak, 2012). The efficacy of reducing side effects and drug resistance has also been proved (Terzi et al., 2019; Feng et al., 2020). Currently, the efficacy of statins has been carried out in both basic studies and clinical trials to evaluate monotherapy and therapies in combination with other chemotherapeutic agents.

While inhibiting cholesterol biosynthesis, statins also inhibit the synthesis of multiple other metabolites. By blocking the MVP pathway, statins halt isoprenoid synthesis, such as GGPP and FPP for GTPase-proteins essential for cancer cells (Takai et al., 2001), which explains the pharmacological effects of statins in antitumor effects (Takai et al., 2001; Buhaescu and Izzedine, 2007; Kidera et al., 2010). Moreover, the antitumor effects of statins may also be related to non-MVP-mediated mechanisms (Okubo et al., 2020).

Since statins have been approved for the treatment of hypercholesterolemia and are one of the most widely used pharmaceutical agents in the world. Thus, their repositioning in the field of oncology is translated more easily and quickly to the clinic. From the first clinical trial of lovastatin combined with cytarabine started in 2001, how statins work and benefit in cancers therapy has been widely evaluated over these 2 decades. When "statins | cancer" are taken as the search term, 223 clinical trials have been found on ClinicalTrial.gov, including 54 phase I studies, 101 phase II studies, 24 phase III studies and 12 phase IV studies from 2005 to 2023. Based on the types of diseases, studies for clinical oncology treatment-related trials were included in the analysis (Table 2).

TABLE 1 Anti-cancer therapies that target cholesterol metabolism.

	Therapeutic class		Mechanism	Cancer type	References
Targeting cholesterol biosynthesis	Stains	Simvastatin Atorvastatin Lovastatin Pravastatin Rosuvastatin Fluvastatin Pitavastatin	Competitive inhibitors of HMGCR	Colorectal, Prostate, Breast, Lung cancer, multiple myeloma, melanoma and other cancers	Nielsen et al. (2012), Osmak. (2012), Fatehi Hassanabad. (2019), Gu et al. (2019), Terzi et al. (2019), Chen Y H et al. (2020), Di Bello et al. (2020), Feng et al. (2020), Lubtow et al. (2020), Okubo et al. (2020)
	Zaragonic acids		Inhibitor of squalene synthase	RMA lymphoma and Lewis lung carcinoma models	Brusselmans et al. (2007), Lanterna et al. (2016)
	TAK-475		Inhibitor of squalene synthase (FDFT1)	Pancreatic ductal adenocarcinoma model	Biancur et al. (2021)
	NB-598		Inhibitor of squalene epoxidase	SCLC lines	Mahoney et al. (2019)
	R048-8071		Inhibitor of OSC	HCT116 CRC, HPAF-II pancreatic adenocarcinoma models and breast cancer lines	Liang et al. (2014), Maione et al. (2015)
Targeting cholesterol uptake	ezetimibe		Selective block of NPC1L1	Breast cancer	Pelton et al. (2014)
Targeting cholesterol efflux	fenofibrate		PPARα agonists	Leukemia, Lymphoma, Multiple Myeloma, endometrial cancer, prostate cancer, breast cancer, oral cancer, pancreatic cancerand and other cancers	Luo et al. (2019), Sun et al. (2019), You et al. (2019), Chen L et al. (2020), Di Bello et al. (2020)
Targeting cholesterol storage	Avasimibe		Inhibitor of ACAT1	Human PC3 prostate cancer, MIA-PaCa2 pancreatic cancer, A549 lung cancer, and HCT116 colon cancer lines	Pal et al. (2013), Lee et al. (2015), Lee et al. (2018), Li et al. (2018)
Targeting cholesterol regulation	Fatostatin		specific inhibitor of SREBP	Prostate cancer, ER-positive breast cancer	Li et al. (2014), Li et al. (2015), Gao et al. (2018), Liu et al. (2020), Yao et al. (2020)
	dipyridamole		inhibit the cleavage of SREBP2	multiple myeloma	Pandyra et al. (2014)
	T0901317		LXR agonists	Breast, lung, prostate cancer and Leukemia	Pommier et al. (2010), El Roz et al. (2012), Flaveny et al. (2015), Villa et al. (2016), Tavazoie et al. (2018), Lou et al. (2019), Brendolan and Russo. (2022)
	GW3965			Leukemia	
	DDA (Dendrogenin A)		LXR partial agonist	Leukemia	
	RGX-104		LXRβ agonist	Advanced solid tumors and lymphomas	
	SR9243		LXR inverse agonist	Colorectal, lung, prostate cancer models	

Of the 15 studies with results released, 5 suggested positive anti-tumor outcomes, including simvastatin: 1 (1/2), pravastatin: 2 (2/7), fluvastatin: 2 (2/2) in NSCLC, breast cancer, prostate cancer, leukemia and HCC.

A phase II study has been carried out to evaluate the efficacy and safety of gefitinib plus simvastatin in patients with advanced non-small cell lung cancer (NSCLC). The result pointed out that there is no superiority of GS (gefitinib plus simvastatin) to G (gefitinib only) was demonstrated in the unselected NSCLC population. But GS showed a higher response rate (RR) and longer progression-free survival (PFS) compared with G alone in patients with wild-type EGFR non-adenocarcinomas (Han J.-Y. et al., 2011). Several studies of simvastatin combination treatment in small cell lung cancer (SCLC) are ongoing. Another study tested the effects of simvastatin on the pharmacokinetics of anastrozole, a potent non-steroidal aromatase inhibitor (AI) that holds promise for breast cancer prevention, on patients with hormone receptor-positive breast cancer suggested that simvastatin is not likely to compromise the activity of anastrozole (Bao et al., 2012). While, a study of simvastatin in patients at higher risk of developing a hormone non-responsive (ER-) breast cancer was carried out in 2011 (NCT01500577). This study included 150 women with a history of estrogen receptor negative ductal intraepithelial neoplasia or lobular intraepithelial neoplasia or atypical hyperplasia, or unaffected subjects carrying a mutation of BRCA1 or with a probability of mutation >10% (according to BRCAPRO) (Lazzeroni et al., 2012) to evaluate the chemoprevention activity of simvastatin compared with nimesulide. And the result of this trial has not yet been released.

A study of breast cancer patients with a 3–6 weeks fluvastatin treatment before surgery suggested measurable biologic changes by reducing tumor proliferation and increasing apoptotic activity in high-grade, stage 0/1 breast cancer (Garwood et al., 2010) (NCT00416403). A phase II study in prostate cancer patients shows that short-term (4–12 weeks) fluvastatin treatment at a

TABLE 2 Clinical trials of statins in cancer.

Drug	Cancer type	Condition	Phase	Combination strategy	References
Simvastatin	Breast Cancer		II	Anastrozole	Bao et al. (2012)
		with dyslipidemia	II	-	
		prevention	II	-	Lazzeroni et al. (2012)
		ER-positive/metastatic	II	Fulvestrant, Metformin	
		metastatic	II	HER2-targeted therapy	
	Prostate Cancer		II	Metformin	
			I	Ezetimibe	Wang et al. (2022)
	Colorectal Cancer	metastatic	II	FOLFIRI (irinotecan, 5-FU, leucovorin)	
		advanced/metastatic	II	Cetuximab/Panitumumab/Bevacizumab	
	Lung cancer SCLC		II	Irinotecan/Albumin Paclitaxel/Irinotecan, Cisplatin	
	NSCLC		II	gefitinib	Han J Y et al. (2011)
Atorvastatin	Breast cancer		II	Letrozole	
		triple negative	II	Zoledronate	
		early stage	III	-	
	Prostate Cancer		II	Celecoxib	
		prevent recurrence	II	-	Jeong et al. (2021)
	Glioblastoma multiforme		II	Temozolomide	Altwairgi et al. (2021)
	Hepatocellular Carcinoma (HCC)	prevent recurrence	II	Metformin	
		advanced	II	Sorafenib	
	Colorectal Cancer	prevention	II	-	
	pancreatic cancer	metastatic	I	Ezetimibe, Evolocumab	
Lovastatin	Ovarian cancer	refractory/relapsed	II	Paclitaxel	
	Breast Cancer	prevention	II	-	Vinayak et al. (2013)
	Melanoma		II	Interferon alfa-2b	
		precancerous lesions	II	-	Linden et al. (2014)
Pravastatin	HCC	advanced	II/III	Sorafenib	Jouve et al. (2019), Riaño et al. (2020), Blanc et al. (2021)
	Leukemia	prevent recurrence	II	Cytarabine/Idarubicin	Advani et al. (2014), Shadman et al. (2015)
		relapsed/refractory	I/II	Cyclosporine, Mitoxantrone Hydrochloride, Etoposide	Chen et al. (2013)
	Lung Cancer SCLC		III	Etoposide, Cisplatin/Carboplatin	Seckl et al. (2017)
Rosuvastatin	Endometrial Carcinoma	Stage I	II	Megestrol Acetate	
	Colorectal Cancer	prevent recurrence/ advanced	II/III	-	
Fluvastatin	Breast Cancer		II	-	Garwood et al. (2010)
	Prostate Cancer		II	-	Longo et al. (2020)
Pitavastatin	Breast Cancer		II/III	-	

cholesterol-lowering dose before radical prostatectomy can increase the percentage of apoptotic prostate cancer cells in the tumor relative to baseline (Longo et al., 2020) (NCT01992042).

A positive result for high dose pravastatin combined with cytarabine and idarubicin in relapsed AML patients' therapy was reported in 2014 (Advani et al., 2014) (NCT00840177). The

recurrence rate has decreased from 75% to 5.5% after the combined treatment, which shows the efficacy of this combined therapy. While another study had been ceased due to the combined drugs did not meet the predefined efficacy criteria for success (Shadman et al., 2015) (NCT01831232).

As for HCC, there are three phase II studies aim to bring out the efficacy of sorafenib combined with statins to select better arms for further clinical trials in patients with advanced hepatocellular carcinoma (HCC), as sorafenib is the preferred drug in the palliative treatment [NCT01418729 (Riaño et al., 2020), NCT01357486 (Blanc et al., 2021), NCT01075555 (Jouve et al., 2019)]. All these three studies showed that adding pravastatin to sorafenib did not improve overall survival (OS) in patients with advanced HCC. However, one of the studies suggested the combination of sorafenib and pravastatin prolonging the time to progression (TTP) of patients with advanced HCC (Blanc et al., 2021).

Despite of the positive outcome of multiple types of Statins drugs in clinical trials mentioned above, there are still some unsatisfactory results. The included studies related to atorvastatin and lovastatin did not suggest a positive outcome. For example, atorvastatin has been evaluated in the prevention of the recurrence of prostate cancer, which has shown that there was no association with a lower risk of disease recurrence compared with placebo (Jeong et al., 2021). While glioblastoma patients treated with atorvastatin in combination with radiotherapy and temozolomide did not show an improvement in progression-free survival (Altwairgi et al., 2021). In addition, evaluation of lovastatin as a prevention drug for its use in the treatment of women at increased risk of breast cancer demonstrated no significant biomarker modulation (NCT00285857) (Vinayak et al., 2013). Besides, there is a study of lovastatin in melanoma, which did not show beneficial changes of lovastatin for precancerous lesions (Linden et al., 2014) (NCT00462280). Some studies had been terminated due to the toxicity of drug combination (Chen et al., 2013) (NCT01342887). There are also trials being recruited or underway, and for those without positive results, longer observation periods and larger sample sizes are needed to determine the therapeutic effects of statins on various types of tumors. Besides, for trials with poor outcomes, distinguishing more subgroups, such as gene polymorphism and smoking (Han J.-Y. et al., 2011; Han J. Y. et al., 2011). May lead to meaningful conclusions. Moreover, the safety of statins still needs to be given enough attention when used in combination with chemotherapeutic drugs, and individual differences in drug use for cancer patients also need to be considered.

4.1.2 Targeting squalene synthase

Squalene protects cancer cells from ferroptotic cell death, providing a growth advantage under conditions of oxidative stress produced by high proliferative rates and in tumor xenografts (Garcia-Bermudez et al., 2019). It has been experimentally demonstrated that Zaragozionic acid, a pharmacological inhibitor of Squalene synthase (SQS), can lead to growth arrest and induction of cytotoxicity in prostate cancer cells (Brusselmans et al., 2007). In addition, using TAK-475, a potent inhibitor of squalene synthase (Fdft1), researcher evaluated the efficacy and tolerability of TAK-475 in a mouse transplant model of pancreatic ductal adenocarcinoma (PDA) and showed significantly reduced tumor growth (Biancur et al., 2021).

4.1.3 Targeting SQLE

A recent study showed that increased squalene production due to the loss of squalene epoxidase (SQLE) in cholesterol nutrient-deficient cells prevents oxidative cell death (Garcia-Bermudez et al., 2019). Mahoney et al. (2019) demonstrated that small cell lung cancer (SCLC) lines display sensitivity to NB-598, a known inhibitor of squalene epoxidase (SQLE). In addition, terbinafine (TB) is an antifungal agent that inhibits squalene epoxidase and has been shown to inhibit tumor growth and angiogenesis (Chien et al., 2012), by the mechanism that TB suppresses *in vitro* and *in vivo* proliferation of various tumor cells, including oral, colon and liver cancer *via* inhibiting DNA synthesis and activating apoptosis, which is related to the p53-dependent signaling pathway (Lee et al., 2003).

4.1.4 Targeting OSC

Oxide squalene cyclase (OSC) is the enzyme that catalyzes the conversion of a 2,3-monoepoxy squalene to a lanosterol. Since lanosterol is a precursor to cholesterol, inhibition of OSC leads to reduced cholesterol synthesis, experimental evidence has demonstrated anti-antitumor effects of OSC inhibitors in human glioblastoma and brain-derived endothelial cells and enhanced antitumor effects in combination with statins (Staedler et al., 2012). Ro 48–8071, an OSC inhibitor, shows anti-tumor effect (Maione et al., 2015), and more importantly, it synergizes with 5-fluorouracil, thus eliciting an enhanced anti-tumor outcome.

4.2 Targeting cholesterol uptake

Administration of a low-cholesterol diet or ezetimibe (an inhibitor of NPC1L1) reduces tumor growth by reducing cholesterol levels (Pelton et al., 2014). In addition, it has been demonstrated that the use of leelamine (a lysosomotropic compound, intercellular cholesterol transport inhibitor) suppresses autophagic flux and induces cholesterol accumulation in lysosomal/endosomal cell compartments, disrupts lysosomal cell compartments, and induces cancer cell death (Kuzu et al., 2014). High dietary cholesterol can bypass the need to enhance endogenous cholesterol synthesis, thus accelerate the development of liver cancer. Moreover, major cholesterol metabolites, such as 27HC, 25HC, 22HC, and 6-oxocholsterol-3β, 5α-diol, can promote tumorigenesis (Nelson, 2018; Riscal et al., 2019). Furthermore, to maintain systemic cholesterol homeostasis and reduce ATP depletion of *de novo* cholesterol biosynthesis, some cancer cells alter mevalonate pathway enzyme expression and deregulate cholesterol influx/efflux genes, such as VLDLR, LDLR, SR-B1 and ABCA1, which in turn may lead to cancer cell resistance to statins (Riscal et al., 2019). Therefore, combining a low cholesterol diet or the use of cholesterol absorption inhibitors (such as ezetimibe) with anticancer drugs may be a promising strategy for clinical treatment of tumors.

Vytorin®, a combination drug which contains ezetimibe (10 mg) and simvastatin (40 mg), was used in an early phase I study to determine whether cholesterol-lowering therapy could slow the growth of prostate cancer (NCT02534376). The result shows that Ki-67 staining decreased in normal prostate tissue and low-grade prostate cancers and there was no significant change in Ki-67 staining in high-grade prostate cancers. This suggests that

TABLE 3 Clinical trials of fibrate in cancer.

Drug	Cancer type	Phase	Combined drug	
Fenofibrate	Central Nervous System Tumor, Pediatric	II	Celecoxib Cyclophosphamide Etoposide Thalidomide	Robison et al. (2014)
	Leukemia			
	Lymphoma			
	Neuroblastoma			
	Sarcoma			
	Multiple Myeloma	II	-	
Bezafibrate	Myelodysplastic Syndromes (MDS)	II	Sodium Valproate Medroxyprogesterone	

cholesterol-lowering therapy may decrease growth in both benign prostate that produces voiding symptoms in older men and low-grade prostate cancer (Wang et al., 2022). An ongoing Phase I trial will evaluate a PCSK9-inhibitors (evolocumab) in combination with atorvastatin and ezetimibe in patients with metastatic pancreatic cancer undergoing standard chemotherapy (NCT04862260).

4.3 Targeting cholesterol efflux

Synthetic bette agonists (including fenofibrate) have been used as lipid-lowering therapeutic agents. In addition to the lipid-lowering effects, drugs targeting PPARα also have therapeutic effects in cancer. In fact, Luo et al. (2019) found that intestinal depletion of PPARα promotes colon carcinogenesis by increasing DNMT1-mediated p21 methylation and PRMT6-mediated methylation of p27. While using fenofibrate activated PPAR and inhibited colon carcinogenesis (Luo et al., 2019). It has been shown that fenofibrate inhibition of cell proliferation simultaneously suppresses the expression of key enzymes in fatty acid metabolism and induces human hepatoma Hep3B cells apoptosis (You et al., 2019). In addition, it has been demonstrated that fenofibrate has anti-cancer effects in endometrial cancer, prostate cancer, triple negative breast cancer, oral cancer and pancreatic cancer (Sun et al., 2019; Chen L et al., 2020). Chen L et al. (2020) demonstrated that fenofibrate could induce mitochondrial reprogramming through activation of the AMPK pathway and inhibition of the HK2 pathway, inhibiting gastric cancer cell proliferation and promoting apoptotic through the PPARα pathway. Therefore, targeting PPARα may be an effective cancer treatment and has been tested in clinical trials. When "fenofibrate/bezafibrate | cancer" are taken as the search term, 18 clinical trials have been found on ClinicalTrial.gov, including a phase I study, 6 phase II studies, 5 phase III studies from 2006 to 2023 (Table 3).

A phase II trial of a multi-agent oral antiangiogenic regimen in children with recurrent or progressive cancer had been carried out in 2006 (NCT00357500). "5-drug" regimen, including celecoxib, cyclophosphamide, etoposide, thalidomide, and fenofibrate, was evaluated in patients with eight diseases. Of 97 patients, 24 patients completed 27 weeks of therapy without progression. As a result, the combination of drugs had shown clinical benefits in patients with low-grade glioma and ependymoma (Robison et al., 2014). And the mitochondrial inhibitory function of fenofibrate was tested in a clinical phase II study in patients with multiple myeloma (NCT01965834).

Of the three included studies, one trial on fenofibrate had results and suggested a positive clinical oncology effect. For now, there are fewer clinical trials of fibrates for oncology treatment. More clinical studies can be conducted to confirm the effectiveness of fibrates in the future.

4.4 Targeting cholesterol storage

High expression of ACAT1 is related to cell proliferation rates, tumor formation and metastasis, and cell resistance (Giacomini et al., 2021). Indeed, treatment of breast cancer cells with ACAT-1 inhibitors resulted in reduced cell proliferation and migration and reduced tumor growth through regulation of cholesterol metabolism (Antalis et al., 2010; Shim et al., 2018). Avasimin, a systemically injectable nanoformulation containing the ACAT-1 inhibitor avasimibe has been developed, which has been used in clinical trials for the treatment of atherosclerosis and shows good human safety (Pal et al., 2013; Lee et al., 2015). The formulation was tested in different human cancer cell lines showing that avasimin reduces lipid droplet accumulation in prostate cancer cells and reduces cellular activity in a variety of tumor cell lines (Lee et al., 2015). ACAT-1 was overexpressed in MIA PaCa-2 human pancreatic cancer cells compared to normal cells, and treatment of cells with avasimibe or knockdown of the ACAT-1 gene results in a block of cholesterol esterification, and a decrease in cell invasion and migration. This may be because ACAT-1 inhibition impairs Wnt/β-catenin signaling, thereby overcoming cancer cell metastasis (Lee et al., 2018). The combination of gemcitabine and avasimbe showed synergistic effects *in vitro* and may overcome gemcitabine resistance for pancreatic ductal adenocarcinoma treatment (Li et al., 2018).

4.5 Targeting cholesterol regulation

4.5.1 Targeting SREBP

Fatostatin, a specific inhibitor binds the SREBP-cleavage activating protein (SCAP) to block cholesterol biosynthesis, is able to inhibit tumor growth *in vivo* in a mouse prostate cancer experiment (Li et al., 2014). In endometrial cancer, Fatostatin reduces cancer cell viability and tumor growth in xenografted

mice and improves their survival rate (Gao et al., 2018). It has also been demonstrated that Fatostatin inhibit the growth and proliferation of human endometrial cancer cells, alter its cell cycle and induce apoptotic (Yao et al., 2020). Furthermore, Fatostatin can induce ER degradation by polyubiquitination of K48 junctions, a key mechanism for tamoxifen to inhibit PI3K-AKT-mTOR signaling in breast cancer, and has a synergistic effect with tamoxifen in reducing cell proliferation *in vitro and in vivo* tumor growth in breast cancer, indicating that Fatostatin may have promising clinical use for ER-positive breast cancer patients (Liu et al., 2020). In addition, the combination of Fatostatin and docetaxel resulted in greater proliferation inhibition and apoptosis induction compared with single agent treatment in PCa cells *in vitro* an,d *in vivo*, especially those with mutant p53s (Li et al., 2015). Of note, dipyridamole was also shown to inhibit the cleavage of SREBP2. The statin–dipyridamole combination was synergistic and induced apoptosis in multiple myeloma and AML cell lines and primary patient samples, whereas normal peripheral blood mononuclear cells were not affected (Pandyra et al., 2014).

4.5.2 Targeting RORγ

The RORγ was identified as an important driver of the cholesterol biosynthesis program. RORγ inhibition would counteract the statin-induced SREBP2-dependent feedback regulation and reduce the tumor cholesterol biosynthesis rate without affecting the host cholesterol homeostasis (Cai et al., 2019). Indeed, ROR inhibitors cooperate with statins to kill TNBC (triple-negative breast cancer) cells, and in addition, ROR-selective antagonists are very effective manifested by leading tumor regression and blocking metastasis in multiple TNBC models (Cai et al., 2019).

4.5.3 Targeting LXR

LXR can be activated by endogenous ligands, such as oxysterol or by agonists. In MCF-7 breast cancer cells, treatment with two LXR agonists (TO901317 and 22 (R) -hydroxycholesterol) can inhibit MCF-7 cells proliferation and induce their apoptosis (El Roz et al., 2012). In prostate cancer, the AKT survival pathway was downregulated by treatment with the LXR agonist T0901317, thereby inducing the apoptotic of LNCaP PCa cells in xenograft nude mice and cell cultures (Pommier et al., 2010). Furthermore, it has been demonstrated that the combination treatment of T0901317 and anticancer drug gefitinib exhibits synergistic effects in lung cancer models, inhibiting lung cancer migration and invasion *in vivo* and *in vitro*, which may be through inhibition of ERK/MAPK signaling pathway (Lou et al., 2019). In hematopoietic malignancies, the agonists of LXR (T0901317, GW3965 and DDA) can induce apoptosis or lethal autophagy in leukemic cells (Brendolan and Russo, 2022). The treatment of primary acute myeloid leukemia (AML) samples with dendrogenin A (DDA), a modulator of LXR, that is, a partial LXR agonist, induces lethal autophagy *in vitro* and *in vivo* (de Medina et al., 2021; Brendolan and Russo, 2022). Meanwhile, exogenous 27-Hydroxycholesterol induces apoptosis in leukemic cells (HL60, KG1α, and K562 cells) through the accumulation of reactive oxygen species (ROS) (Woo et al., 2022). In addition, because LXR is a transcription factor towards to different targets including genes associated with glycolysis and lipogenesis, targeting this receptor may be a promising approach for cancer therapy. Interestingly, a reverse agonist SR9243 was designed, and SR9243 inhibits LXR activation by enhancing LXR-corepressor recruitment (Flaveny et al., 2015). It has been demonstrated that SR9243 can induce apoptosis in leukemic cells. In contrast, as was previously described, the activation of LXR by different agonists has also been shown to reduce cancer cell survival by promoting cholesterol efflux, especially in glioblastoma (Villa et al., 2016).

Very recently, the latest trial was just posted on *Clinicaltrials* (ClinicalTrials.gov) on 23 January 2023 which is initiated in 2016 (ClinicalTrials.gov Identifier: NCT02922764). This is a phase I, dose escalation and expansion study of RGX-104, an oral small molecule targeting the LXR. By depleting both myeloid-derived suppressor cells (MDSCs) and tumor blood vessels, it exerts its anti-tumor activity (Tavazoie et al., 2018). This trial will evaluate single agents or combinations in patients with advanced solid tumors and lymphomas. Combinations include nivolumab, ipilimumab, docetaxel, or pembrolizumab plus carboplatin/pemetrexed. In the expansion stage, the study will provide further characterization of the safety, efficacy, PK, and pharmacodynamics. Immunological activity and biomarkers of LXR target activation will also be evaluated.

The statins, as well as ezetimibe and fibrates mentioned in the above clinical trials, are all approved in the blood cholesterol guideline, which demonstrate their safety and feasibility for oncology treatment (Grundy et al., 2019). In the last 3 years, there were 29 ongoing phase II or III clinical trials for oncology treatment with statins alone or in combination with other drugs, 13 of which were first posted in these 3 years. Other targeted drugs related to cholesterol metabolism are also gaining attention. These trials focus on the prevention of cholesterol metabolism-related drugs in patients at high risk for cancer, the treatment of further disease progression, and the prevention of recurrence in cancer patients, and are primarily focused on breast, prostate, small cell lung, intestinal and uterine cancers. Furthermore, other trials focusing on the prevention and treatment of side effects of chemotherapy and radiotherapy for tumors, such as heart failure, hearing loss (Fernandez et al., 2021), and metabolic syndrome, which are not selected for analysis but show the promise of this class of drugs in oncology treatment.

5 Conclusion and perspectives

Cholesterol is one of the important nutrients for normal physiological function, the latest Dietary Guidelines for Americans and Chinese removed the restriction for dietary cholesterol. However, we should think calmly about dietary cholesterol and health. Restricted dietary cholesterol intake in people at high risk of cardiovascular disease is recommended in many guidelines. In addition, dietary cholesterol is just one aspect of a healthy diet. Population health is closely related to the overall dietary pattern. We should not only pay attention to a separate aspect of the food, but also consider the interactive effects of multiple foods. Besides, Current dietary guidelines limit saturated fatty acids to 10% of total energy, and dietary cholesterol intake is generally not too high if people meet this requirement.

Cholesterol is normally linked to cardiovascular diseases. Recently, there has been extensive evidence demonstrating that cardiovascular disease and cancer are intertwined. Firstly,

cardiovascular disease and cancer share several common risk factors, including diabetes, dyslipidemia, cachexia, and an impaired immune response. Secondly, Anticancer therapies can induce CVD *via* several mechanisms, including direct cardiotoxicity, effects on the vasculature, and perturbations to cardiovascular and immune homeostasis (Curigliano et al., 2012; Karlstaedt et al., 2022). Thirdly, patients with cardiovascular disease have higher cancer risk compared with individuals from the general population (a concept referred to as reverse cardio-oncology) (Aboumsallem et al., 2020; Karlstaedt et al., 2022; Koelwyn et al., 2022).

In this review, it is evident that cholesterol metabolism is critical for cancer progression and targeted drugs including statins and fibrates are widely used in clinical trials (Huang et al., 2020; Xu et al., 2020). However, there are still a number of outstanding questions in the field need to be further addressed. Firstly, in cholesterol metabolism targeted therapy, the maintenance of cholesterol homeostasis is more important than just lowers the level of cholesterol. Secondly, the accurate metabolic subtypes of cancers should be established for better applying metabolic therapy. Thirdly, it is not so clear that the effect of cholesterol metabolism on immune microenvironment which also plays the key roles upon tumor therapy. So far, the efficacy of targeted cholesterol metabolism therapy largely depends on cancer types and all targeted drugs are not used as first-line drugs but used in combination with other therapy. Besides directly targeting cholesterol metabolism, bile acid, the main product of cholesterol transformation, directly affects the intestinal microflora, and the microecology is closely related to the occurrence and prognosis of cancers. Therefore, we should also focus on the microecology of intestinal microflora while detecting cholesterol levels inside and outside tumor cells. Nevertheless, all these progressions from bench to bed make targeting cholesterol metabolism therapy a fascinating field to work in, and targeted therapy which is more effectively, safely, precisely and comprehensively should be further investigated.

Author contributions

WX and HW contributed equally to this work, and drafted the manuscript. XZ and YW wrote the part of targeted drugs in clinical trials and tables. LX, BC, and JS conceived and designed the study, reviewed the manuscript. LX, JS, and WX were responsible for the final review of the manuscript. All authors read and approved the final manuscript.

Funding

The study was supported by the National Natural Science Foundation of China (No. 81972966, No. 82001248, and No. 81902840), Beijing Natural Science Foundation (No. 7214269) and the study was supported by the State Key Laboratory of Natural and Biomimetic Drugs (No. K202226).

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

Aboumsallem, J. P., Moslehi, J., and de Boer, R. A. (2020). Reverse cardio-oncology: Cancer development in patients with cardiovascular disease. *J. Am. Heart Assoc.* 9, e013754. doi:10.1161/JAHA.119.013754

Advani, A. S., McDonough, S., Copelan, E., Willman, C., Mulford, D. A., List, A. F., et al. (2014). SWOG0919: A phase 2 study of idarubicin and cytarabine in combination with pravastatin for relapsed acute myeloid leukaemia. *Br. J. Haematol.* 167, 233–237. doi:10.1111/bjh.13035

Aguirre-Portoles, C., Feliu, J., Reglero, G., and Ramirez de Molina, A. (2018). ABCA1 overexpression worsens colorectal cancer prognosis by facilitating tumour growth and caveolin-1-dependent invasiveness, and these effects can be ameliorated using the BET inhibitor apabetalone. *Mol. Oncol.* 12, 1735–1752. doi:10.1002/1878-0261.12367

Allott, E. H., Howard, L. E., Cooperberg, M. R., Kane, C. J., Aronson, W. J., Terris, M. K., et al. (2014). Serum lipid profile and risk of prostate cancer recurrence: Results from the SEARCH database. *Cancer Epidemiol. Biomarkers Prev.* 23, 2349–2356. doi:10.1158/1055-9965.EPI-14-0458

Altmann, S. W., Davis, H. R., Jr., Zhu, L. J., Yao, X., Hoos, L. M., Tetzloff, G., et al. (2004). Niemann-Pick C1 like 1 protein is critical for intestinal cholesterol absorption. *Science* 303, 1201–1204. doi:10.1126/science.1093131

Altwairgi, A. K., Alghareeb, W. A., AlNajjar, F. H., Alhussain, H., Alsaeed, E., Balbaid, A. A. O., et al. (2021). Atorvastatin in combination with radiotherapy and temozolomide for glioblastoma: A prospective phase II study. *Invest. New Drugs* 39, 226–231. doi:10.1007/s10637-020-00992-5

Antalis, C. J., Arnold, T., Rasool, T., Lee, B., Buhman, K. K., and Siddiqui, R. A. (2010). High ACAT1 expression in estrogen receptor negative basal-like breast cancer cells is associated with LDL-induced proliferation. *Breast Cancer Res. Treat.* 122, 661–670. doi:10.1007/s10549-009-0594-8

Baek, A. E., Yu, Y.-R. A., He, S., Wardell, S. E., Chang, C.-Y., Kwon, S., et al. (2017). The cholesterol metabolite 27 hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells. *Nat. Commun.* 8, 864. doi:10.1038/s41467-017-00910-z

Bao, T., Blackford, A. L., and Stearns, V. (2012). Effect of simvastatin on the pharmacokinetics of anastrozole. *Breast Cancer Res. Treat.* 131, 709–711. doi:10.1007/s10549-011-1859-6

Biancur, D. E., Kapner, K. S., Yamamoto, K., Banh, R. S., Neggers, J. E., Sohn, A. ;S. W., et al. (2021). Functional genomics identifies metabolic vulnerabilities in pancreatic cancer. *Cell Metab.* 33, 199–210.e8. doi:10.1016/j.cmet.2020.10.018

Blanc, J. F., Khemissa, F., Bronowicki, J. P., Monterymard, C., Perarnau, J. M., Bourgeois, V., et al. (2021). Phase 2 trial comparing sorafenib, pravastatin, their combination or supportive care in HCC with Child-Pugh B cirrhosis. *Hepatol. Int.* 15, 93–104. doi:10.1007/s12072-020-10120-3

Brendolan, A., and Russo, V. (2022). Targeting cholesterol homeostasis in hematopoietic malignancies. *Blood* 139, 165–176. doi:10.1182/blood.2021012788

Brusselmans, K., Timmermans, L., Van de Sande, T., Van Veldhoven, P. P., Guan, G., Shechter, I., et al. (2007). Squalene synthase, a determinant of Raft-associated cholesterol and modulator of cancer cell proliferation. *J. Biol. Chem.* 282, 18777–18785. doi:10.1074/jbc.M611763200

Buhaescu, I., and Izzedine, H. (2007). Mevalonate pathway: A review of clinical and therapeutical implications. *Clin. Biochem.* 40, 575–584. doi:10.1016/j.clinbiochem.2007.03.016

Cai, D., Wang, J., Gao, B., Li, J., Wu, F., Zou, J. X., et al. (2019). RORγ is a targetable master regulator of cholesterol biosynthesis in a cancer subtype. *Nat. Commun.* 10, 4621. doi:10.1038/s41467-019-12529-3

Campion, O., Al Khalifa, T., Langlois, B., Thevenard-Devy, J., Salesse, S., Savary, K., et al. (2020). Contribution of the low-density lipoprotein receptor family to breast cancer progression. *Front. Oncol.* 10, 882. doi:10.3389/fonc.2020.00882

Cerqueira, N. M., Oliveira, E. F., Gesto, D. S., Santos-Martins, D., Moreira, C., Moorthy, H. N., et al. (2016). Cholesterol biosynthesis: A mechanistic overview. *Biochemistry* 55, 5483–5506. doi:10.1021/acs.biochem.6b00342

Chang, T. Y., Chang, C. C., Ohgami, N., and Yamauchi, Y. (2006). Cholesterol sensing, trafficking, and esterification. *Annu. Rev. Cell Dev. Biol.* 22, 129–157. doi:10.1146/annurev.cellbio.22.010305.104656

Chen L, L., Peng, J., Wang, Y., Jiang, H., Wang, W., Dai, J., et al. (2020). Fenofibrate-induced mitochondrial dysfunction and metabolic reprogramming reversal: The anti-tumor effects in gastric carcinoma cells mediated by the PPAR pathway. *Am. J. Transl. Res.* 12, 428–446.

Chen, T. L., Estey, E. H., Othus, M., Gardner, K. M., Markle, L. J., and Walter, R. B. (2013). Cyclosporine modulation of multidrug resistance in combination with pravastatin, mitoxantrone and etoposide for adult patients with relapsed/refractory acute myeloid leukemia: A phase 1/2 study. *Leukemia Lymphoma* 54, 2534–2536. doi:10.3109/10428194.2013.777836

Chen Y H, Y. H., Chen, Y. C., Lin, C. C., Hsieh, Y. P., Hsu, C. S., and Hsieh, M. C. (2020). Synergistic anticancer effects of gemcitabine with pitavastatin on pancreatic cancer cell line MIA PaCa-2 *in vitro* and *in vivo*. *Cancer Manag. Res.* 12, 4645–4665. doi:10.2147/CMAR.S247876

Chien, M. H., Lee, T. S., Kao, C., Yang, S. F., and Lee, W. S. (2012). Terbinafine inhibits oral squamous cell carcinoma growth through anti-cancer cell proliferation and anti-angiogenesis. *Mol. Carcinog.* 51, 389–399. doi:10.1002/mc.20800

Cruz, P. M., Mo, H., McConathy, W. J., Sabnis, N., and Lacko, A. G. (2013). The role of cholesterol metabolism and cholesterol transport in carcinogenesis: A review of scientific findings, relevant to future cancer therapeutics. *Front. Pharmacol.* 4, 119. doi:10.3389/fphar.2013.00119

Curigliano, G., Cardinale, D., Suter, T., Plataniotis, G., de Azambuja, E., Sandri, M. T., et al. (2012). Cardiovascular toxicity induced by chemotherapy, targeted agents and radiotherapy: ESMO clinical practice guidelines. *Ann. Oncol. Official J. Eur. Soc. Med. Oncol.* 23 (7), vii155–vii166. vii155-vii166. doi:10.1093/annonc/mds293

de Gonzalo-Calvo, D., Lopez-Vilaro, L., Nasarre, L., Perez-Olabarria, M., Vazquez, T., Escuin, D., et al. (2015). Intratumor cholesteryl ester accumulation is associated with human breast cancer proliferation and aggressive potential: A molecular and clinicopathological study. *BMC Cancer* 15, 460. doi:10.1186/s12885-015-1469-5

de Medina, P., Diallo, K., Huc-Claustre, E., Attia, M., Soulès, R., Silvente-Poirot, S., et al. (2021). The 5,6-epoxycholesterol metabolic pathway in breast cancer: Emergence of new pharmacological targets. *Br. J. Pharmacol.* 178, 3248–3260. doi:10.1111/bph.15205

Di Bello, E., Zwergel, C., Mai, A., and Valente, S. (2020). *The Innovative Potential of Statins in Cancer*, 8.*New Targets New Ther.*

Ehmsen, S., Pedersen, M. H., Wang, G., Terp, M. G., Arslanagic, A., Hood, B. L., et al. (2019). Increased cholesterol biosynthesis is a key characteristic of breast cancer stem cells influencing patient outcome. *Cell Rep.* 27, 3927–3938.e6. doi:10.1016/j.celrep.2019.05.104

El Roz, A., Bard, J. M., Huvelin, J. M., and Nazih, H. (2012). LXR agonists and ABCG1-dependent cholesterol efflux in MCF-7 breast cancer cells: Relation to proliferation and apoptosis. *Anticancer Res.* 32, 3007–3013.

Fatehi Hassanabad, A. (2019). Current perspectives on statins as potential anti-cancer therapeutics: Clinical outcomes and underlying molecular mechanisms. *Transl. Lung Cancer Res.* 8, 692–699. doi:10.21037/tlcr.2019.09.08

Feng, J., Dai, W., Mao, Y., Wu, L., Li, J., Chen, K., et al. (2020). Simvastatin re-sensitizes hepatocellular carcinoma cells to sorafenib by inhibiting HIF-1α/PPAR-γ/PKM2-mediated glycolysis. *J. Exp. Clin. Cancer Res. CR* 39, 24. doi:10.1186/s13046-020-1528-x

Fernandez, K. A., Allen, P., Campbell, M., Page, B., Townes, T., Li, C. M., et al. (2021). Atorvastatin is associated with reduced cisplatin-induced hearing loss. *J. Clin. Invest.* 131, e142616. doi:10.1172/JCI142616

Flaveny, C. A., Griffett, K., El-Gendy Bel, D., Kazantzis, M., Sengupta, M., Amelio, A. L., et al. (2015). Broad anti-tumor activity of a small molecule that selectively targets the warburg effect and lipogenesis. *Cancer Cell* 28, 42–56. doi:10.1016/j.ccell.2015.05.007

Freed-Pastor, W. A., Mizuno, H., Zhao, X., Langerod, A., Moon, S. H., Rodriguez-Barrueco, R., et al. (2012). Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. *Cell* 148, 244–258. doi:10.1016/j.cell.2011.12.017

Gallagher, E. J., Zelenko, Z., Neel, B. A., Antoniou, I. M., Rajan, L., Kase, N., et al. (2017). Elevated tumor LDLR expression accelerates LDL cholesterol-mediated breast cancer growth in mouse models of hyperlipidemia. *Oncogene* 36, 6462–6471. doi:10.1038/onc.2017.247

Gao, S., Shi, Z., Li, X., Li, W., Wang, Y., Liu, Z., et al. (2018). Fatostatin suppresses growth and enhances apoptosis by blocking SREBP-regulated metabolic pathways in endometrial carcinoma. *Oncol. Rep.* 39, 1919–1929. doi:10.3892/or.2018.6265

Garcia-Bermudez, J., Baudrier, L., Bayraktar, E. C., Shen, Y., La, K., Guarecuco, R., et al. (2019). Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death. *Nature* 567, 118–122. doi:10.1038/s41586-019-0945-5

Garwood, E. R., Kumar, A. S., Baehner, F. L., Moore, D. H., Au, A., Hylton, N., et al. (2010). Fluvastatin reduces proliferation and increases apoptosis in women with high grade breast cancer. *Breast Cancer Res. Treat.* 119, 137–144. doi:10.1007/s10549-009-0507-x

Geng, F., Cheng, X., Wu, X., Yoo, J. Y., Cheng, C., Guo, J. Y., et al. (2016). Inhibition of SOAT1 suppresses glioblastoma growth via blocking SREBP-1-mediated lipogenesis. *Clin. Cancer Res.* 22, 5337–5348. doi:10.1158/1078-0432.CCR-15-2973

Giacomini, I., Gianfanti, F., Desbats, M. A., Orso, G., Berretta, M., Prayer-Galetti, T., et al. (2021). Cholesterol metabolic reprogramming in cancer and its pharmacological modulation as therapeutic strategy. *Front. Oncol.* 11, 682911. doi:10.3389/fonc.2021.682911

Grabacka, M., and Reiss, K. (2008). Anticancer properties of PPARalpha-effects on cellular metabolism and inflammation. *PPAR Res.* 2008, 930705. doi:10.1155/2008/930705

Graf, G. A., Yu, L., Li, W. P., Gerard, R., Tuma, P. L., Cohen, J. C., et al. (2003). ABCG5 and ABCG8 are obligate heterodimers for protein trafficking and biliary cholesterol excretion. *J. Biol. Chem.* 278, 48275–48282. doi:10.1074/jbc.M310223200

Grundy, S. M., Stone, N. J., Bailey, A. L., Beam, C., Birtcher, K. K., Blumenthal, R. S., et al. (2019). 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: A report of the American college of cardiology/American heart association task force on clinical practice guidelines. *Circulation* 139, e1082–e1143. doi:10.1161/CIR.0000000000000625

Gu, L., Saha, S. T., Thomas, J., and Kaur, M. (2019). Targeting cellular cholesterol for anticancer therapy. *FEBS J.* 286, 4192–4208. doi:10.1111/febs.15018

Guo, D., Reinitz, F., Youssef, M., Hong, C., Nathanson, D., Akhavan, D., et al. (2011). An LXR agonist promotes glioblastoma cell death through inhibition of an EGFR/AKT/SREBP-1/LDLR-dependent pathway. *Cancer Discov.* 1, 442–456. doi:10.1158/2159-8290.CD-11-0102

Han, J.-Y., Lim, K. Y., Yu, S. Y., Yun, T., Kim, H. T., and Lee, J. S. (2011). A phase 2 study of irinotecan, cisplatin, and simvastatin for untreated extensive-disease small cell lung cancer. *Cancer* 117, 2178–2185. doi:10.1002/cncr.25790

Han, J. Y., Lee, S. H., Yoo, N. J., Hyung, L. S., Moon, Y. J., Yun, T., et al. (2011). A randomized phase II study of gefitinib plus simvastatin versus gefitinib alone in previously treated patients with advanced non-small cell lung cancer. *Clin. Cancer Res.* 17, 1553–1560. doi:10.1158/1078-0432.CCR-10-2525

He, J., Shin, H., Wei, X., Kadegowda, A. K., Chen, R., and Xie, S. K. (2015). NPC1L1 knockout protects against colitis-associated tumorigenesis in mice. *BMC Cancer* 15, 189. doi:10.1186/s12885-015-1230-0

Horton, J. D., Shah, N. A., Warrington, J. A., Anderson, N. N., Park, S. W., Brown, M. S., et al. (2003). Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. U. S. A.* 100, 12027–12032. doi:10.1073/pnas.1534923100

Huang, B., Song, B.-L., and Xu, C. (2020). Cholesterol metabolism in cancer: Mechanisms and therapeutic opportunities. *Nat. Metab.* 2, 132–141. doi:10.1038/s42255-020-0174-0

Huang, J., Li, L., Lian, J., Schauer, S., Vesely, P. W., Kratky, D., et al. (2016). Tumor-induced hyperlipidemia contributes to tumor growth. *Cell Rep.* 15, 336–348. doi:10.1016/j.celrep.2016.03.020

Hulce, J. J., Cognetta, A. B., Niphakis, M. J., Tully, S. E., and Cravatt, B. F. (2013). Proteome-wide mapping of cholesterol-interacting proteins in mammalian cells. *Nat. Methods* 10, 259–264. doi:10.1038/nmeth.2368

Iwayanagi, Y., Takada, T., and Suzuki, H. (2008). HNF4alpha is a crucial modulator of the cholesterol-dependent regulation of NPC1L1. *Pharm. Res.* 25, 1134–1141. doi:10.1007/s11095-007-9496-9

Jeong, I. G., Lim, B., Yun, S. C., Lim, J. H., Hong, J. H., and Kim, C. S. (2021). Adjuvant low-dose statin use after radical prostatectomy: The PRO-STAT randomized clinical trial. *Clin. Cancer Res.* 27, 5004–5011. doi:10.1158/1078-0432.CCR-21-0480

Jouve, J. L., Lecomte, T., Bouché, O., Barbier, E., Khemissa Akouz, F., Riachi, G., et al. (2019). Pravastatin combination with sorafenib does not improve survival in advanced hepatocellular carcinoma. *J. Hepatol.* 71, 516–522. doi:10.1016/j.jhep.2019.04.021

Karlstaedt, A., Moslehi, J., and de Boer, R. A. (2022). Cardio-onco-metabolism: Metabolic remodelling in cardiovascular disease and cancer. *Nat. Rev. Cardiol.* 19, 414–425. doi:10.1038/s41569-022-00698-6

Kennedy, M. A., Barrera, G. C., Nakamura, K., Baldan, A., Tarr, P., Fishbein, M. C., et al. (2005). ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. *Cell Metab.* 1, 121–131. doi:10.1016/j.cmet.2005.01.002

Kidera, Y., Tsubaki, M., Yamazoe, Y., Shoji, K., Nakamura, H., Ogaki, M., et al. (2010). Reduction of lung metastasis, cell invasion, and adhesion in mouse melanoma by statin-induced blockade of the Rho/Rho-associated coiled-coil-containing protein kinase pathway. *J. Exp. Clin. Cancer Res. CR* 29, 127. doi:10.1186/1756-9966-29-127

Kim, W. Y. (2019). Therapeutic targeting of lipid synthesis metabolism for selective elimination of cancer stem cells. *Arch. Pharm. Res.* 42, 25–39. doi:10.1007/s12272-018-1098-z

Koelwyn, G. J., Aboumsallem, J. P., Moore, K. J., and de Boer, R. A. (2022). Reverse cardio-oncology: Exploring the effects of cardiovascular disease on cancer pathogenesis. *J. Mol. Cell. Cardiol.* 163, 1–8. doi:10.1016/j.yjmcc.2021.09.008

Kopecka, J., Godel, M., and Riganti, C. (2020a). Cholesterol metabolism: At the cross road between cancer cells and immune environment. *Int. J. Biochem. Cell Biol.* 129, 105876. doi:10.1016/j.biocel.2020.105876

Kopecka, J., Trouillas, P., Gasparovic, A. C., Gazzano, E., Assaraf, Y. G., and Riganti, C. (2020b). Phospholipids and cholesterol: Inducers of cancer multidrug resistance and therapeutic targets. *Drug Resist Updat* 49, 100670. doi:10.1016/j.drup.2019.100670

Kuzu, O. F., Gowda, R., Sharma, A., and Robertson, G. P. (2014). Leelamine mediates cancer cell death through inhibition of intracellular cholesterol transport. *Mol. Cancer Ther.* 13, 1690–1703. doi:10.1158/1535-7163.MCT-13-0868

Kuzu, O. F., Noory, M. A., and Robertson, G. P. (2016). The role of cholesterol in cancer. *Cancer Res.* 76, 2063–2070. doi:10.1158/0008-5472.CAN-15-2613

Lanterna, C., Musumeci, A., Raccosta, L., Corna, G., Moresco, M., Maggioni, D., et al. (2016). The administration of drugs inhibiting cholesterol/oxysterol synthesis is safe and increases the efficacy of immunotherapeutic regimens in tumor-bearing mice. *Cancer Immunol. Immunother.* 65, 1303–1315. doi:10.1007/s00262-016-1884-8

Lazzeroni, M., Guerrieri-Gonzaga, A., Serrano, D., Cazzaniga, M., Mora, S., Casadio, C., et al. (2012). Breast ductal lavage for biomarker assessment in high risk women: Rationale, design and methodology of a randomized phase II clinical trial with nimesulide, simvastatin and placebo. *BMC Cancer* 12, 575. doi:10.1186/1471-2407-12-575

Lee, H. J., Li, J., Vickman, R. E., Li, J., Liu, R., Durkes, A. C., et al. (2018). Cholesterol esterification inhibition suppresses prostate cancer metastasis by impairing the wnt/β-catenin pathway. *Mol. Cancer Res.* 16, 974–985. doi:10.1158/1541-7786.MCR-17-0665

Lee, S. S., Li, J., Tai, J. N., Ratliff, T. L., Park, K., and Cheng, J. X. (2015). Avasimibe encapsulated in human serum albumin blocks cholesterol esterification for selective cancer treatment. *ACS Nano* 9, 2420–2432. doi:10.1021/nn504025a

Lee, W. S., Chen, R. J., Wang, Y. J., Tseng, H., Jeng, J. H., Lin, S. Y., et al. (2003). *In vitro* and *in vivo* studies of the anticancer action of terbinafine in human cancer cell lines: G0/G1 p53-associated cell cycle arrest. *Int. J. Cancer* 106, 125–137. doi:10.1002/ijc.11194

Lewis, C. A., Brault, C., Peck, B., Bensaad, K., Griffiths, B., Mitter, R., et al. (2015). SREBP maintains lipid biosynthesis and viability of cancer cells under lipid- and oxygen-deprived conditions and defines a gene signature associated with poor survival in glioblastoma multiforme. *Oncogene* 34, 5128–5140. doi:10.1038/onc.2014.439

Li, J., Qu, X., Tian, J., Zhang, J. T., and Cheng, J. X. (2018). Cholesterol esterification inhibition and gemcitabine synergistically suppress pancreatic ductal adenocarcinoma proliferation. *PLoS One* 13, e0193318. doi:10.1371/journal.pone.0193318

Li, X., Chen, Y. T., Hu, P., and Huang, W. C. (2014). Fatostatin displays high antitumor activity in prostate cancer by blocking SREBP-regulated metabolic pathways and androgen receptor signaling. *Mol. Cancer Ther.* 13, 855–866. doi:10.1158/1535-7163.MCT-13-0797

Li, X., Wu, J. B., Chung, L. W., and Huang, W. C. (2015). Anti-cancer efficacy of SREBP inhibitor, alone or in combination with docetaxel, in prostate cancer harboring p53 mutations. *Oncotarget* 6, 41018–41032. doi:10.18632/oncotarget.5879

Liang, J. Q., Teoh, N., Xu, L., Pok, S., Li, X., Chu, E. S. H., et al. (2018). Dietary cholesterol promotes steatohepatitis related hepatocellular carcinoma through dysregulated metabolism and calcium signaling. *Nat. Commun.* 9, 4490. doi:10.1038/s41467-018-06931-6

Liang, Y., Besch-Williford, C., Aebi, J. D., Mafuvadze, B., Cook, M. T., Zou, X., et al. (2014). Cholesterol biosynthesis inhibitors as potent novel anti-cancer agents: Suppression of hormone-dependent breast cancer by the oxidosqualene cyclase inhibitor RO 48-8071. *Breast Cancer Res. Treat.* 146, 51–62. doi:10.1007/s10549-014-2996-5

Lin, C.-Y., Huo, C., Kuo, L.-K., Hiipakka, R. A., Jones, R. B., Lin, H.-P., et al. (2013). Cholestane-3β, 5α, 6β-triol suppresses proliferation, migration, and invasion of human prostate cancer cells. *PloS One* 8, e65734. doi:10.1371/journal.pone.0065734

Linden, K. G., Leachman, S. A., Zager, J. S., Jakowatz, J. G., Viner, J. L., McLaren, C. E., et al. (2014). A randomized, double-blind, placebo-controlled phase II clinical trial of lovastatin for various endpoints of melanoma pathobiology. *Cancer Prev. Res. (Phila)* 7, 496–504. doi:10.1158/1940-6207.CAPR-13-0189

Liu, W., Chakraborty, B., Safi, R., Kazmin, D., Chang, C.-Y., and McDonnell, D. P. (2021). Dysregulated cholesterol homeostasis results in resistance to ferroptosis increasing tumorigenicity and metastasis in cancer. *Nat. Commun.* 12, 5103. doi:10.1038/s41467-021-25354-4

Liu, Y., Zhang, N., Zhang, H., Wang, L., Duan, Y., Wang, X., et al. (2020). Fatostatin in combination with tamoxifen induces synergistic inhibition in ER-positive breast cancer. *Drug Des. Devel Ther.* 14, 3535–3545. doi:10.2147/DDDT.S253876

Longo, J., Hamilton, R. J., Masoomian, M., Khurram, N., Branchard, E., Mullen, P. J., et al. (2020). A pilot window-of-opportunity study of preoperative fluvastatin in localized prostate cancer. *Prostate Cancer Prostatic Dis.* 23, 630–637. doi:10.1038/s41391-020-0221-7

Lou, R., Cao, H., Dong, S., Shi, C., Xu, X., Ma, R., et al. (2019). Liver X receptor agonist T0901317 inhibits the migration and invasion of non-small-cell lung cancer cells *in vivo* and *in vitro*. *Anticancer Drugs* 30, 495–500. doi:10.1097/CAD.0000000000000758

Lubtow, M. M., Oerter, S., Quader, S., Jeanclos, E., Cubukova, A., Krafft, M., et al. (2020). *In vitro* blood-brain barrier permeability and cytotoxicity of an atorvastatin-loaded nanoformulation against glioblastoma in 2D and 3D models. *Mol. Pharm.* 17, 1835–1847. doi:10.1021/acs.molpharmaceut.9b01117

Luo, J., Yang, H., and Song, B. L. (2020). Mechanisms and regulation of cholesterol homeostasis. *Nat. Rev. Mol. Cell Biol.* 21, 225–245. doi:10.1038/s41580-019-0190-7

Luo, Y., Xie, C., Brocker, C. N., Fan, J., Wu, X., Feng, L., et al. (2019). Intestinal PPARα protects against colon carcinogenesis via regulation of methyltransferases DNMT1 and PRMT6. *Gastroenterology* 157, 744–759.e4. doi:10.1053/j.gastro.2019.05.057

Luu, W., Sharpe, L. J., Capell-Hattam, I., Gelissen, I. C., and Brown, A. J. (2016). Oxysterols: Old tale, new twists. *Annu. Rev. Pharmacol. Toxicol.* 56, 447–467. doi:10.1146/annurev-pharmtox-010715-103233

Mahoney, C. E., Pirman, D., Chubukov, V., Sleger, T., Hayes, S., Fan, Z. P., et al. (2019). A chemical biology screen identifies a vulnerability of neuroendocrine cancer cells to SQLE inhibition. *Nat. Commun.* 10, 96. doi:10.1038/s41467-018-07959-4

Maione, F., Oliaro-Bosso, S., Meda, C., Di Nicolantonio, F., Bussolino, F., Balliano, G., et al. (2015). The cholesterol biosynthesis enzyme oxidosqualene cyclase is a new target to impair tumour angiogenesis and metastasis dissemination. *Sci. Rep.* 5, 9054. doi:10.1038/srep09054

McDonnell, D. P., Park, S., Goulet, M. T., Jasper, J., Wardell, S. E., Chang, C.-Y., et al. (2014). Obesity, cholesterol metabolism, and breast cancer pathogenesis. *Cancer Res.* 74, 4976–4982. doi:10.1158/0008-5472.CAN-14-1756

Nelson, E. R. (2018). The significance of cholesterol and its metabolite, 27-hydroxycholesterol in breast cancer. *Mol. Cell Endocrinol.* 466, 73–80. doi:10.1016/j.mce.2017.09.021

Nielsen, S. F., Nordestgaard, B. G., and Bojesen, S. E. (2012). Statin use and reduced cancer-related mortality. *N. Engl. J. Med.* 367, 1792–1802. doi:10.1056/NEJMoa1201735

Nohturfft, A., and Zhang, S. C. (2009). Coordination of lipid metabolism in membrane biogenesis. *Annu. Rev. Cell Dev. Biol.* 25, 539–566. doi:10.1146/annurev.cellbio.24.110707.175344

Okubo, K., Isono, M., Miyai, K., Asano, T., and Sato, A. (2020). Fluvastatin potentiates anticancer activity of vorinostat in renal cancer cells. *Cancer Sci.* 111, 112–126. doi:10.1111/cas.14225

Osmak, M. (2012). Statins and cancer: Current and future prospects. *Cancer Lett.* 324, 1–12. doi:10.1016/j.canlet.2012.04.011

Ouimet, M., Barrett, T. J., and Fisher, E. A. (2019). HDL and reverse cholesterol transport. *Circ. Res.* 124, 1505–1518. doi:10.1161/CIRCRESAHA.119.312617

Pal, P., Gandhi, H., Giridhar, R., and Yadav, M. R. (2013). ACAT inhibitors: The search for novel cholesterol lowering agents. *Mini Rev. Med. Chem.* 13, 1195–1219. doi:10.2174/1389557511313080007

Pandyra, A., Mullen, P. J., Kalkat, M., Yu, R., Pong, J. T., Li, Z., et al. (2014). Immediate utility of two approved agents to target both the metabolic mevalonate pathway and its restorative feedback loop. *Cancer Res.* 74, 4772–4782. doi:10.1158/0008-5472.CAN-14-0130

Pelton, K., Coticchia, C. M., Curatolo, A. S., Schaffner, C. P., Zurakowski, D., Solomon, K. R., et al. (2014). Hypercholesterolemia induces angiogenesis and accelerates growth of breast tumors *in vivo*. *Am. J. Pathol.* 184, 2099–2110. doi:10.1016/j.ajpath.2014.03.006

Petan, T., Jarc, E., and Jusovic, M. (2018). Lipid droplets in cancer: Guardians of fat in a stressful world. *Molecules* 23.

Pommier, A. J., Alves, G., Viennois, E., Bernard, S., Communal, Y., Sion, B., et al. (2010). Liver X Receptor activation downregulates AKT survival signaling in lipid rafts and induces apoptosis of prostate cancer cells. *Oncogene* 29, 2712–2723. doi:10.1038/onc.2010.30

Raccosta, L., Fontana, R., Maggioni, D., Lanterna, C., Villablanca, E. J., Paniccia, A., et al. (2013). The oxysterol-CXCR2 axis plays a key role in the recruitment of tumor-promoting neutrophils. *J. Exp. Med.* 210, 1711–1728. doi:10.1084/jem.20130440

Riaño, I., Martín, L., Varela, M., Serrano, T., Núñez, O., Mínguez, B., et al. (2020). *Efficacy and safety of the combination of pravastatin and sorafenib for the treatment of advanced hepatocellular carcinoma*. Cancers (Basel): ESTAHEP Clinical Trial, 12.

Riscal, R., Skuli, N., and Simon, M. C. (2019). Even cancer cells watch their cholesterol. *Mol. Cell* 76, 220–231. doi:10.1016/j.molcel.2019.09.008

Robison, N. J., Campigotto, F., Chi, S. N., Manley, P. E., Turner, C. D., Zimmerman, M. A., et al. (2014). A phase II trial of a multi-agent oral antiangiogenic (metronomic) regimen in children with recurrent or progressive cancer. *Pediatr. Blood Cancer* 61, 636–642. doi:10.1002/pbc.24794

Rosenson, R. S., Brewer, H. B., Jr., Davidson, W. S., Fayad, Z. A., Fuster, V., Goldstein, J., et al. (2012). Cholesterol efflux and atheroprotection: Advancing the concept of reverse cholesterol transport. *Circulation* 125, 1905–1919. doi:10.1161/CIRCULATIONAHA.111.066589

Seckl, M. J., Ottensmeier, C. H., Cullen, M., Schmid, P., Ngai, Y., Muthukumar, D., et al. (2017). Multicenter, phase III, randomized, double-blind, placebo-controlled trial of pravastatin added to first-line standard chemotherapy in small-cell lung cancer (LUNGSTAR). *J. Clin. Oncol. Official J. Am. Soc. Clin. Oncol.* 35, 1506–1514. doi:10.1200/JCO.2016.69.7391

Shadman, M., Mawad, R., Dean, C., Chen, T. L., Shannon-Dorcy, K., Sandhu, V., et al. (2015). Idarubicin, cytarabine, and pravastatin as induction therapy for untreated acute myeloid leukemia and high-risk myelodysplastic syndrome. *Am. J. Hematol.* 90, 483–486. doi:10.1002/ajh.23981

Shim, S. H., Sur, S., Steele, R., Albert, C. J., Huang, C., Ford, D. A., et al. (2018). Disrupting cholesterol esterification by bitter melon suppresses triple-negative breast cancer cell growth. *Mol. Carcinog.* 57, 1599–1607. doi:10.1002/mc.22882

Smith, B., and Land, H. (2012). Anticancer activity of the cholesterol exporter ABCA1 gene. *Cell Rep.* 2, 580–590. doi:10.1016/j.celrep.2012.08.011

Sorrentino, G., Ruggeri, N., Specchia, V., Cordenonsi, M., Mano, M., Dupont, S., et al. (2014). Metabolic control of YAP and TAZ by the mevalonate pathway. *Nat. Cell Biol.* 16, 357–366. doi:10.1038/ncb2936

Staedler, D., Chapuis-Bernasconi, C., Dehmlow, H., Fischer, H., Juillerat-Jeanneret, L., and Aebi, J. D. (2012). Cytotoxic effects of combination of oxidosqualene cyclase inhibitors with atorvastatin in human cancer cells. *J. Med. Chem.* 55, 4990–5002. doi:10.1021/jm300256z

Sun, J., Zheng, Z., Chen, Q., Pan, Y., Quan, M., and Dai, Y. (2019). Fenofibrate potentiates chemosensitivity to human breast cancer cells by modulating apoptosis via AKT/NF-κB pathway. *Onco Targets Ther.* 12, 773–783. doi:10.2147/OTT.S191239

Takai, Y., Sasaki, T., and Matozaki, T. (2001). Small GTP-binding proteins. *Physiol. Rev.* 81, 153–208. doi:10.1152/physrev.2001.81.1.153

Tavazoie, M. F., Pollack, I., Tanqueco, R., Ostendorf, B. N., Reis, B. S., Gonsalves, F. C., et al. (2018). LXR/ApoE activation restricts innate immune suppression in cancer. *Cell* 172.

Terzi, H., Altun, A., and Şencan, M. (2019). *In vitro* comparison of the cytotoxic effects of statins on U266 myeloma cell line. *Indian J. Med. Res.* 150, 630–634. doi:10.4103/ijmr.IJMR_672_18

van Reyk, D. M., Brown, A. J., Hult'en, L. M., Dean, R. T., and Jessup, W. (2006). Oxysterols in biological systems: Sources, metabolism and pathophysiological relevance. *Redox Biol.* 11 (6), 255–262. doi:10.1179/135100006X155003

Villa, G. R., Hulce, J. J., Zanca, C., Bi, J., Ikegami, S., Cahill, G. L., et al. (2016). An LXR-cholesterol Axis creates a metabolic Co-dependency for brain cancers. *Cancer Cell* 30, 683–693. doi:10.1016/j.ccell.2016.09.008

Vinayak, S., Schwartz, E. J., Jensen, K., Lipson, J., Alli, E., McPherson, L., et al. (2013). A clinical trial of lovastatin for modification of biomarkers associated with breast cancer risk. *Breast Cancer Res. Treat.* 142, 389–398. doi:10.1007/s10549-013-2739-z

Wang, Y., You, S., Su, S., Yeon, A., Lo, E. M., Kim, S., et al. (2022). Cholesterol-lowering intervention decreases mTOR complex 2 signaling and enhances antitumor immunity. *Clin. Cancer Res.* 28, 414–424. doi:10.1158/1078-0432.CCR-21-1535

Weinglass, A. B., Kohler, M., Schulte, U., Liu, J., Nketiah, E. O., Thomas, A., et al. (2008). Extracellular loop C of NPC1L1 is important for binding to ezetimibe. *Proc. Natl. Acad. Sci. U. S. A.* 105, 11140–11145. doi:10.1073/pnas.0800936105

Willinger, T. (2019). Oxysterols in intestinal immunity and inflammation. *J. Intern. Med.* 285, 367–380. doi:10.1111/joim.12855

Woo, S. Y., Lee, H., Park, S. M., Choi, H. S., Kim, J., Kwon, M., et al. (2022). Role of reactive oxygen species in regulating 27-hydroxycholesterol-induced apoptosis of hematopoietic progenitor cells and myeloid cell lines. *Cell Death Dis.* 13, 916. doi:10.1038/s41419-022-05360-0

Wu, G., Wang, Q., Xu, Y., Li, J., Zhang, H., Qi, G., et al. (2019). Targeting the transcription factor receptor LXR to treat clear cell renal cell carcinoma: Agonist or inverse agonist? *Cell Death Dis.* 10, 416. doi:10.1038/s41419-019-1654-6

Xu, H., Zhou, S., Tang, Q., Xia, H., and Bi, F. (2020). *Cholesterol metabolism: New functions and therapeutic approaches in cancer, biochimica et biophysica acta*. Reviews On Cancer, 1874, 188394.

Yamanaka, K., Urano, Y., Takabe, W., Saito, Y., and Noguchi, N. (2014). Induction of apoptosis and necroptosis by 24(S)-hydroxycholesterol is dependent on activity of acyl-CoA: cholesterol acyltransferase 1. *Cell Death Dis.* 5 (1), e990. doi:10.1038/cddis.2013.524

Yang, Y. F., Jan, Y. H., Liu, Y. P., Yang, C. J., Su, C. Y., Chang, Y. C., et al. (2014). Squalene synthase induces tumor necrosis factor receptor 1 enrichment in lipid rafts to promote lung cancer metastasis. *Am. J. Respir. Crit. Care Med.* 190, 675–687. doi:10.1164/rccm.201404-0714OC

Yao, L., Chen, S., and Li, W. (2020). Fatostatin inhibits the development of endometrial carcinoma in endometrial carcinoma cells and a xenograft model by targeting lipid metabolism. *Arch. Biochem. Biophys.* 684, 108327. doi:10.1016/j.abb.2020.108327

You, B. J., Hour, M. J., Chen, L. Y., Luo, S. C., Hsu, P. H., and Lee, H. Z. (2019). Fenofibrate induces human hepatoma Hep3B cells apoptosis and necroptosis through inhibition of thioesterase domain of fatty acid synthase. *Sci. Rep.* 9, 3306. doi:10.1038/s41598-019-39778-y

Yue, S., Li, J., Lee, S. Y., Lee, H. J., Shao, T., Song, B., et al. (2014). Cholesteryl ester accumulation induced by PTEN loss and PI3K/AKT activation underlies human prostate cancer aggressiveness. *Cell Metab.* 19, 393–406. doi:10.1016/j.cmet.2014.01.019

Zhong, C., Fan, L., Li, Z., Yao, F., and Zhao, H. (2019). SREBP2 is upregulated in esophageal squamous cell carcinoma and cooperates with cMyc to regulate HMGCR expression. *Mol. Med. Rep.* 20, 3003–3010. doi:10.3892/mmr.2019.10577

Frontiers in Pharmacology

Explores the interactions between chemicals and living beings

The most cited journal in its field, which advances access to pharmacological discoveries to prevent and treat human disease.

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