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Progress in antitumor mechanisms and applications of phenformin (Review)

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Abstract. Phenformin, a biguanide compound, has attracted increased attention due to its prominent antitumor activity. As a multi-target agent, the antitumor effects of phenformin involve a wide range of factors, including inhibition of mitochondrial complex I, activation of AMP-activated protein kinase, impact on the tumor microenvironment, suppression of cancer stem cells and others. In addition, phenformin has been shown to markedly augment the effectiveness of various clinical treatment methods, including radiotherapy, chemotherapy, targeted therapy and immunotherapy. It is noteworthy that breakthrough progress has been made in the treatment of cancer with phenformin with application in clinical trials for the treatment of melanoma. Phenformin not only reduces the lesion area of patients, but also enhances the efficacy of dalafinib/trimetinib. In the present review, the novel breakthroughs in the antitumor effects and mechanisms of phenformin were discussed. In addition, the current review focuses on the clinical development value of phenformin, striving to provide new insights into the future research direction of phenformin in the field of tumor treatment.

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1. Introduction

Metformin, with the title of 'magic drug', plays an important role in treating diseases such as diabetes, cancer, anxiety, obesity, cardiovascular diseases and others (1). However, due to the high concentration required for its antitumor effect, the clinical trial results of metformin in treating cancer are not optimal (2,3). The derivative of metformin, phenformin, has improved antitumor activity compared with metformin and has attracted widespread attention. One of the main reasons for its high antitumor activity is that phenformin has a benzene ring, making it more hydrophobic and easier to penetrate cell membranes than metformin (4). A study on the molecular mechanism of biguanides targeting mammalian respiratory chain complex I suggested that the benzene ring structure enables it to stably bind to Q channel of mitochondrial complex I through van der Waals interactions, resulting in a stronger inhibitory effect on mitochondrial complex I (5). Another important factor is that metformin requires the organic cation transporters (OCT) in order to enter cells, while phenformin does not (4). This expands the application scope of phenformin, making it still effective in some cell types with low expression of OCT, such as in melanoma cell lines (6). Therefore, due to its improved activity and adaptability, phenformin is considered to have more clinical development value than metformin.

It is well known that phenformin is a typical agonist of AMP-activated protein kinase (AMPK). The effect of phenformin on cancer cell proliferation is mainly achieved by inhibiting complex I of the mitochondrial respiratory chain to activate AMPK and block the mammalian target of rapamycin (mTOR) pathway, thereby affecting protein synthesis, tumor angiogenesis, epithelial-mesenchymal transition (EMT), cell cycle arrest and proliferation inhibition (4,7). In addition, another category of molecular mechanisms that is not dependent on AMPK has been reported (8,9) (Fig. 1). For instance, phenformin blocks the mTOR signaling pathway by inhibiting Rag GTPase without the involvement of AMPK (9). Moreover, phenformin exerts antitumor effects by acting on the tumor microenvironment (TME) (10,11) or self-renewal of cancer stem cells (CSCs) (12). The latest research has also revealed a new mechanism through which phenformin induces autophagic cell death in cancer cells by inducing endoplasmic reticulum (ER) stress without relying on AMPK (13). The

complex diversity of antitumor mechanisms of phenformin further highlights its notable clinical application potential.

Resistance and low response rates to antineoplastic drugs are common in clinical therapy (14,15). It has been shown that phenformin enhances the sensitivity or alleviates resistance of drugs, including chemotherapy drugs (16,17), radiotherapy (18), targeted therapeutic drugs (19,20) and immune checkpoint inhibitors (10). More specifically, marked breakthroughs of phenformin combined with dalafinib/trimetinib have been made in clinical trials of melanoma (21). In addition to considering its efficacy, the lactic acid toxicity of phenformin has also been a concern. Although phenformin was withdrawn from the market in the late 1970s due to the risk of lactic acidosis (22), phenformin has relatively low toxicity (64 cases of lactate acidosis per 100,000 patients) compared with other cancer treatments (23). In addition, some achievements have been made in reducing the lactate toxicity of phenformin. For example, the administration of 2-deoxyglucose (24) or oxamate (25) can markedly alleviate the symptoms of lactic acid poisoning caused by phenformin. In several epidemiological studies, a relationship between phenformin and diminished incidence and mortality of cancer in patients with type 2 diabetes has been revealed (26,27). These studies indicated that phenformin is an anticancer agent with great clinical value.

The present review primarily presents novel advancement in the antitumor mechanism of phenformin, summarizes the current treatment status of monotherapy and combination therapy of phenformin in various tumors, and analyzes and discusses the future direction of phenformin in the field of cancer treatment, aiming to provide theoretical foundations and insights for the successful application of phenformin in the clinical treatment of cancers.

2. Antitumor mechanisms of phenformin

The direct target of phenformin remains unclear, and its antitumor effects are principally achieved through disrupting mitochondrial function, influencing TME and CSCs. Next, an in-depth analysis of the antitumor mechanisms of phenformin is presented.

Regulating TME. The TME refers to the living environment around tumors, encompassing adjacent blood vessels, immune cells, fibroblasts, signaling molecules and extracellular matrix components (28). According to their different functions, the immune cells in the TME can be categorized into tumor-promoting and -inhibiting cells, which play distinct roles in various stages of tumor progression (29). The prototypical representatives of tumor-promoting immune cells are myeloid-derived suppressor cells (MDSCs), which possess the ability to inhibit immune cell response. MDSCs not only promote tumor angiogenesis by mediating JAK2/STAT3 signaling to activate VEGFA and MMP9 production (30), but also induce EMT in cancer cells by mediating the TGF-β, EGF and HGF signaling pathways, thereby promoting cancer cell metastasis (31,32).In a murine melanoma model, phenformin selectively decreased the accumulation of G-MDSCs in the spleen and tumor and downregulated the expression levels of Arg-1 and S100A8/9 in MDSC. The combination therapy of phenformin and anti-programmed death-1 (PD-1) antibody exhibits a synergistic effect by inducing $CD8^+$T cell infiltration (10). This work has received the attention of researchers and has been reviewed (33). Due to the prominent performance of phenformin in inhibiting melanoma cells in preclinical studies, researchers conducted a phase I clinical trial of phenformin combined with other drugs to treat patients with melanoma. In line with preclinical investigations, a reduction in tumor-infiltrating MDSCs was also observed in patients treated with phenformin, indicating that phenformin may enhance the immune recognition of melanoma cells (21). Phenformin plays a particularly vital role in the tumor immune microenvironment (TIME) based on preclinical studies and clinical trials.

Tumor cell-derived exosomal microRNAs (miRNAs/miR), a marked substance in TME, serve as messengers to transmit signals between cells (34). Exosomal miRNAs are important in regulating tumor growth, invasion, metastasis and angiogenesis (35). Therefore, tumor cell-derived exosomal miRNAs are currently popular antitumor targets. Targeting exosomal miRNAs is also deemed an effective avenue for tumor therapy. Phenformin plays a crucial role in regulating exosomal miRNA. For instance, Zhuang *et al* (11) discovered that phenformin markedly upregulated oral squamous cell carcinoma cells (OSCC)-derived exosomal miR-1246 and miR-205, subsequently mediating the ACE signaling pathway and downregulating VEGFA expression, thereby inhibiting angiogenesis in vascular endothelial cells. These studies indicated that phenformin is an effective anticancer strategy by inhibiting tumor angiogenesis affecting TME.

In conclusion, the aforementioned studies have indicated that the target of action of phenformin is not only focused on cancer cells, but also extends to the extracellular environment, including the TIME and intercellular communication (Fig. 2). Both preclinical and clinical trials demonstrated that phenformin effectively reduces tumor-promoting immune cells known as MDSCs, highlighting the marked regulatory mechanism of phenformin in the TIME. However, the TME is intricate, and it remains unclear whether phenformin exerts potential effects on other constituents, such as macrophages. In addition, exosomal miRNAs derived from tumor cells are considered promising targets for disease diagnosis and treatment as they can accurately reflect crucial information originating from the tumor cells. However, the limited abundance and diversity of exosomal miRNAs is challenging for precise quantification in tumor diagnosis. If they are implemented as diagnostic and therapeutic targets in clinical practice, numerous issues remain to be addressed such as the urgent need for diagnostic methods with heightened sensitivity and fidelity and appropriate drug delivery systems to introduce drugs into extracellular vesicles.

Inhibiting the self-renewal of CSCs. CSCs are a subgroup of cancer cells. Despite limited quantity, they possess the ability to regenerate, multiply infinitely and differentiate in multiple directions. There is a correlation between CSCs and tumor invasion, metastasis, drug resistance, as well as relapse post-treatment (36). Therefore, inhibiting self-renewal of CSCs is considered a potential treatment for tumors. Phenformin has been shown to impede the self-renewal of CSCs effectively, and its mechanism of action primarily involves non-coding

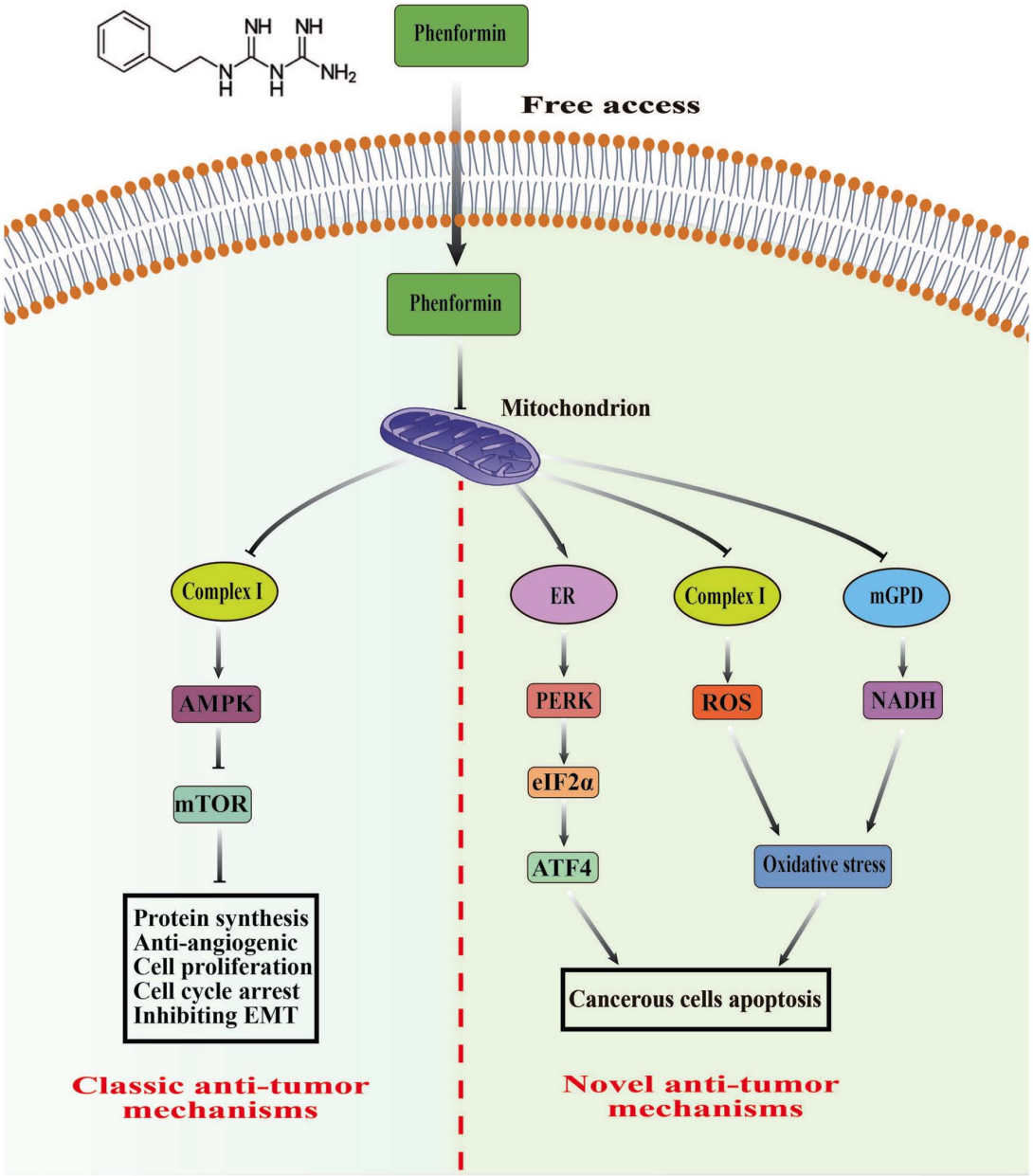

Figure 1. Main antitumor mechanisms of phenformin. The classic antitumor mechanism of phenformin is to activate AMPK and block the mTOR pathway by inhibiting mitochondrial respiratory chain complex I, thereby affecting protein synthesis, tumor angiogenesis, EMT, cell cycle arrest and inhibition of proliferation. In addition, phenformin can promote cancer cell apoptosis by inducing ER stress or oxidative stress. AMPK, AMP-activated protein kinase; mTOR, mammalian target of rapamycin; EMT, epithelial mesenchymal transition; ER, endoplasmic reticulum; mGPD, mitochondrial glycerol-3-phosphate dehydrogenase; ROS, reactive oxygen species; NADH, nicotinamide adenine dinucleotide.

RNA and stem cell markers (12,37). According to a study by Jiang *et al* (12), phenformin effectively restrained the self-renewal of glioma stem cells by directly upregulating the miR-124 pathway or activating let-7, a tumor suppressor miRNA, to inhibit the HMGA2 pathway. SOX2, a stem cell marker, was downregulated by phenformin, thereby inhibiting melanoma stem cell properties (37). In addition, researchers have also shown that high expression of SOX2 was associated with aldehyde dehydrogenase (ALDH) overexpression in melanoma cells, and downregulating ALDH by phenformin notably inhibited the self-renewal of CSCs (37,38). This indicates that phenformin is an effective tumor metabolism regulator, and targeting cancer cell metabolism is another strategy to inhibit self-renewal of CSCs.

Taken together, phenformin inhibits the self-renewal of CSCs mainly by affecting non-coding RNA and stem cell markers (Fig. 3). Additionally, cancer cells often activate alternative signaling pathways to sustain their survival during targeted drug treatment. For instance, in melanoma cells treated with BRAF or MEK inhibitors, it was shown that cancer cells were resistant to drugs by producing higher levels of ALDH (39). As it was aforementioned, phenformin downregulates ALDH and inhibits the self-renewal of CSCs. This indicates that combining phenformin with BRAF or

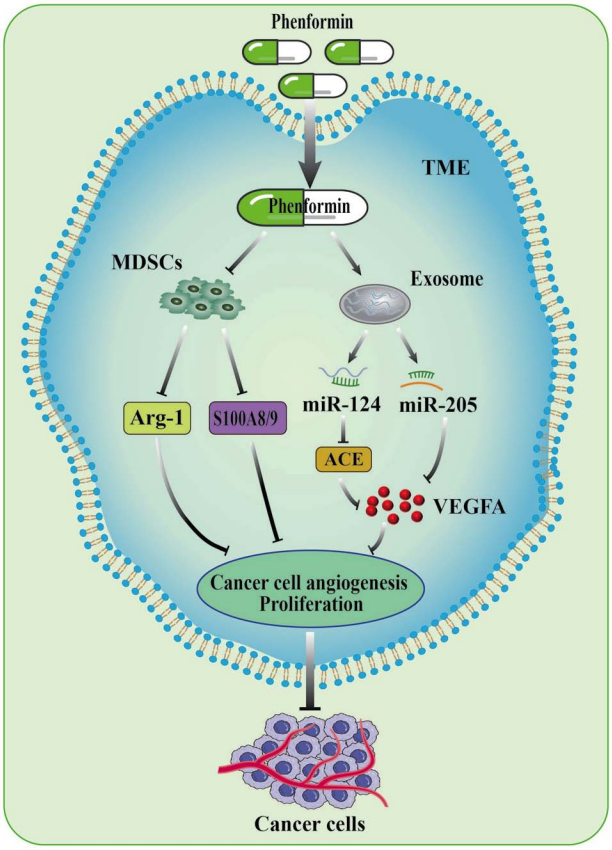

Figure 2. Phenformin regulates the TME. Phenformin affects the molecular mechanisms by which TME prevents tumor development. TME, tumor microenvironment; MDSCs, myeloid-derived suppressor cells; ACE, angiotensin converting enzyme; VEGFA, vascular endothelial growth factor A.

MEK inhibitors may be a promising strategy for overcoming resistance to BRAF or MEK inhibition in melanoma cells.

Inducing cellular stress response. During the malignant growth process of tumor cells, environmental factors continuously trigger cellular stress responses, thereby affecting their growth status (40). Research has found that phenformin can induce stress responses in cancer cells, such as oxidative stress and ER stress, thereby inhibiting the growth and proliferation of tumor cells. Nicotinamide adenine dinucleotide (NADH), a reduced form of nicotinamide adenine dinucleotide, is closely associated with maintaining cell proliferation, differentiation, energy metabolism and cell protection (41). Studies have shown that phenformin regulates NADH production by acting on mitochondria, inducing cell apoptosis (42,43). According to Kim *et al* (43), phenformin exerted antiproliferative effects in cancer cells by inhibiting mitochondrial complex I and subsequently downregulating the NAD/NADH ratio, thereby disrupting redox homeostasis and reducing intracellular aspartic acid levels. In addition, phenformin inhibits mitochondrial mGPD, a component of glycero-phosphate shuttling, prompting the cell to synthesize NADH, which induces the formation of the Gli1/CtBP2 complex, further inhibiting transcription and translation of Hedgehog and ultimately suppressing cancer cell proliferation (8). These studies indicated that the antitumor mechanism of phenformin is closely related to the disruption of cellular redox status.

The formation of reactive oxygen species (ROS), unstable molecules containing oxygen, occurs as a byproduct of oxygen consumption and cellular metabolism (44,45). The overabundance of ROS in tumor cells results in oxidative stress, thereby causing cytotoxic consequences (45). Phenformin inhibits mitochondrial complex I and induces excessive production of ROS, resulting in oxidative stress and DNA damage, ultimately triggering cell apoptosis (46,47). In glioma cells, Wang *et al* (48) also found that phenformin could induce the production of ROS, resulting in ROS imbalance. By contrast, the ROS inhibitor NAC attenuates the induced apoptosis ability of phenformin. These findings suggested a close association between the anticancer effects of phenformin and the generation of ROS. Hence, using phenformin to enhance ROS production and induce oxidative stress holds promise as a potential approach for cancer treatment.

Phenformin not only induces oxidative stress, but also inhibits tumor growth by inducing ER stress. The ER stress is involved in various important biochemical processes of tumor cells, such as cell cycle, DNA damage and repair, cell apoptosis and autophagy, through pathways such as IRE1, PERK and ATF6 (49,50). The latest research shows that phenformin promotes OSCC autophagy by inducing ER response. From a mechanistic perspective, phenformin induces ER stress activation of the PERK/eIF2 α/ATF4 axis pathway to enhance DDIT4 and NIBAN1 expression without relying on AMPK, leading to mTOR inhibition to promote autophagy and inhibit OSCC cell proliferation (13). This indicates that targeting the ER stress pathway to induce cancer cell apoptosis is another novel antitumor mechanism of phenformin, providing new ideas for innovative tumor treatment.

Affecting cancer cell metabolism. Cancer cells often maintain high value-added rates by altering their metabolism, such as increasing macromolecular biosynthesis, accelerating ATP production (51). Phenformin affects cancer cell metabolism by acting on different signaling pathways (52). As a central regulatory factor of metabolism, the mTOR signaling pathway is overactivated in most cancers (53). The activation of mTOR contributes to sustaining cellular survival by promoting anabolic metabolism or suppressing catabolic processes (54). Phenformin plays an essential role in modulating anabolic and catabolic processes by phosphorylating downstream proteins of AMPK or regulating gene expression (55). For instance, phenformin activates AMPK and reduces S6 protein phosphorylation to block the mTOR signaling pathway, suppressing protein synthesis and inducing autophagy, further leading to cell cycle arrest and apoptosis (56). Beyond that, previous research has demonstrated that metformin augments lipolysis by activating AMPK, thereby impeding the proliferation of cancer cells (57). However, whether phenformin can mediate AMPK activation and affect the lipid metabolism of cancer cells needs to be further verified. At present, it is unclear whether phenformin exerts antitumor effects by affecting glucose metabolism. It is noteworthy that respiratory stress induced by phenformin promotes aerobic glycolysis and glucose-dependent states, enabling cells to resist mitochondrial dysfunction (58). In this case, phenformin mediates an increase in glycolytic flux, thereby reducing the sensitivity of cancer cells to phenformin. Therefore, the combination of phenformin

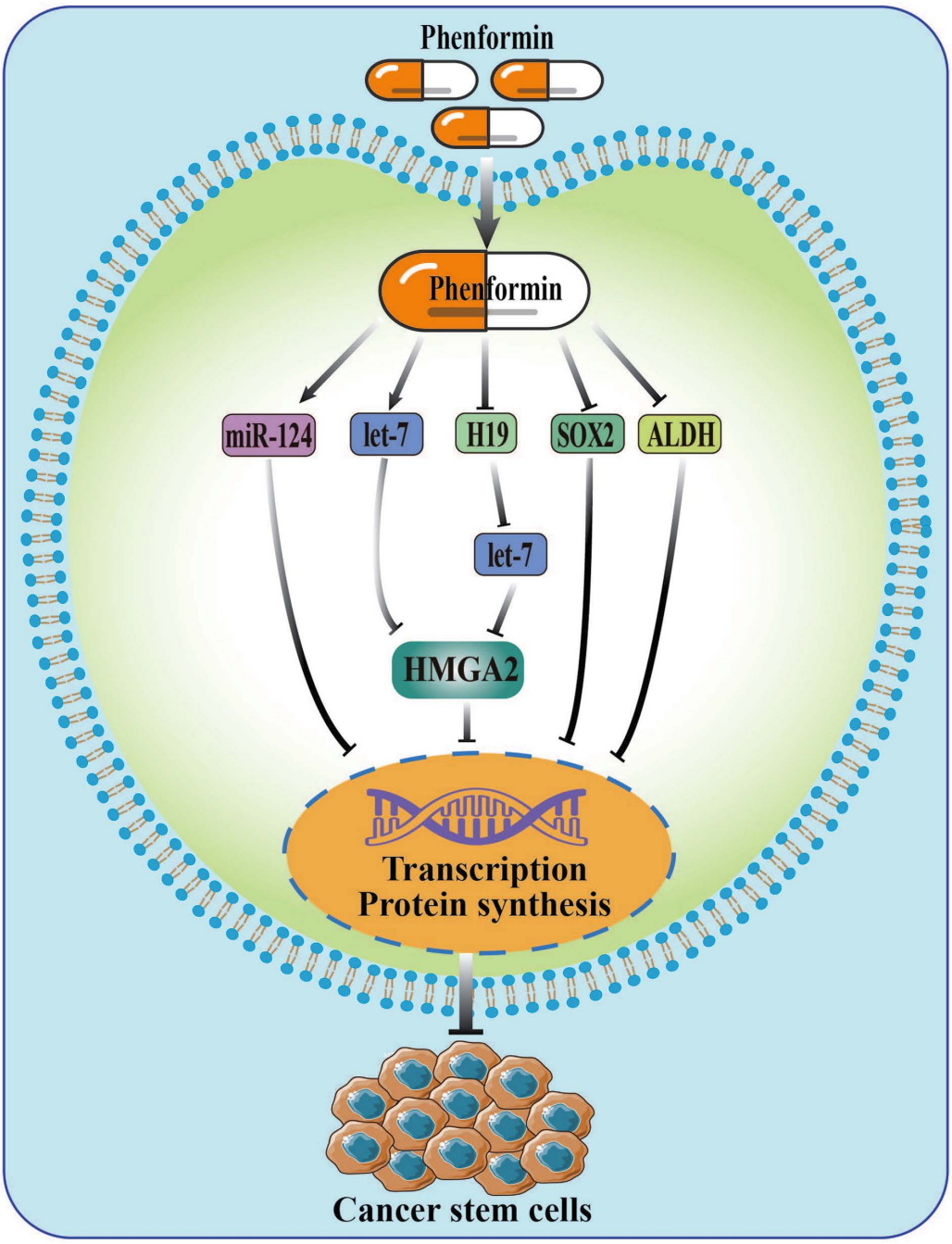

Figure 3. Phenformin inhibits the self-renewal of CSCs. Phenformin exerts its inhibitory effects on CSCs by modulating various miRNAs, transcription factors such as SOX2 and ALDH. ALDH, aldehyde dehydrogenase, CSCs, cancer stem cells; miRNA, microRNA.

and glycolytic flux inhibitors such as dichloroacetate (12) or oxamate (46) is a promising antitumor strategy.

Except for material metabolism, phenformin also affects the energy metabolism of cancer cells. The inhibition of mitochondrial complex I by phenformin reduces ATP production, consequently inducing the indirect activation of AMPK to regulate energy metabolism in tumor cells (59,60). In certain tumors where the specific tumor suppressor LKB1 is mutated or inactivated, such as non-small cell lung cancer (NSCLC) (61,62) and lymphoma (63), obstruction of AMPK activation by phenformin induces energy metabolic stress, giving rise to apoptosis through ATP depletion in tumor cells. Therefore, phenformin can be used as an ATP-depleting cytotoxic agent to eradicate LKB1-deficient tumors efficiently. This cytotoxicity exclusively targets tumors with LKB1 mutations while it does not affect healthy tissue, which contributes to enhancing the therapeutic index and mitigating toxic side effects (64). These studies suggested that the induction of energy metabolic stress in cancer cells by phenformin may represent a feasible cancer treatment option.

In summary, phenformin exerts a distinctive role in the metabolic processes of tumor cells. However, tumor metabolism constitutes an intricate network system. When a metabolic pathway of tumor cells is inhibited, it can maintain

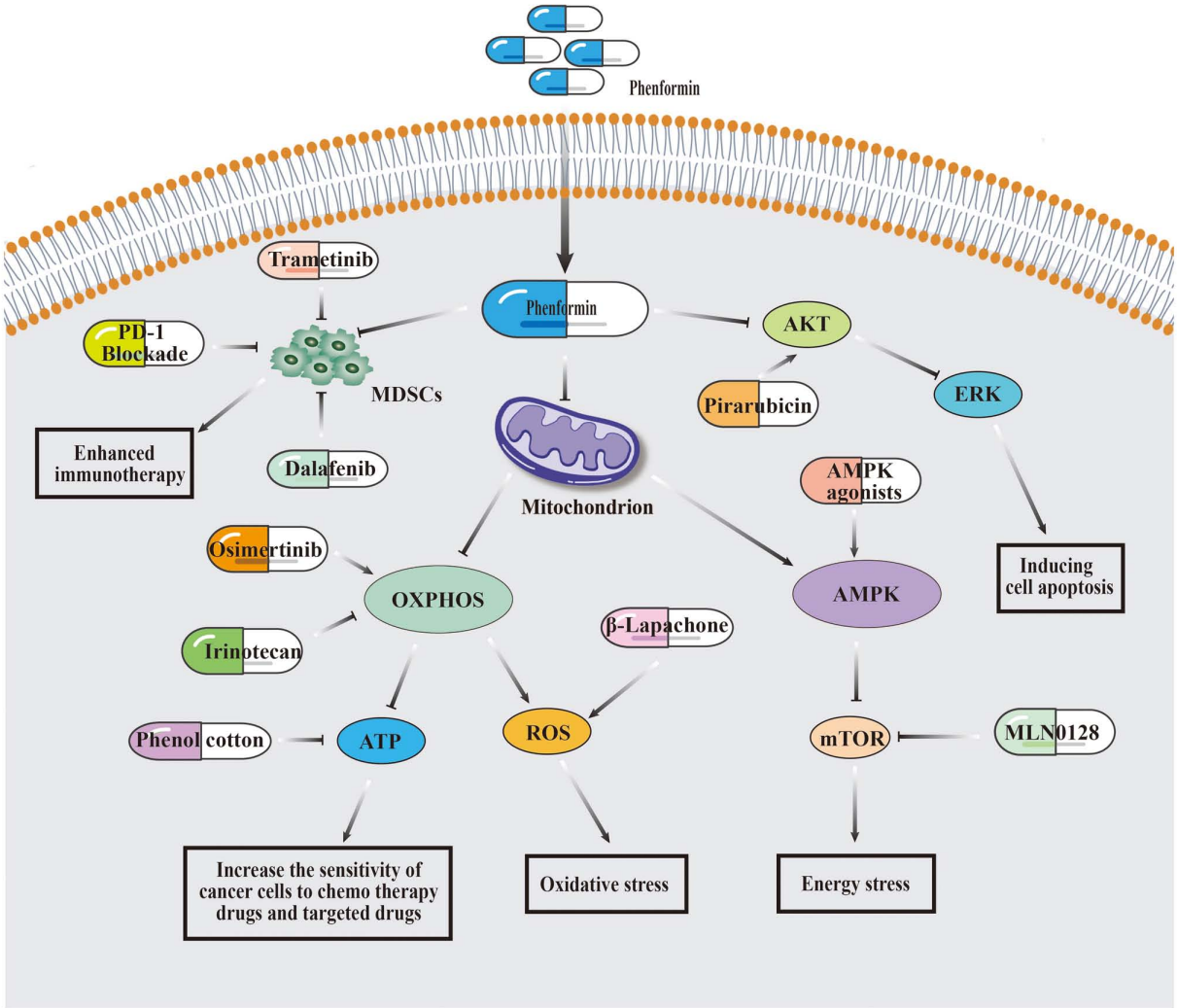

Figure 4. Strategies and mechanisms of combination therapy with phenformin. By inhibiting OXPHOS or activating AMPK, phenformin induces energy stress or oxidative stress in cancer cells, thereby enhancing the sensitivity of cancer cells to chemotherapy drugs, targeted drugs, or other clinical agents. Additionally, phenformin suppresses MDSCs in immune cells to augment the antitumor efficacy of immunotherapy. Moreover, phenformin exhibits the potential to overcome cancer cell resistance to chemotherapy agents through its inhibitory effects on the AKT/ERK pathway. MDSCs, myeloid-derived suppressor cells; ERK, extracellular signal-regulated kinase; AMPK, AMP-activated protein kinase; mTOR, mammalian target of rapamycin; OXPHOS, oxidative phosphorylation.

growth by activating other compensatory metabolic pathways. The application of phenformin in metabolic combination therapy leads to complementary inhibition of tumor metabolism processes, such as phenformin combined with MCT1 inhibitor AZD3965 (65) or glycolytic inhibitor Gnetin H (66). This is a potential strategy for cancer treatment, which can achieve multi-channel blockade of tumor substances and energy metabolism pathways. Furthermore, it is imperative to devise strategies that avoid the potential harm inflicted on healthy cells. The integration of rapidly advancing technologies, such as metabolomics, spatial metabolomics, lipidomics, proteomics and others are crucial for elucidating the mechanisms underlying aberrant tumor metabolism and identifying specific molecular targets.

Blocking the cancer cell cycle. An eminent characteristic of cancer development is the aberrant activation of cyclins that instigate the unbridled proliferation of cancer cells. Hence, cell cycle regulators have become attractive targets for anticancer therapy (67). It is noteworthy that phenformin exhibits the ability to inhibit cyclins or cyclin kinases, thereby impeding the progression of the tumor cell cycle. For instance, phenformin induces the upregulation of P21, a cyclin-dependent kinase suppressor protein, leading to cell cycle arrest in glioblastoma (GBM) cells (68). In addition, phenformin downregulates cyclin D1 by inhibiting the MAPK/ERK signaling pathway, leading to the increase of breast cancer cells in the G1 phase (69). Jackson *et al* (56) also observed a notable downregulation of cyclin D1 and CDK4, and an upregulation of P21 in ovarian cancer cells upon treatment with phenformin, giving rise to alterations in the cell cycle of ovarian cancer. These studies suggested that phenformin exhibits potential as an antitumor agent by regulating cell cycle factor-induced cell cycle arrest.

Inhibiting tumor angiogenesis. Tumor blood vessels, as conduits for the transportation of tumor nutrients, facilitate the escape of tumor cells and make tumor angiogenesis a pivotal indicator

of solid tumor growth, invasion and metastasis (70). Hypoxia and VEGF are essential factors affecting angiogenesis. Solid tumors frequently exhibit elevated proangiogenic factor VEGF expression levels (71). In addition, mTORC1 facilitates tumor angiogenesis by promoting HIF-1α synthesis through signal transduction pathways involving S6K1, 4E-BP1 and STAT3. AMPK activator effectively suppresses mTOR signaling and inhibits tumor angiogenesis (72). Jaidee *et al* (73) demonstrated that phenformin effectively restrained mTOR activity through the activation of AMPK, consequently impeding the HIF-1α signal pathway and reducing VEGF expression; this ultimately inhibited angiogenesis in bile duct cancer, but did not affect normal angiogenesis. Beyond that, phenformin has shown the ability to impede the expression of proangiogenic factors and suppress angiogenesis in both *in vitro* and *in vivo* models of KRAS-mutated NSCLC by effectively suppressing the ERK pathway (74). These positive results indicated that inhibition of tumor angiogenesis by phenformin offers remarkable therapeutic advantages in the treatment of tumors.

Suppressing EMT. The process by which epithelial tumor cells lose their adhesion ability, then gain the migration ability of mesenchymal cells and promote metastasis and drug resistance is called EMT, which is closely associated with the initiation, development and metastasis of tumor cells (75). The TGF-β/Smad and insulin-like growth factor (IGF) signaling pathway play a pivotal role in inducing EMT (76,77). TGF-β receptor 2 signaling is widely expressed in numerous types of cancer and prominently influences the EMT process in cancer cells. Lin *et al* (78) found that phenformin effectively suppressed TGF-β-induced EMT by activating AMPK. Furthermore, Park *et al* (79) confirmed that phenformin decreases the expression of intermediate mesenchymal markers such as N-cadherin and vimentin, and EMT regulators including Snail, Twist, Slug and Zeb1 in colorectal cancer cells. The observed downregulation of these markers and regulators further impedes TGF-β-induced EMT. In addition, it has been reported that IGF facilitated EMT by mediating the PI3K and MAPK pathways, enhancing the invasive ability of breast cancer cells (77). In ErbB2-overexpressing breast cancer cells, phenformin can block the IGF1 receptor signaling pathway by activating AMPK, thus impeding the EMT process (76). These studies suggested that phenformin can inhibit the EMT process by activating AMPK to regulate TGF-β and IGF signal transduction, which is a new strategy to treat tumors.

3. Applications of phenformin in various cancers

The antitumor effects of phenformin have been extensively validated through *in vitro* and *in vivo* studies across versatile cancer types, including, lung cancer (74,80-82), skin cancer (83), hepatoma (84), breast cancer (26), pancreatic cancer (85), ovarian cancer (86), prostate cancer (87), colon adenocarcinoma (88) and others. Next, a comprehensive review of the recent research progress of phenformin in various cancer types is presented, and the therapeutic effects and underlying mechanisms of phenformin in combination with clinical drugs in the treatment of tumors are systematically summarized (Fig. 4).

Lung cancer. Lung cancer is one of the most common malignancies, and has relatively high morbidity and mortality rates. The treatment efficacy for lung cancer remains unsatisfactory owing to drug resistance and specific gene mutations in the treatment process (89,90). Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are a primary class of targeted therapeutics for the management of lung cancer. However, prolonged usage can lead to the emergence of drug resistance (91). It has previously been established that in NSCLCs, which have acquired resistance to EGFR-TKIs, there is a metabolic shift from glycolysis to oxidative phosphorylation (OXPHOS) (19,43). Notably, phenformin-induced inhibition of OXPHOS resulted in redox imbalance, suppressing tumor metabolic abnormalities, and ultimately triggering the death of acquired EGFR-TKI-resistant NSCLC cells (43). Furthermore, Martin *et al* (19) discovered that combining osimertinib with phenformin delayed the resistance of tumor cells to osimertinib. In addition to mitigating drug resistance, phenformin enhances the sensitivity of chemotherapy drugs. For instance, Lee *et al* (16) identified that the co-administration of phenol cotton and phenformin synergistically inhibited OXPHOS and reduced intracellular ATP levels in irinotecan-resistant NSCLC cells, thereby inducing cell death and enhancing the sensitivity of cells to irinotecan. Additionally, a small number of preclinical studies have certified that phenformin exhibited the potential to augment the sensitivity of NSCLC towards radiation therapy (18), as well as reinforce the sensitivity of Ym155, a mitochondrial inhibitor (92). These studies indicated that phenformin can reverse drug resistance in NSCLC and enhance efficacy by inhibiting OXPHOS, providing new ideas for addressing issues such as drug resistance and low efficacy in clinical cancer treatment.

KRAS represents the most frequently mutated gene in lung adenocarcinoma, but no effective KRAS-targeted therapy has been developed (93). As it was aforementioned, it has been discovered that phenformin displayed inhibitory effects on the proliferation of lung cancer cells harboring KRAS mutations or LKB1 deficiency (62,94). Momcilovic *et al* (62) revealed that the combination of phenformin and mTOR kinase inhibitor MLN0128 hindered further the survival of KRAS/LKB1 co-mutated NSCLC cells. Knockout of the ALDH1L1 gene enhances the inhibitory effect of phenformin on KRAS mutation-driven lung cancer cell proliferation (94). In addition, LKB1 deficiency attenuates the sensitivity of cancer cells to the MEK inhibitor selumetinib in KRAS-mutated NSCLC. The antitumor effect is markedly enhanced when phenformin is combined with selumetinib (95). These preclinical studies suggested that whether administered alone or in combination, phenformin may be a promising drug for the treatment of specific gene-mutated lung cancer.

In conclusion, phenformin primarily enhances the susceptibility of lung cancer cells to chemoradiation, targeted therapies and other clinical drugs by impeding mitochondrial OXPHOS. This indicates that the dual treatment of targeting tumorigenic growth signals and cancer metabolism stands for a novel and efficacious strategy. Although phenformin is emerging in lung cancer treatment, current studies have primarily focused on lung cancer cells and small animal models. Further investigations involving large model animals and human subjects are imperative to address drug resistance mechanisms and

Table I. The combination strategies of phenformin for tumor treatment.

First author, year	Combined drugs	Cancer type	Antitumor mechanism	References
Martin *et al*, 2016	Osimertinib	NSCLC	Inhibit OXPHOS, induce REDOX imbalance, and accelerate cell death in osimertinib-resistant NSCLC cells.	(19)
Lee *et al*, 2018	Phenol cotton + Irinotecan	NSCLC	The synergistic inhibition of OXPHOS gives rise to a reduction in ATP production and triggers cellular apoptosis.	(16)
Wang *et al*, 2015	Radiotherapy	NSCLC	Activation of AMPK or enhancement of endoplasmic reticulum stress enhances NSCLC response to ionizing radiation treatment.	(18)
Mondal *et al*, 2022	BMP inhibitor + Ym155	NSCLC	Synergistically induce AIF caspase-independent cell death in lung cancer cells by activating AMPK.	(92)
Zhang *et al*, 2017	Selumetinib	NSCLC	Synergistically inhibit phosphorylated ERK and S6 levels, leading to apoptosis induction.	(95)
Momcilovic *et al*, 2015	Kinase inhibitor MLN0128	NSCLC	The induction of energy stress promotes the acceleration of cellular apoptosis.	(62)
Yuan *et al*, 2013	BRAF inhibitor PLX4720	Melanoma	The activation of AMPK suppresses mTOR signaling and triggers apoptosis in BRAF mutant melanoma cells.	(6)
Trousil *et al*, 2017	ERK inhibitor SCH772984	Melanoma	The activation of AMPK suppresses mTOR signaling, thereby impeding proliferation and promoting apoptosis in NF1 mutant melanoma cells.	(99)
Chapman *et al*, 2023	Dabrafenib + Trametinib	Melanoma	Activation of AMPK tempts ROS, selectively inhibiting MDSCs in mice and enhancing the sensitivity of Dabrafenib/Trametinib in patients with BRAF V600 mutant melanoma.	(21)
Li and Xiang, 2022	Anti-PD-1 antibody	Melanoma	Reduce the population of G-MDSCs and potentiate the antitumor efficacy of anti-PD-1 antibodies in melanoma cells.	(33)
Huang *et al*, 2021	Sorafenib	HCC	Simultaneous targeting of the CRAF/ERKand PI3K/AKT/mTOR pathways exhibits a more pronounced inhibitory effect on the proliferation of HCC cells.	(20)
Veiga *et al*, 2018	mTOR dual inhibitor	HCC	Induce mitochondrial dysfunction, trigger compensatory glycolytic transformation, and markedly augment the susceptibility of HCC cells to dual mTOR inhibitors.	(102)
Totten *et al*, 2021	NQO1 inhibitor β-Lapachone	Breast cancer	Increase the level of ROS, thereby exerting further inhibitory effects on the proliferation of breast cancer cells.	(47)
Peng *et al*, 2019	Pirarubicin	BC	Reversal of pirarubicin-induced AKT and ERK phosphorylation, circumvention of pirarubicin resistance in BC cells, and facilitation of cellular apoptosis.	(17)
Kong *et al*, 2020	MitoTEMPO	Leukemia	Induce comprehensive stress response and decrease proliferation of cell proliferation.	(106)
Rosilio *et al*, 2013	Metformin + AICAR	Leukemia	The activation of AMPK suppresses mTOR signaling and impedes the proliferation of PTEN-deficient T-cell lymphoma in mice and human T-ALL/T-LL cancer cells.	(107)
Masoud *et al*, 2020	Gefitinib	PDAC	Inhibition of mitochondrial complex I results in a transition of cells to a low OXPHOS state, thereby augmenting the antitumor efficacy of gemcitabine against high OXPHOS tumors.	(59)
Lee *et al*, 2021 Park *et al*, 2018	Phenol cotton	PDAC GMB	Reduce ATP and accelerate cell apoptosis.	(109,110)

NCSLC, non-small cell lung cancer; HCC, hepatocellular carcinoma; BC, breast cancer; OXPHOS, oxidative phosphorylation; BMP, bone morphogenetic protein; AIF, apoptosis-inducing factor; PDAC, pancreatic ductal adenocarcinoma; T-ALL, T acute lymphoblastic leukemia; T-LL, T lymphoblastic leukemia; PTEN, phosphatase and tensin homolog deleted on chromosome ten.

enhance the sensitization effects of phenformin. Regardless, phenformin manifests promising potential for the treatment of advanced lung cancer.

Skin cancer. Melanoma is an infrequent and perilous form of skin cancer (96). Some mutated genes in melanoma, such as BRAF, NRAS and NF1, activate the RAS/RAF/MEK/ERK

signaling pathway, resulting in uncontrolled tumor growth and proliferation (97). However, prolonged use of inhibitors targeting these signaling pathways increases the chances of developing drug resistance. It has been reported that phenformin can suppress RAF/MEK/ERK signaling by activating AMPK, thereby impeding the proliferation of melanoma cells (6,98). The combination of phenformin and the BRAF inhibitor PLX4720 displays a synergistic effect in suppressing the viability of BRAF mutant melanoma cells (6). In addition, phenformin and ERK inhibitor SCH772984 also synergistically inhibit the proliferation of NF1 mutant melanoma cells and accelerate apoptosis of cells (99). These inhibitors, along with phenformin, work together to inhibit the cell growth and proliferation signaling pathway mTOR, thereby synergistically exerting antitumor effects. Additionally, melanoma cells exposed to BRAF and ERK inhibitors chronically exhibit heightened sensitivity towards OXPHOS (100). Consequently, when used with these inhibitors, phenformin demonstrates superior inhibitory efficacy compared with its standalone usage.

Recent preclinical studies and clinical trials have shown that the combination of phenformin and immune checkpoint inhibitors is a favorable strategy for treating melanoma. Phenformin selectively reduces the accumulation of G-MDSCs in the spleen and tumors of melanoma mouse models, thereby enhancing the anti PD-1 antibody combination therapy effect (33). The aforementioned study has shed light on the potential impact of phenformin on the TME. Building upon the efficacy of phenformin in inhibiting melanoma cells, a phase I b clinical trial was conducted to assess the safety and effectiveness of combining phenformin with dalafenib/trametinib (BRAF inhibitor/MEK inhibitor) in patients diagnosed with BRAFV600E/K mutant melanoma (NCT03026517). In this study, shrinkage of tumor lesions was found in 56% of patients. In addition, phenformin was also observed to reduce tumor-promoting immune cells MDSCs and enhance immune recognition of melanoma cells, confirming the results of preclinical studies (21). The occurrence of lactate toxicity, accompanied by symptoms of vomiting/nausea, was observed in 2 out of the 18 treated patients, and it disappeared upon discontinuation of medication. Consequently, considering the potential toxicity of phenformin, further investigation is warranted to determine the optimal dosage.

The aforementioned studies reveal the considerable role of phenformin in the treatment of skin cancer, particularly melanoma. The clinical trial provides recommendations for the dosage of phenformin in subsequent phase II clinical trials. It suggests that the combination of phenformin and immune checkpoint inhibitors is a potential treatment for melanoma. This will advance the progress of phenformin towards clinical application.

Hepatoma. The high heterogeneity exhibited by hepatocellular carcinoma (HCC) brings a substantial challenge to its treatment (101). A low response rate is a prevalent phenomenon observed in current therapeutic approaches. Notably, the efficacy of phenformin in enhancing the sensitivity of clinical drugs and mitigating drug resistance in HCC has been observed. Huang *et al* (20) demonstrated that sorafenib and phenformin suppressed HCC cell proliferation by concurrently targeting the CRAF/ERK and PI3K/AKT/mTOR signaling pathways. More importantly, the combination of the two did not result in weight loss or liver and kidney toxicity in mice, suggesting that the combination of sorafenib and phenformin is a safe and effective strategy for the treatment of HCC. Furthermore, Veiga *et al* (102) also provided evidence supporting the role of phenformin in inducing mitochondrial dysfunction, enhancing the sensitivity of HCC cells to mTOR inhibitors, and effectively controlling tumor burden in mouse models. These studies indicated that phenformin primarily increases its inhibitory effect on liver cancer cells by inhibiting the mTOR signaling pathway and inducing mitochondrial damage. The liver is a crucial metabolic organ, and phenformin has a potential role in regulating tumor metabolism. Thus, the combination of phenformin with inhibitors targeting critical enzymes involved in metabolic pathways like PKM2 and ALDH may potentially enhance the therapeutic efficacy against HCC.

Breast cancer. The incidence rate of breast cancer ranks highest among female malignancies; the mortality rate is second, while the treatment options for breast cancer remain limited (103,104). Previous studies have confirmed that phenformin showed inhibitory effects on the development and progression of breast tumors by modulating angiogenesis, cell proliferation, apoptosis and EMT in breast cancer cells and animal models (69,105). The antitumor safety of phenformin was verified in xenotransplantation of breast cancer mice (26). Furthermore, phenformin is also used in conjunction with other pharmaceutical agents for the management of breast cancer. For instance, Totten *et al* (47) demonstrated that inflammatory mediators in cancer induced the activation of STAT1 signaling, thereby upregulating the expression of reactive oxygen scavenger NQO1 and attenuating the inhibitory effect of phenformin on breast cancer cells. The combination of the NQO1 inhibitor β-lapadone and phenformin evoked an elevation in oxidative stress, rendering the breast cancer xenograft tumor model susceptible to phenformin. The findings suggested that phenformin manifests antitumor efficacy in both cellular and murine models, offering valuable insights for the development of breast cancer treatment.

Other types of cancers. In recent years, phenformin has been found to have potential inhibitory effects on various types of cancer, including bladder cancer (17), leukemia (106,107), brain tumor (25,108), pancreatic cancer (59,85,109,110) and others. The chemotherapeutic agent pirarubicin stimulated Akt and ERK phosphorylation, giving rise to resistance in bladder cancer. Peng *et al* (17) demonstrated that phenformin effectively sensitized bladder cancer cells to pirarubicin by inhibiting AKT and ERK signaling pathways. In a mouse model of T cell acute lymphoblastic leukemia, the combination of mitochondrial antioxidants and phenformin efficiently reduced the leukemia burden (106). Furthermore, in mouse models of PTEN-deficient T-cell lymphoma and human T-all/T-LL cancer cells, the combined administration of phenformin, metformin and the AMPK agonist AICAR synergistically activated AMPK, while inhibiting the mTOR signaling pathway, thereby exhibiting potent anti-leukemic properties (107). In pancreatic ductal adenocarcinoma with high OXPHOS, phenformin induces a transition to a low OXPHOS state by inhibiting mitochondria, and acts as a sensitization agent for the chemotherapy drug gemcitabine,

further enhancing the antitumor effect of gemcitabine (59). In addition, the combination of phenformin and phenol cotton displays a double inhibitory effect in GBM, pancreatic cancer and other malignancies (118-110). In particular, Park *et al* (108) also discovered that the combination of phenformin, phenol cotton and the chemotherapy drug temozolomide in the treatment of GBM resulted in intracellular energy deficiency and induced apoptosis, offering a novel strategy for addressing resistance and recurrence issues in GBM. The aforementioned studies indicate that phenformin mainly reverses tumor resistance or synergizes efficacy by inhibiting OXPHOS or mTOR signaling pathways in various cancers.

In summary, based on preclinical investigations of multiple types and levels of tumor, phenformin exhibits robust antitumor activity. In combination therapy specifically, phenformin demonstrates unique advantages in mitigating drug resistance and enhancing drug sensitivity. The latest strategies for the combination therapy of phenformin are summarized in Table I.

4. Discussion

In recent years, phenformin has gradually become a 'star' antitumor drug, which has marked clinical significance. Phenformin has been used to treat type 2 diabetes, and it has the basis of pharmacokinetics and relatively complete human data. In addition, phenformin showed notable tumor inhibition effect in a variety of tumors, and its lactic acid toxicity was controllable, which met the requirements of clinical drugs with high efficiency and low toxicity. From the analysis of the results of preclinical studies and clinical trials, compared with a single drug regimen, the combined application of phenformin as an adjunct drug with clinical treatment regimens, such as chemotherapy, radiotherapy, targeted therapy and immunotherapy, ensures safety and efficacy by decreasing the dosage of phenformin, which further enriched and optimized clinical cancer treatment strategies and provided more selectivity for cancer treatment.

Despite the positive outcomes of phenformin as an anticancer agent in numerous preclinical trials, there is still a long way to go to promote the successful use of phenformin in clinical oncology treatment. Therefore, the future can be optimized from the following two aspects discussed below. From a pharmaceutical perspective, by optimizing how phenformin enters cells, its antitumor activity can also be considerably enhanced. For instance, the use of CD147 nanoparticles loaded with phenformin (81) or graphene drug carriers for targeted delivery of phenformin (111) can effectively and expeditiously transport phenformin to the site of lesion, thereby facilitating enhancement in the antitumor efficacy of phenformin. Furthermore, to enhance the antitumor efficacy of phenformin, it is vital to consider structural modifications of phenformin from a pharmacochemical perspective. Over the past few years, researchers have focused on synthesizing highly effective and low-toxic derivatives of phenformin to optimize the antitumor activity of phenformin. The novel phenformin derivatives 2-(2-chlorophenyl) ethyl biguanide (2-Cl-phen) (112-114) and IM156 (115) exhibited superior efficacy in inhibiting tumor growth. IM156 has undergone a phase I clinical trial (NCT03272256) in patients with advanced solid tumors and lymphoma. Consequently, from the perspectives of pharmaceutics and pharmaco-chemistry, developing specific tumor-targeting drug delivery materials and optimizing the structure of phenformin are future research directions to enhance the antitumor activity of phenformin.

To sum up, in order to promote the application and clinical treatment of tumors with phenformin, efforts need to be made in the following aspects: i) The delivery system of phenformin requires optimization or use of novo dosage forms for precise packaging and delivering it to tumor lesions, aiming to mitigate toxic side effects ; ii) the structure of the target of phenformin needs to be analyzed to refine the synthesis of high-efficiency and low-toxicity phenformin derivatives; iii) the mechanisms underlying drug resistance and insensitivity in tumors need to be investigated from a clinical perspective, and novel combination therapy strategies for phenformin need to be identified; and iv) despite positive anticancer effects observed in preclinical studies and phase I trials, it is urgent to augment the sample size for phase II and III clinical trials to comprehensively assess lactic acid toxicity and therapeutic efficacy at recommended dosages.

In conclusion, as a member of the biguanide family, phenformin has shown superior antitumor effects than metformin in various cancer cell lines and xenograft animal models, garnering considerable attention from researchers. Phenformin principally affects a series of processes in tumor occurrence and development by inducing mitochondrial dysfunction or acting on TME and CSCs, such as affecting cancer cell metabolism, inducing cancer cell stress response, inhibiting tumor angiogenesis, EMT, blocking cell cycle and others. The multifaceted diversity of antitumor mechanisms exhibited by phenformin further broadens its range of applications. It is noteworthy that phenformin reverses tumor resistance to clinical drugs or synergistically increases efficacy by inhibiting oxidative OXPHOS or mTOR signaling pathways, providing a new strategy for clinical cancer treatment. Moreover, it has been observed in clinical trials that the combination of phenformin with clinical drugs effectively reduced MDSCs in patients, which will further promote the study of the combined regimen of phenformin and immunotherapy. In conclusion, substantial evidence supports the promising potential of phenformin as an effective anticancer drug, and it is worth further studying its mechanism of action, which will pave the way for phenformin to enter the clinical treatment of tumors and provide a broader strategy for cancer treatment.

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Authors' contributions

QZ reviewed the literature and drafted the original manuscript. DL designed and reviewed the manuscript. XY reviewed the manuscript and provided funding acquisition. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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