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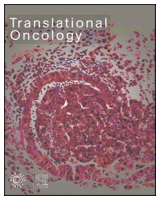

Review Article

Metformin as anticancer agent and adjuvant in cancer combination therapy: Current progress and future prospect

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ABSTRACT

Metformin, as the preferred antihyperglycemic drug for type 2 diabetes, has been found to have a significant effect in inhibiting tumor growth in recent years. However, metformin alone in cancer treatment has the disadvantages of high dose concentrations and few targeted cancer types. Increasing studies have confirmed that metformin can be used in combination with conventional anticancer therapy to obtain more promising clinical benefits, which is expected to be rapidly transformed and applied in clinic. Some combination therapy strategies including metformin combined with chemotherapy, radiotherapy, targeted therapy and immunotherapy have been proven to have more significant antitumor effects and longer survival time than monotherapy. In this review, we summarize the synergistic antitumor effects and mechanisms of metformin in combination with other current conventional anticancer therapies. In addition, we update the research progress and the latest prospect of the metformin-combined application in the cancer treatment. This work could provide more evidence and future direction for the clinical application of metformin in antitumor.

Introduction

Metformin (N, N-dimethylbiguanide), the first-line drug for the treatment of type 2 diabetes mellitus (T2DM), was first used in clinical application in 1957 [1]. Metformin could lower blood glucose through inhibiting hepatic gluconeogenesis [2]. A large number of clinical trials have proven the safety and high effectiveness of metformin. In recent years, the particular role of metformin in cancer treatment has been demonstrated in extensive preclinical studies and clinical trials [3]. Firstly, tumor cells often use different glucose and energy metabolism pathways from normal cells [4]. However, diabetes is a metabolic disease, which just provides a hyperglycemia environment for cancer cells. A number of studies have reported a fairly strong association between diabetes and cancer. One study involving more than 400,000 people suggests that T2DM may lead to an increased risk of many cancers [5]. Diabetes is considered as a risk factor for cancer development, and metformin as a potent antihyperglycemic drug can reduce this risk in patients with T2DM [6]. Metformin is expected to exert potent antitumor capacity in patients with different cancer types. Some studies have demonstrated the same potential antitumor effects of metformin in non-diabetic patients [7]. Metformin can treat cancer through various mechanisms, but it exhibits disadvantages in its individual treatment such as high effective concentration and few targeted cancer types, so we hope to seek more effective therapeutic strategies in terms of combination. The correct and appropriate combination of drug regimens can maximize the effectiveness of anti-cancer therapy.

In this review, we summarize and update the recent research advances in metformin combination antitumor drugs and therapies. This review will show the latest potential preclinical studies, cohort studies and clinical trials of metformin and different types of antitumor drugs, treatments, or related targets to evaluate metformin as a new partner for antitumor drug combinations in various cancer environments. This work could provide a more effective and safe therapeutic reference program for the clinic and a direction for future metformin treatment of cancer.

Functions and mechanisms of metformin in the treatment of cancer

Metformin, the preferred glucose-lowering drug for patients with T2DM, is typically an adenosine monophosphate-activated protein

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kinase (AMPK) activator (Fig. 1). AMPK is an important kinase for regulating energy homeostasis, which is responsible for regulating cell physiological activities and maintaining cell metabolic balance [8]. However, there is a strong link between diabetes and cancer development. AMPK restores the normal function of the liver and other tissues in diabetic patients as well as stops the metabolism of rapidly proliferating tumors [9]. Therefore, metformin can be used as a potential tumor-suppressing drug mainly through the activation of AMPK.

Metformin mainly acts through the indirect route of insulin dependence and the direct route of non-insulin dependence [10]. Metformin can alter the endocrine metabolic environment of the host and thus affect the tumor growth [11]. Based on the Warburg effect, cancer cells require large amounts of glucose for nourishment and are highly dependent on glycolysis for proliferation [12]. As an insulin sensitizer, metformin can promote hepatic glycogen synthesis, glucose catabolism and insulin transport by activating AMPK in the liver to reduce the levels of insulin and insulin-like growth factor-1 (IGF-1) in serum [13]. Metformin activates AMPK in the liver, producing an indirect anti-tumor mechanism. Meanwhile, metformin directly inhibits the expression of insulin receptor (IR) and insulin-like growth factor receptor 1 (IGFR1) [14]. It leads to the reduction of IR-mediated signaling in cancer cells and acts on the PI3K pathway, thus inhibiting the growth of tumor cells. IGFR is abnormally expressed in many tumors, which promotes tumor transformation and survival of malignant tumor cells [15].

Metformin can also act directly through insulin non-dependent mechanisms. Firstly, metformin enters tumor cells via organic cation transporters (OCT) and partially inhibits the mitochondrial respiratory chain complex1 [16]. This leads to a decrease in intracellular ATP and an increase in AMP levels, which inhibits gluconeogenesis and further activates AMPK. Activated AMPK can exert anticancer effects by inhibiting the mammalian target of rapamycin (mTOR) signaling pathway, in which tuberous sclerosis complex 2 (TSC2) is activated or phosphorylated [17]. The mTOR is an important regulator of cell growth and proliferation. The over-activation of mTOR is related to tumor progression, drug resistance and worse prognosis [18]. In addition, activated AMPK can inhibit angiogenesis in tumor tissues by inhibiting vascular endothelial growth factor (VEGF) and plasminogen activator inhibitor-1 (PAI-1), thus blocking the nutritional supply of tumor cells [19]. AMPK activation can also inhibit fatty acid synthesis by inhibiting acetyl-coenzyme A carboxylase (ACC) and fatty acid synthase (FASN) activity. The anti-proliferation effect of metformin on cancer cells is related to the inhibition of lipogenesis. Cancer cell proliferation requires fatty acids to synthesize membranes and signal molecules [20]. It has been proved that FASN can be used as a therapeutic target for breast cancer, prostate cancer, colorectal cancer and other cancers [21]. Furthermore, AMPK can induce cell cycle arrest and autophagy by down-regulating cyclin D1 and up-regulating p53, resulting in the inhibition of cell proliferation [22]. In addition to AMPK activation mechanism, metformin can also promote reactive oxygen species (ROS) production by inhibiting mitochondrial respiratory-chain complex I, which can lead to DNA damage and gene mutation [23].

Metformin Combined with Chemotherapy Exerts Synergistic Effects

Chemotherapy mainly refers to the traditional chemotherapeutic treatments represented by cytotoxic drugs that stop cancer cells from proliferating, infiltrating and metastasizing. Traditional chemotherapeutic drugs such as platinum, which is commonly used, can kill any fast-growing cell, including normal cells. This results in serious side effects of chemotherapy, which leads to poor tolerance of patients and aggravates cancer pain. However, how to diminish or eliminate the side

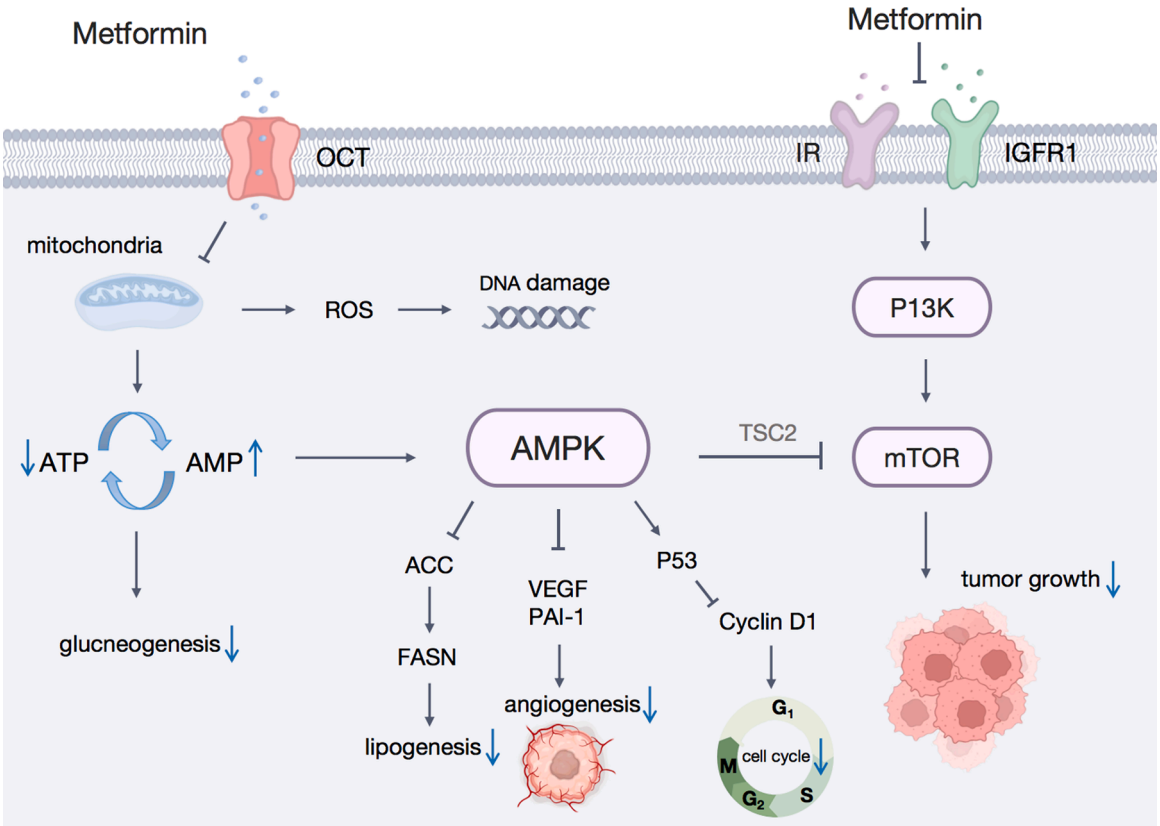

Fig. 1. Mechanism of metformin in the treatment of cancer. OCT, organic cation transporters; IR, insulin receptor; IGFR1, insulin-like growth factor receptor 1; ROS, reactive oxygen species; ACC, acetyl-coa carboxylase; FASN, fatty acid synthase; VEGF, vascular endothelial growth factor; PAI-1, plasminogen activator inhibitor -1; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin.

effects of chemotherapy, increase the efficacy of chemotherapy, and eliminate the resistance of cancer cells to chemotherapy will be an important issue to be addressed. Metformin combined with chemotherapy is a feasible strategy to treat cancer, which can increase the effect of drugs, reduce the toxic side effects of drugs and improve the prognosis of patients.

A large number of studies have shown that the combination strategy of metformin and chemotherapy could be used to treat many types of cancer. In studies on ovarian cancer, the combination of chemotherapy and metformin significantly inhibited pAKT and AKT (protein kinase B, PKB) downstream effector, pmTOR, more strongly than treatment with chemotherapy alone (carboplatin, 50 μM) or metformin (0.5 mM), with the resulting synergistic effect associated with inhibition of the AKT/mTOR pathway [24] (Fig. 2). Moreover, the investigators also demonstrated that neoadjuvant and concurrent metformin use had a stronger synergistic antitumor effect than adjuvant metformin. The neoadjuvant and concurrent metformin regimen may promote the regulation of glucose metabolism to keep blood glucose at lower levels or in an environment that is not conducive to cancer cell growth. Subsequent chemotherapy would then produce better tumor suppression efficacy. Therefore, metformin should be used prior to or concurrently with chemotherapy to obtain better efficacy. Metformin may also make a difference in chemotherapy resistance. Metformin induces autophagy in SKOV3/DDP ovarian cancer cells resistant to cisplatin, and improves the sensitivity of ovarian cells to chemotherapy [25]. In a phase I study of patients with advanced epithelial ovarian cancer, the recommended phase II dose (RP2D) of metformin combined with carboplatin or paclitaxel was evaluated and determined to be 1000mg three times daily (TDS) [26]. Thus, it seems feasible to add metformin to the chemotherapy regimen for ovarian cancer for combination therapy.

In addition, metformin combined with chemotherapy has shown great promise in leukemia. Metformin may show more significant antitumor effects in acute myeloid leukemia (AML) in conjunction with cytarabine (ara-C) which is associated with inhibition of the mTORC1/P70S6K pathway [27]. Acute promyelocytic leukemia (APL) belongs to a subtype of AML. A study demonstrated that metformin in combination with paclitaxel (combined dose, 34 nM & 4 μM) induced apoptosis in the APL cell line HL-60, which was associated with the down-regulation of related genes of the TNF family [28]. The combination of chemotherapy and metformin can be equally effective against lymphoma. In a retrospective study of patients with diffuse large B-cell lymphoma (DLBCL) treated with chemotherapeutic agents (rituximab and anthracyclines) between 1997 and 2013, there were 49 diabetic and 215 non-diabetic patients among 264 patients with DLBCL [29]. When treated with chemotherapeutic drugs, patients with DLBCL using metformin had significantly improved PFS (90 months vs. 60 months, $P = 0.036$) and OS (100 months vs. 71 months, $P = 0.039$) compared with patients using other hypoglycemic drugs. Furthermore, this study has further proved that metformin can enhance the anti-DLBCL effect of doxorubicin and rituximab. However, a limitation of this experiment is that the metformin concentrations in the study do not perfectly correspond to the plasma concentrations of metformin in real patients. Depending on the duration of metformin administration in different patients, the range of plasma concentrations maybe even longer.

There have also been many studies on breast cancer. The advantage of metformin combined with chemotherapy is related to killing cancer

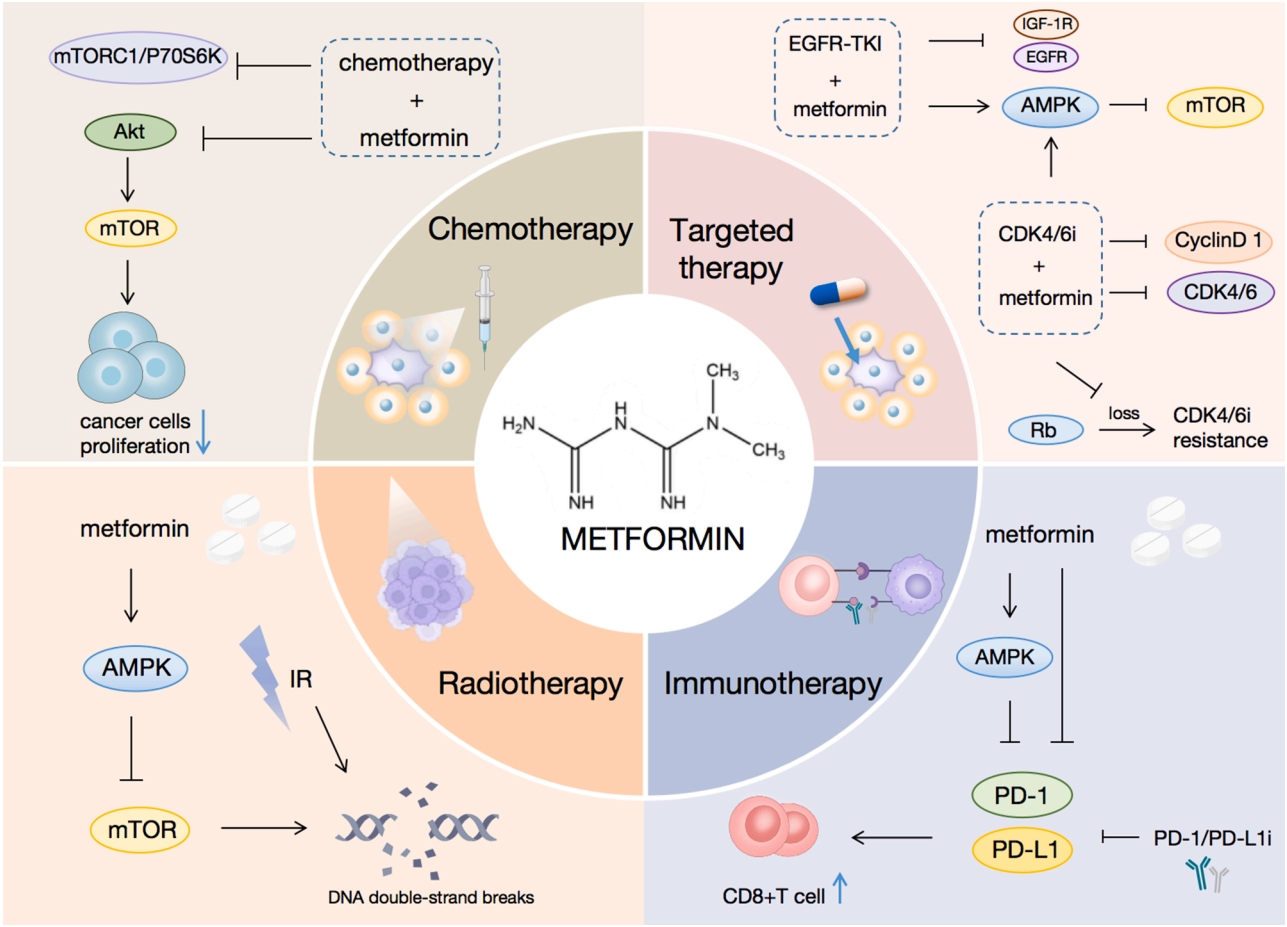

Fig. 2. Mechanisms of metformin in combination with conventional antitumor therapy. Akt, protein kinase B; mTOR, mammalian target of rapamycin; AMPK, adenosine monophosphate activated protein kinase; IGF-1R, Insulin-like growth factor 1 receptor; EGFR, epidermal growth factor receptor; Rb, retinoblastoma protein; IR, Infrared Radiation; PD-1, programmed death-1; PD-L1, programmed death ligand-1

stem cells [30]. Metformin selectively kills cancer stem cells in nude mice bearing MCF10A ER-Src cell tumor xenografts. It can inhibit tumor growth and eliminate tumor in synergy with doxorubicin, and the combination of the two is more effective than monotherapy. In addition, the Institute of Medicine of Alexandria University conducted a prospective study on patients with stage II and III non-diabetic breast cancer who received metformin combined with neoadjuvant chemotherapy from May 2017 to March 2019 [31]. The results showed that patients treated with metformin in combination with chemotherapy had significantly higher rates of pathological complete response (pCR, 14.8 % vs 6.3 %). The combination therapy group had a lower proportion of patients with a residual cancer burden score (RCB) class 3 (40.7 % vs 68.8 %). The researchs have shown that adding metformin to chemotherapy is more effective in patients with breast cancer. Fortunately, a randomized controlled study has shown that metformin could also reduce the toxicity induced by neoadjuvant chemotherapy (Adriamycin + Cyclophosphamide + Paclitaxel, AC-T) in nondiabetic breast cancer patients [32]. The results of a phase II clinical trial in breast cancer patients showed that metformin could improve the adverse effects of neuropathy (PN) in paclitaxel-treated breast cancer patients [33]. The prognosis was also better in patients using metformin. Thus, metformin may be able to be used as a chemoprotective agent, reducing the toxicity of chemotherapy and ameliorating adverse effects. The safety and tolerability of metformin were confirmed, but a large number of phase III clinical trials are still needed to follow up the study. Based on the anti-tumor effect of metformin, metformin may be a suitable partner for chemotherapy to achieve significant curative effect and inhibit tumor growth.

Metformin sensitization and radioprotection in radiotherapy

Radiotherapy mainly produces ionizing radiation through specific rays, which destroy nuclear DNA, and inhibit or kill tumor cells to remove tumor lesions. However, radiotherapy has side effects. While radiation kills tumor cells, normal cells are also harmed. We hoped to find a safe and effective way to reduce the side effects of general radiotherapy and improve its curative effect. Recently, it has been found that metformin, as a classical antihyperglycemic drug, can have various effects on radiotherapy. The role of metformin in radiotherapy is mainly in the enhancement of radiotherapy sensitization and as a radioprotective agent.

The sensitizing and adjuvant properties of metformin for radiation therapy have been demonstrated by various studies. Metformin radiosensitizes ductal breast cancer MCF7 cells by increasing intracellular reactive oxygen species (ROS) production through decreased thioredoxin (Trx) expression, but has no effect on other basal-like breast cancer (MDA-MB-231) [34]. In addition, metformin may act in combination with the aspirin metabolite salicylic acid to enhance the proliferation inhibition of radiotherapy on prostate cancer [35]. This was associated with activation of AMPK, inhibition of the mTOR pathway and reduction of hypoxia inducible factor 1 (HIF1α) levels, which inhibits DNA replication in tumor cells (Fig. 2). Similarly, metformin enhanced the radiosensitizing effect of H460 cells in cisplatin-sensitive sarcoma and non-small cell lung cancer (NSCLC) cells, which was associated with AMPK dependence, but this was not effective in A549 cells [36]. Additionally, acute or chronic toxicity to the intestine from radiotherapy was found to be mitigated by metformin, which was related to metformin-induced AMPK-dependent mitochondrial autophagy [37]. Thus, metformin enhances the sensitivity of P53-mutated colorectal tumors to radiotherapy, as demonstrated in a mice xenograft model. A study found that the addition of metformin 4, 24 or 72 h after radiotherapy significantly induced A549 tumor necrosis (almost up to 50 %-70 % of the tumor) compared to radiotherapy alone [38]. Its effect of enhancing sensitivity to radiotherapy was the same as that of adding metformin 1h before radiotherapy. Moreover, the radiosensitization mechanism of metformin is related to the tumor microenvironment and tumor necrosis is most effective in a low oxygen and low glucose environment. Although radiotherapy can effectively kill tumor cells, normal tissues and cells are also inevitably harmed. It was found that mice exposed to 6 Gy or 8 Gy radiation can be effectively protected from radiation damage if they are pretreated with metformin [39]. The survival time of mice treated with metformin before irradiation was significantly prolonged, and the hematopoietic injury and intestinal injury caused by radiation were also reduced. Furthermore, radiotherapy can cause cytotoxicity and genotoxicity in human peripheral blood lymphocytes [40]. Metformin pretreatment could effectively inhibit X-ray radiation-induced lymphocyte apoptosis and DNA damage, providing radioprotection and reducing side effects in cancer patients. However, the study was limited to in vitro experiments and could not demonstrate whether the effect was synergistic in vivo. More data from preclinical and clinical trials are needed to further confirm the role that metformin plays in radiotherapy.

Currently, only few clinical trials have been completed evaluating the value of metformin in radiotherapy, and most clinical trials are ongoing or the results remain unspecified. The specific effects of metformin on radiotherapy still need to be verified in a large number of clinical trials. An interesting phase III clinical trial evaluated metformin in pediatric brain tumor survivors treated with intracranial radiation [41]. Those using metformin had better declarative memory and working memory than the placebo group. Metformin promotes cognitive recovery and brain repair in pediatric brain tumor patients after radiotherapy, and the safety and tolerability of metformin are well validated. Therefore, metformin may be able to reduce radiotherapy side effects and toxicity as a protective agent for radiotherapy. We expect more research results to be translated into clinical applications to avoid radiotherapy toxicity and side effects. Metformin is expected to be widely used in clinical treatment as a radioprotective and sensitizing agent for radiation therapy.

Metformin synergizes targeted therapy to inhibit tumor progression

Targeted therapy acts on specific tumor-related targets, accurately locates and specifically identifies tumor cells and strikes them. Targeted therapy is mainly based on targeted drugs, which are divided into small molecule drugs and monoclonal antibodies. Targeted drug therapy has the advantages of accurate administration and remarkable curative effect, but it inevitably produces side effects and drug resistance. We can look for new solutions in terms of drug combinations. The combination of metformin and targeted drugs is mainly seen in metformin combination with cyclin-dependent kinase 4/6 (CDK4/6) inhibitors and epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs).

Metformin combined with CDK4/6 inhibitors inhibits tumor growth

CDKs are a family of serine/threonine kinases, which are involved in regulating cell cycle, transcription and RNA splicing, and are often overexpressed or structurally activated in tumor cells [42,43]. Therefore, by inhibiting the CDK of tumor cells, the cycle progression of tumor cells can be effectively prevented to control the proliferation of tumor cells, achieving the purpose of treating tumors. CDK4/6 are key kinases to regulate cell cycle progression and cell proliferation [44]. Although CDK4/6 inhibitors are effective in breast cancer patients, acquired resistance is inevitable. This may be attributed to mutations in the tumor suppressor retinoblastoma protein (RB) and its downstream transcription factors E2F amplification, CDK4 and CDK6 amplification, overexpression of CCNE1/2, activation of the PI3K/AKT/mTOR signaling pathway, and other cell cycle mechanisms [45]. A new treatment or strategy needs to be found to overcome such problems and enhance the efficacy of CDK inhibitors. Recent studies demonstrated that metformin could inhibit tumor growth by activating AMPK to inhibit mTOR [46].

Metformin also reduces the expression of CCNE1/2 and CDK4/6 in tumors, which is associated with the AMPKα/Yap1 pathway [47]. Metformin may synergize with CDK4/6 inhibitors to inhibit tumor cells. In conclusion, metformin in combination with CDK4/6 inhibitors may maximize therapeutic efficacy in cancer patients.

In recent years, the research on the combined use of CDK inhibitors and metformin has made a new breakthrough in the treatment of various cancers. The synergistic effect of CDK4/6 inhibitor palbociclib and metformin can show high anti-ovarian cancer efficacy, which may be related to the cell cycle arrest controlled by cyclin D [48]. The combination therapy of metformin and CB-839 (Glutamine 1 inhibitor) overcame the drug resistance of CDK4/6 inhibitor in esophageal squamous cell carcinoma (ESCC) cells, which was related to the fact that the lack of Rb, the drug resistance mechanism of CDK4/6 inhibitor, made tumor cells sensitive to the combination therapy [49] (Fig. 2). The combination of ribociclib and metformin will further enhance AMPK phosphorylation and induce human hepatocellular carcinoma cell (Hep3B) death [50]. In addition, the study found that the application of the CDK 4/6 inhibitor ribociclib promoted the M2-type polarization of macrophages, while metformin works together with ribociclib to exert the anti-tumor metastasis effect by inhibiting the M2-type polarization of macrophages [51]. These findings suggest that metformin combined with ribociclib can be used in clinical treatment to reduce the occurrence of tumor metastasis and inhibit tumor growth. Metformin also showed synergistic antiproliferative effects with the CDKs inhibitor abemaciclib in cancer cell lines such as U251 cells (human glioma cells), A549 cells (human alveolar basal epithelial cells), and MCF7 cells (human breast cancer cells) [52]. However, it has been confirmed that the synergistic mechanism did not seem to be associated with cell cycle inhibition and apoptosis, but with inhibition of the mTOR pathway. Additional research has shown that metformin combined with CDK4/6 inhibitor LY2835219 (abemaciclib) can inhibit head and neck squamous cell carcinoma (HNSCC) by inducing cell cycle arrest [53]. Meanwhile, metformin could modulate LY2835219-induced senescence-associated secretory phenotype (SASP) by inhibiting LY2835219-activated mTOR and stat3 pathways, enhancing the anti-HNSCC effect of CDK4/6 inhibitors. Moreover, on the basis of this study, the experimenter further verified the effect of metformin on the up-regulation of IL1-α, IL1-β, TGF-β and CCL5 cytokines related to aging maintenance and tumor inhibition in MCF7 cells by LY2835219. Therefore, the effect of metformin combined with abemaciclib on breast cancer can be explored. The combination of CDK inhibitors and metformin may be considered as a new targeted therapeutic strategy to achieve better anticancer efficacy.

Metformin has also been shown to inhibit CDK4 and CDK6. The addition of metformin reduced the expression levels of cyclin D1, CDK4, CDK6, cyclin E, and CDK2 in gastric cancer cells [54]. The expressions of cyclin D1 and CDK4 in acute myeloid leukemia (AML) cell line SKM-1 were down-regulated by metformin [55]. Metformin also inhibited the proliferation of esophageal cancer cells by down-regulating cyclin D1, CDK4 and CDK6, which blocked the cell cycle transition from G0 to G1 [56] (Fig. 2). The mechanism of action of the combination of metformin and CDK4/6 inhibitors requires more research. There are currently no clinical trials of CDK4/6 inhibitors in combination with metformin, and the efficacy and toxicity of this combination therapy are expected to be further evaluated in more cancer patients and further validated in clinical studies.

Metformin combined with EGFR-TKIs targets tumors and reduces drug resistance

EGFR is a member of the ErbB receptor family and a tyrosine kinase-type receptor, which plays an important role in cell proliferation, survival and differentiation. TKI can enter the cell and act directly on the intracellular region of the EGFR receptor, interfering with ATP binding and blocking the autophosphorylation of kinase, thus blocking the abnormal signaling and producing tumor suppressive effect. EGFR-TKI is the first-line treatment for non-small-cell lung cancer (NSCLC) patients carrying EGFR mutations [57]. However, most patients with EGFR mutations eventually develop resistance or insensitivity to EGFR-TKI therapy, and combining EGFR-TKI with other drugs can delay resistance and enhance its efficacy [58]. Some research has shown that metformin could address the problem of EGFR-TKIs resistance, increase drug sensitivity and reduce EGFR-TKIs-induced side effects [59]. Due to its high safety and tolerability, metformin may play a synergistic role as a partner of EGFR-TKIs in cancer treatment.

There have been a lot of results from the combination of EGFR-TKI and metformin. In terms of apoptosis, metformin alone was able to induce 113-kDa PARP cleavage to the 89-kDa fragment in all NSCLC cell lines tested, whereas gefitinib induced an increase in procaspase-3 cleavage only in the CALU-3 (gefitinib-sensitive) cell line [60]. However, when metformin and gefitinib are combined, it is accompanied by continuous cleavage of procaspase-3 and 113-kDa PARP in CALU-3, CALU-3 GEF-R (gefitinib-resistance) and H1299 (gefitinib-hyposensitive) cell lines. In terms of autophagy, it has been reported that metformin inhibits autophagy and enhances the sensitivity of osimertinib by inducing AMPK activation in a time-dependent manner [61]. These findings strongly suggest the use of metformin as a combination therapy or adjunct to enhance the tumor-killing potential of EGFR-TKI.

Although the efficacy of EGFR-TKI is remarkable compared with chemotherapy, drug resistance inevitably appears and leads to disease progression. The mechanisms of EGFR-TKI resistance are complex and diverse, and identifying a strategy for combination therapy may improve resistance. Combination therapy has provided a new way out for TKI resistance. Metformin reversed and delayed the acquired drug resistance of EGFR mutant lung cancer cells to EGFR-TKI by activating AMPK and inhibiting downstream ERK/NF-κB signal transduction [62]. In addition, Metformin increased the sensitivity of primary drug-resistant cells (H1975) to gefitinib primarily by downregulating the IGF-1R pathway [63] (Fig. 2). The study found that the combination therapy of metformin effectively blocked the tumor growth in xenografts with TKI-resistant cancer cells, which was related to the decrease of interleukin 6 (IL-6) secretion and expression, epithelial–mesenchymal transition (EMT) reversal and IL-6 signal activation in vivo [64]. Metformin sensitized afatinib therapy in TKI-resistant lung cancer cells, possibly by reducing the expression and activation of the EGFR signaling pathway. Metformin/EGFR-TKI combination therapy can reduce EMT biomarkers and increase E- cadherin, an epithelial marker in NSCLC cells, which can modify glycolytic phenotype in a state-dependent manner [65].

At present, clinical trials of EGFR-TKI combined with metformin are being carried out continuously. A retrospective study evaluated the prognostic impact of metformin in combination with EGFR-TKI in patients with NSCLC with T2DM [66]. Patients treated with EGFR-TKI plus metformin had significantly higher median progression-free survival (PFS) and median overall survival (OS), objective remission rate (ORR) and disease control rate (DCR) than the EGFR-TKI plus other classes of glucose-lowering agents group (PFS: 19.0 vs. 8.0 months; OS: 32.0 vs. 23.0 months; ORR: 70.5 % vs. 45.7 %; DCR: 97.7 % vs. 80.4 %). The research shows that metformin and EGFR-TKI have synergistic effects in the treatment of T2DM patients with NSCLC who carry EGFR activation mutation. Unfortunately, the researchers performed the analysis only in patients with T2DM and lacked data on cancer patients without diabetes. Therefore, an attempt can be made to compare monotherapy with adjuvant metformin therapy in non-diabetic conditions to study whether it can provide benefits to NSCLC patients. The National Cancer Institute of Mexico launched a randomized phase II clinical trial in patients with advanced lung adenocarcinoma with EGFR mutation positive [67]. Among 139 patients with lung adenocarcinoma, the experimental group was treated with metformin plus EGFR-TKI (gefitinib, erlotinib and afatinib), while the control group was treated with EGFR-TKI only. The results showed that the PFS (13.3 months vs 9.9 months), ORR (71.0 % vs 54.3 %), and OS (31.3 months vs 17.5 months) were significantly better in the combination group than in the monotherapy group. This

means that the combination strategy can extend the survival of patients with EGFR-mutated advanced lung adenocarcinoma by up to one year. Because the participants in the experiment were not diabetic patients, the observed treatment effects can be attributed to the treatment of cancer rather than to the control of diabetes. In addition, the researchers investigated the incidence and severity of treatment-related adverse events, and there was no difference between the two groups, which proved the safety of combined therapy. Although this is only a phase II clinical trial, it is significant and has the potential to change clinical treatment options for lung cancer. It can be concluded that the first-line treatment research of metformin combined with targeted drugs is very promising, and its specific mechanisms of action and biomarkers are still to be explored.

Metformin combined with immunotherapy enhances effectiveness against cancer

Immunotherapy mainly restores the body's own immune system to resist and attack cancer cells. Once it works, it can enable patients with advanced cancer to survive for a long time or even achieve clinical cure. Compared with chemotherapy, its overall side effects are less. Immune checkpoint inhibitors (ICI) targeting the programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) pathway are among the most established immunotherapeutic drugs, which kill tumor cells by liberating immune cells suppressed by cancer cells [68]. In fact, the drug still has some limitations, especially its low effectiveness and unclear treatment-related markers, which still cannot meet the current clinical needs. In clinical trials, it has been found that only about 20 % of patients will respond to immunotherapy, and some of them even lose their response after a period of treatment [69]. Moreover, some tumor types are generally less effective for immunotherapy, such as breast cancer, prostate cancer, and colon cancer [70]. A number of studies have shown that metformin combination enhances the immune efficacy of PD-1/PD-L1 inhibitors and may act as an adjuvant therapy with immune drugs to suppress tumors, and its related mechanism of action needs to be further investigated.

The combination of metformin and PD-1/PD-L1 inhibitors is considered as a promising strategy for cancer treatment. Wabitsch et al. [71] found that nonalcoholic steatohepatitis (NASH) diminished the effect of anti-PD-1 treatment in mice with liver tumors and even exacerbated disease progression. NASH causes critical alterations in CD8+ T cell metabolism in mice, which affects the efficacy of ICI, but metformin can save the day. The researchers injected CT26 (mice colorectal carcinoma cell line) or RIL-175 tumor (hepatocellular carcinoma, HCC) into BALB/c and C57BL/6 mice, and fed them methionine/choline deficiency (MCD) diet and western diet (WD). Anti-PD-1 or metformin monotherapy did not alter tumor growth in NASH mice, but adding metformin to the combined therapy can restore the anti-PD-1 effect of NASH mice with cancer. Similarly, the experimenter tested that metformin also showed synergistic effect in NASH mice treated with anti-PD-L1 and anti-VEGFR 2. Chen et al. [72] demonstrated that a dual blockade strategy of lactate/ G-protein coupled receptor 81 (GPR81) and PD-1/PD-L1 pathway could significantly enhance the antitumor effects of metformin and even lead to tumor regression. Scharping et al. [73] found that metformin alleviated tumor hypoxia by modulating the oxygen tension of the tumor microenvironment and altering hypoxia-driven T cell phenotypic changes, while resulting in increased sensitivity of the tumor microenvironment to PD-1 blockade, increased T cell function, and tumor regression. The results of Zhang et al. [74] showed that metformin promoted the differentiation of memory CD8+ T cells through the AMPK-miR-107-Eomes-PD-1 pathway, enhanced the antitumor activity of CD8+ T lymphocytes, and prevented the depletion of CD8+ T cells in patients (Fig. 2). Munoz et al. [75] team added metformin to tumor membrane vesicle (TMV) vaccine treatment and found that metformin downregulated PD-L1 expression through the AMPK pathway. Metformin enhanced TMV vaccine-induced 4T1 tumor growth inhibition compared to TMV vaccine alone. Metformin might serve as an excellent adjuvant in combination with immunotherapy to target tumors.

Combination therapy with metformin and PD-1/PD-L1 inhibitors has been carried out in clinical trials. In a unique case study of a patient with small cell lung cancer (SCLC) who was treated with metformin in combination with nivolumab, the patient demonstrated partial remission according to the Response Evaluation Criteria in Solid Tumours (RECIST 1.1) [76]. The patient showed significant lasting response for more than 6 months without any side effects. Prior to the addition of metformin, this patient's course showed multiple progressions in multiple modalities, including surgery, chemoradiotherapy, and immunotherapy (nivolumab alone). The incorporation of metformin reversed disease progression with nivolumab monotherapy. This is strong evidence that metformin helps to enhance the efficacy of PD-1 inhibitors. A retrospective cohort study investigated the efficacy of metformin in combination with immune check point inhibitors (anti--PD-1/anti-CTLA-4) for the treatment of metastatic malignant melanoma [77]. Patients treated with immunotherapy in combination with metformin had higher ORR and DCR, longer median PFS and OS. Patients treated with immunotherapy alone without metformin had more sites of tumor metastasis during treatment. However, favorable treatment-related outcomes were obtained with only a small sample size. Besides, data from 50 patients with stage IV NSCLC treated with nivolumab, pembrolizumab or atezolizumab in combination with metformin or without metformin were subjected to electronic chart review to extract data [78]. The results showed higher ORR, DCR, OS, and PFS in the combination treatment, and better disease outcomes in patients treated with the combination treatment as compared with those in the control group. However, the current studies all have drawbacks due to the still small sample size. A large number of prospective clinical trials are still needed to study the effects and clinical benefits of metformin in combination with immunotherapy before the most appropriate combination therapy can be applied to conventional clinical treatment. These studies will promote the clinical use of metformin and immunotherapy, and provide strong evidence for further research in the future.

Discussion

Currently, a large number of studies focus on clinical trials and preclinical studies of metformin combination therapy for cancer in vitro and in vivo. The combination of these different treatment modalities may allow control of tumor growth for a longer period of time than conventional drugs alone in patients with advanced disease. However, there are still some difficulties in the application of metformin in combination. Firstly, drug combinations at different doses and concentrations may produce interactions or drug toxicity. Metformin often brings gastrointestinal side effects, which may aggravate discomfort and reduce compliance in patients already on anticancer therapy. The effect of the applicable drug combinations may also vary in patients with different degrees of comorbidity. How to choose the appropriate drug combination to maximize the effect of metformin and combination drugs and at the same time safe and non-toxic is the key issue in the study of metformin combinations. Along with the personalization of oncology treatment regimens, metformin combination therapy can be matched flexibly. In addition, metformin is commonly used in patients with T2DM, where its combined anticancer effect is demonstrated. The antineoplastic efficacy of metformin in diabetic and non-diabetic patients needs to be evaluated. In normal patients who have never used glucose-lowering drugs, it is an open question whether the anticancer effect of metformin can still be demonstrated or can be achieved by combining other drugs. Patients with diabetes have longer cycles of metformin use, which may result in the length and order of metformin dosing having different effects on efficacy. Although there are still some shortcomings in the use of metformin in cancer, it is promising. We would expect that the therapeutic effect of metformin in combination

with anticancer therapy will be superior to its treatment alone. We hope that our review can provide more references for the realization of metformin's anticancer effects in clinical applications in the future, and also promote the faster development of metformin combination therapy for cancer treatment in the clinic.

Conclusion

In this review, we proved the powerful effect of metformin combined with anti-tumor drugs, and determined the feasibility of metformin combined with anti-tumor drugs and treatment. Metformin still needs the latest and more cumulative evidence in the study of combining different types of anti-tumor drugs, treatments or related targets to evaluate metformin as a new partner of anti-tumor drug combination in various cancer environments. It is hoped that it can provide a more effective and safer treatment reference scheme for clinic, and also provide a direction for metformin to treat cancer in the future.

CRediT authorship contribution statement

Lin Zhu: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Kaiqing Yang:** Writing – review & editing, Visualization, Validation. **Zhe Ren:** Writing – original draft, Visualization. **Detao Yin:** Writing – review & editing, Supervision, Conceptualization. **Yubing Zhou:** Writing – review & editing, Supervision, Resources, Investigation, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

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