Research progress on the therapeutic effect and mechanism of metformin for lung cancer (Review)

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Received May 6, 2022; Accepted August 25, 2022

DOI: 10.3892/or.2022.8440

Abstract. Lung cancer is the most common type of cancer and the leading cause of cancer-associated death worldwide. Despite the availability of various treatments such as surgery, chemoradiotherapy, targeted drugs and immunotherapy, treatment is expensive and the prognosis remains poor. At present, lung cancer drugs and treatment programs remain in a state of continuous exploration and research to improve the prognosis, and to reduce the pain and economic burden for the patients. Type 2 diabetes is a common chronic disease in middle-aged and elderly patients, leading to significantly increased complications of cardiovascular and cerebrovascular diseases. Epidemiology shows that type 2 diabetes also increases the incidence of malignant tumors, including lung, liver, colorectal and pancreatic cancer. Metformin is a biguanide, widely used as a first-line oral drug in treating type 2 diabetes. Metformin has a hypoglycemic effect and a biological antitumor impact, reducing the incidence of various tumors, including lung cancer, and improving the prognosis of patients with tumors. The anti-lung cancer effect of metformin involves a variety of mechanisms that can improve the therapeutic effect and prognosis of lung cancer, as a single drug or in combination with other therapies. The present study aims to review the associated literature and the therapeutic effects of metformin on lung cancer.

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Key words: lung cancer, metformin, antitumor effect, mechanism

1. Introduction

Metformin is a biguanide developed from galegine, a guanidine derivative; it is a hydrophilic base, present as a cationic species at physiological pH. Metformin is absorbed mainly in the small intestine, and then taken up by the liver to play an antihyperglycemic role. Metformin is excreted unchanged in the urine (1). As the primary drug for treating type 2 diabetes, it controls blood glucose by reducing glycogen decomposition, inhibiting gluconeogenesis, inhibiting intestinal glucose absorption and increasing insulin sensitivity in the surrounding tissues (2,3). The molecular target of metformin is presenilin enhancer protein 2 (PEN2). Metformin binds PEN2 and initiates a signaling pathway that interacts with the glucose-sensing pathway via V-type proton ATPase subunit S1 to activate the lysosomal 5'-adenosine monophosphate-activated protein kinase (AMPK) pathway, which is an indispensable mechanism that enables metformin to inhibit hepatic gluconeogenesis and improve insulin sensitivity (4,5). Recent studies have found that metformin can inhibit the occurrence and development of lung cancer in addition to its hypoglycemic effect (Fig. 1).

An extensive cohort study published in 2009 discovered lower cancer morbidity rates (6), while another study discovered lower mortality rates (7), including those for lung cancer, in patients with type 2 diabetes treated with metformin compared with patients who had never used metformin. Cancer was diagnosed in 7.3% of 4,085 metformin users compared with 11.6% of 4,085 comparators (6). In patients taking metformin compared with patients not taking metformin at baseline, the adjusted hazard ratio (HR) for cancer mortality was 0.43 (95% CI, 0.23-0.80) (7). Several studies have indicated that metformin inhibits tumor growth (6,8-10). A meta-analysis found that metformin increased the survival time of patients with lung cancer plus type 2 diabetes, suggesting that metformin may improve the prognosis of these patients (11). A study using Surveillance, Epidemiology, and End Results public data included 750 mergers of patients with type 2 diabetes plus stage IV non-small cell lung cancer (NSCLC), with 61% patients on

metformin. After controlling for social-demographic characteristics, the types of lung cancer and treatment, the results showed that patients with advanced lung cancer undergoing metformin treatment had higher survival rates compared with patients not treated with metformin (12). Therefore, certain researchers began to study metformin in lung cancer treatment and its mechanism, using animal tests and clinical studies. Studies have shown that metformin has cytotoxic effects on human lung cancer cell lines (including squamous cell carcinoma (13), adenocarcinoma (14), large cell carcinoma (15), small cell carcinoma and non-transformed cell lines (16). Tan *et al* (17) conducted clinical trials to compare the efficacy of metformin with insulin and other hypoglycemic drugs in the treatment of NSCLC, and discovered that patients treated with metformin had longer overall survival (OS) (P=0.007) and progression-free survival (PFS) (P=0.002) times. In a meta-analysis of 14 randomized controlled trials (RCTs), Stevens *et al* (18) discovered no significant effect of metformin on cancer mortality. Therefore, the effect of metformin on the treatment and prognosis of lung cancer remains controversial, requiring confirmation from further studies.

2. Mechanisms of metformin in the treatment of lung cancer

Antitumor effects through liver kinase B1 (LKB1)-dependent AMPK kinase pathways. Metformin can produce antitumor effects through the LKB1-AMPK kinase pathway. LKB1 is a tumor suppressor gene; its encoding product, LKB1 protein, is a serine/threonine kinase that can regulate various cell physiological and pathological processes. Somatic LKB1 gene mutations exist in numerous malignant tumors, including lung cancer, colon cancer, breast cancer and Peutz-Jeghers syndrome (a cancer susceptibility disease) (19,20). Gene mutations in the somatic LKB1-AMPK pathway increase the risk of precancerous lesions (21). In NSCLC, 13% of adenocarcinomas and 5% of squamous cell carcinomas have LKB1 mutations. However, the LKB1-AMPK pathway can still be activated by metformin and inhibit tumor growth (22). These results suggest that metformin may have other mechanisms to inhibit tumor genesis and development. Mammalian target of rapamycin (mTOR), the downstream target of the LKB1-AMPK pathway, is an important target of metformin in tumor inhibition. mTOR is the catalytic subunit of two multi-protein complexes, mTOR complex 1 (mTORC1) and mTORC2, regulating cell growth and integrating input signals from various hormonal and energy-sensing pathways (23). These signals include insulin and insulin-like growth factor 1 (IGF-1), IGF-2 and AMPK (24). AMPK activates tumor suppressor gene binding sclerosis complex 1 (TSC1) and TSC2/mTORC1 to form mTOR inhibitory complex, leading to mTORC1 downregulation. AMPK can also directly inhibit the positive regulator of mTOR, namely regulatory-associated protein of mTOR, leading to its downregulation (25). Metformin can also inhibit the IGF-1 insulin signaling pathway through the AMPK-dependent insulin receptor substrate 1 (IRS-1) phosphorylation pathway, inhibiting the phosphatidylinositol 3-kinase (PI3K)/AKT pathway and downregulating the mTOR signaling pathway to interfere with protein synthesis, affecting tumor cell proliferation (26). As the target molecule of PI3K/AKT/mTOR signaling, mTOR contributes to the control of protein synthesis. There is a positive correlation between protein synthesis rates and proliferation rates. In turn, the production of mitochondrial ATP is needed to fuel protein synthesis and proliferation. The production of mitochondrial energy, protein synthesis and proliferation are co-regulated processes, and mTORC1 stimulates the synthesis of numerous nuclear-encoded mitochondrial regulators, such as TFAM, mitochondrial ribosomal proteins and complex I and V components via the upregulated translation of corresponding mRNAs (27). mTOR plays a major part in coupling mitochondrial functions and translation. As well as regulating nuclear-encoded mitochondrial regulator synthesis, mTOR regulates the translation of mRNAs encoding proteins that promote proliferation, including cyclins, ornithine decarboxylase (ODC) and Myc. Through the aforementioned mechanisms, the synthesis of cyclins, ODC and Myc, which can promote tumor cell proliferation, are inhibited by the downregulation of PI3K/AKT/mTOR signaling (28). Therefore, tumor cell proliferation is inhibited.

Metformin in the treatment of type 2 diabetes can improve type 1 diabetes insulin resistance and the inflammatory response through the p53/RAP2A pathway, and regulate the p53/RAP2A pathway to improve insulin resistance (29). Similarly, AMPK-dependent p53 activation has been associated with the antitumor effects of metformin. p53, a tumor suppressor protein and one of the downstream targets of AMPK, achieves its antitumor function by increasing transcriptional expression of proteins involved in DNA repair, apoptosis and the prevention of cell proliferation, alteration and senescence. When DNA suffers oxidative damage, intracellular damage recognition signals activate p53 and its transcriptional targets, protecting genomic integrity and regulating cell metabolism and the cell cycle. p53 inhibits tumors by activating multiple genes that inhibit the AKT and mTORC1 pathways; it can be phosphorylated by serine and activated by AMPK (30-33). According to a previous study, metformin-treated p53 mutant cells had significantly higher apoptosis rates than wild-type colon cancer cells (34). However, another study has shown that metformin can enhance the efficacy of radiotherapy, independent of AMPK and p53 status (35). Metformin can inhibit mTOR and slow cell cycle progression through regulated in DNA damage and development 1 independently of AMPK (36). In addition, metformin selectively inhibits tumor growth and triggers apoptosis in p53-deficient HCT116 xenografts (34). Therefore, metformin can act through AMPK or p53, but the specific pathway is complex. In addition, AMPK can induce endoribonuclease DICER (DICER) expression, an enzyme involved in microRNA (miRNA/miR) synthesis, whose change and loss of function can lead to complex tumor syndrome, suggesting that metformin-induced DICER expression may be one of the antitumor mechanisms (37). DICER belongs to the double-stranded RNA-specific endonuclease family, which are able to convert the miRNA precursor forms into their mature forms through a stepwise process. The methylation levels of DICER are significantly higher in patients with lung cancer. Methylation analysis of the first region of the DICER can distinguish patients with NSCLC from healthy individuals (38). In one study, DICER was regulated by MDA-7/interleukin (IL)-24 through the downregulation of microphthalmia-associated transcription factor

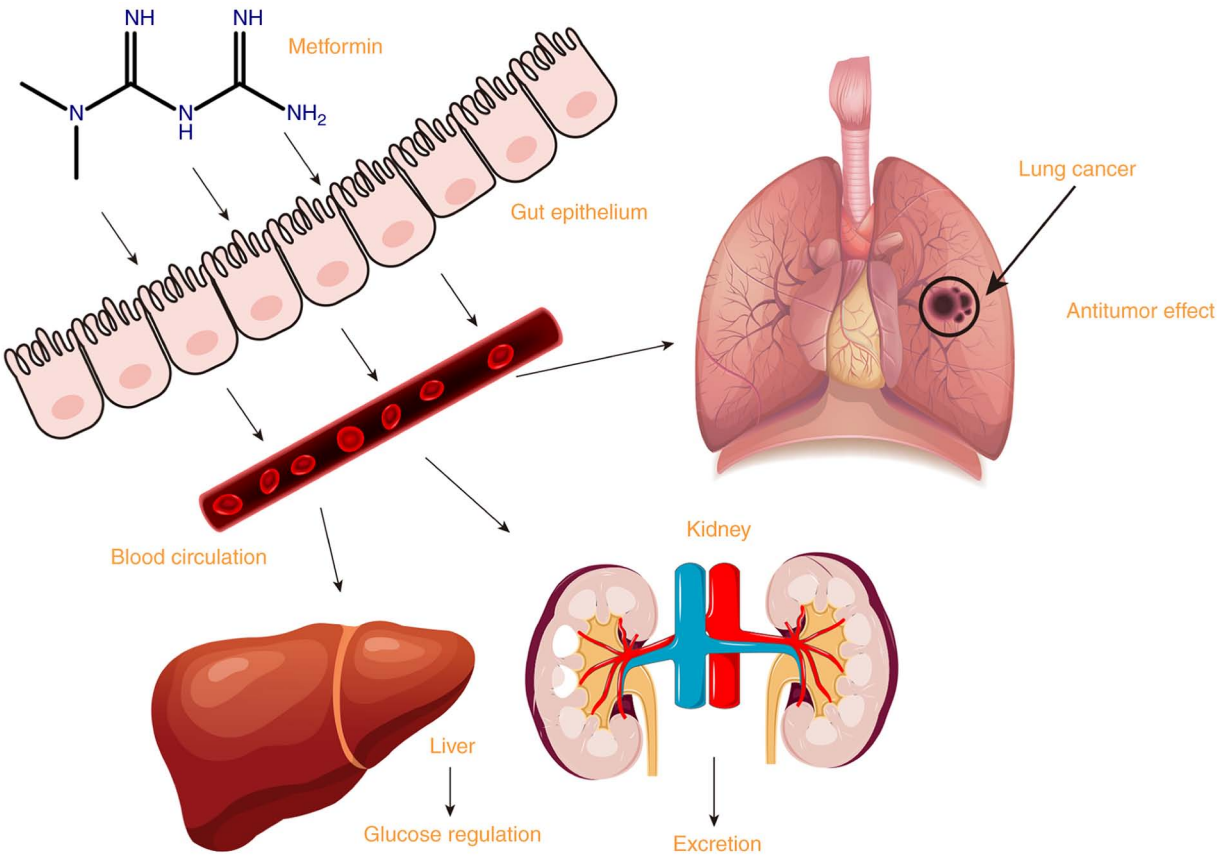

Figure 1. Absorption, uptake, excretion and antitumor effect of metformin.

in lung cancer cells (39). Dicer expression of NSCLC was significantly increased in stage II tumors compared with that in stage I tumors, and in stage III tumors compared with that in stage II and I tumors (40). Dicer contributes to the resistance to gefitinib in lung cancer (41), and can promote autophagy and cisplatin resistance in NSCLC by downregulating let-7i-5p, as well as inhibiting the activation of the PI3K/AKT/mTOR pathway (42). Activated AMPK can also trigger UNC-51-like kinase 1 (a serine/threonine kinase that is an autophagy promoter in mammals), inducing apoptosis, cell cycle arrest and autophagy to exert antitumor effects (43). Metformin also inhibits proto-oncogene c-Myc and hypoxia-inducible factor 1α (HIF-1α) via AMPK (44). HIF-1α is a transcription factor that promotes the expression of glycolysis enzyme glucose transporter 1 (GLUT1) and monocarboxylic acid transporter 4, both of which play a key role in the metabolic transformation of cancer. There is no expression of HIF-1α in human normal lung tissues, while HIF-1α is highly expressed in lung cancer tissues. HIF-1α is mainly expressed in the nucleus and cytoplasm of lung cancer cells, presenting with obvious heterogeneity (45). In a previous study, the expression of HIF-1α around the tumor necrosis area and the infiltrating edge of the tumor was significantly increased, and the expression intensity in SCLC with a high degree of malignancy, strong invasion and early metastasis was significantly higher than that of squamous cell carcinoma and adenocarcinoma (46). The expression intensity of HIF-1α was also closely associated with the degree of differentiation and postoperative survival of lung cancer cells. HIF-1α promotes the increase in vascular endothelial growth factor (VEGF) production, angiogenesis and permeability, provides more oxygen and nutrients for tumor cells, and ensures the proliferation of tumor cells (47). On the other hand, HIF-1α can also induce anti-apoptotic factors to make cells resist apoptosis, or increase the transcription of other enzymes associated with glycolysis and glycogen generation, in order to increase the proliferation activity of lung cancer cells, enable invasion and metastasis, and shorten the survival period of affected patients (48). These studies indicate that the AMPK signaling pathway is a promising new target for tumor therapy. However, another study has found that AMPK may lose its regulatory role in cancer cells due to mutations/deletions of its upstream regulatory kinase Lkb1/Stk11 or ubiquitination of the MAGE-A3/6-TRIM28 E3 ligase complex. This results in autophagy inhibition, mTOR signaling pathway activation and metformin hypersensitivity (49). Therefore, further animal experiments and clinical studies are required to explore the role of this pathway in the antitumor effect of metformin.

Downregulating the GRB/IRS-1/PI3K/AKT/mTOR pathway. IGFs are multifunctional cell proliferation regulators that promote cell differentiation, proliferation and individual growth and development. IGFs include IGF-1 and IGF-2. Type 1 IGF receptor (IGF-1R) belongs to the receptor tyrosine kinases (RTKs) family. It can be activated by IGF-1 or IGF-2, causing phosphorylation of its tyrosine kinase domain and initiating intracellular signal transduction, thereby regulating cell growth and differentiation, development, senescence and other life activities (50). Studies have revealed that IGF-1R can

regulate blood glucose and stimulate the growth of NSCLC cell lines, promoting carcinogenic transformation, growth and survival of tumor cells (51,52). In addition, IGF can activate the Ras/Raf/ERK signaling pathway through growth factor receptor-bound protein 2 and promote tumor cell proliferation (53). Downstream activation of IGF-1R upregulates the PI3K/AKT/mTOR pathway and the mitogen-activated protein kinase (MAPK)/ERK pathway (also known as the KRAS-Raf-MEK-ERK pathway) to enhance cell proliferation. IGFs are associated with the occurrence, development and metastasis of tumors. Metformin can downregulate IGF-1 by inhibiting the PI3K/AKT and MEK/ERK signaling pathways, regulating lung cancer cell metabolism and inhibiting cell proliferation (54,55). In NSCLC, activating the PI3K/AKT/mTOR signaling pathway leads to more aggressive lung cancer and a worse prognosis, especially in squamous cell lung cancer. Metformin can block the IGF-1-insulin signaling pathway by phosphorylating IRS-1, inhibiting the IRS-1/PI3K/AKT and PI3K/AKT/mTOR signaling pathways, thereby preventing mTOR activation and inhibiting NSCLC (55). Other studies have demonstrated that abnormal activation of the PI3K/AKT/mTOR signaling pathway is one of the mechanisms of acquired imported epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) resistance in patients with adenocarcinoma and EGFR activation mutation (56). Inhibiting the PI3K/AKT/mTOR signaling pathway may also overcome radiotherapy and chemotherapy resistance, as well as immune escape in NSCLC (57). Animal experiments have indicated that nicotine derivatives can reduce the number of tumor-related regulatory T cells (Tregs) by inhibiting mTOR in tumor cells (58) and creating a favorable environment for the occurrence and development of nicotine-induced lung tumors (59). Rapamycin activation by metformin can prevent the occurrence of lung tumors. Metformin mildly inhibits the mTOR pathway in tumors, which can reduce the occurrence of lung tumors by 40-50%. One study discovered that mTOR inhibition in lung tissue is associated with lower circulating IGF-1 and insulin levels rather than lower AMPK (60).

A variety of anti-IGF-1R monoclonal antibody drugs have been developed. A study has disclosed that metformin alone or combined with figitumumab (anti-IGF-1R monoclonal antibody) can achieve antitumor effects on NSCLC by inhibiting the PI3K/AKT and MEK/ERK signaling pathways, and downregulating the IGF-1R signaling pathway. It has been suggested that metformin combined with figitumumab may have a good therapeutic value in treating NSCLC (54).

Inhibiting mTORC1 to regulate glucose and amino acid concentration. Metformin can inhibit the mTORC1 signal by inhibiting and regulating the activity of the Rag GTPases complex. A number of environmental signaling factors, including nutrition and growth factors, activate the mTORC1 pathway to regulate the growth of organisms (61). Cell-based studies showed that mTORC1 senses amino acids through the RagA-D family of GTPases (also known as RRAGA, B, C and D) (62). However, their importance in mammalian physiology is unclear. In animal experiments, mice expressed the active form of RagA ($RagA^{GTP}$) by inserting endogenous promoters through a gene knock-in method. Fasted $RagA^{GTP/GTP}$ newborn mice could not trigger autophagy and produce amino acids used to convert glucose, resulting in an imbalance in glucose homeostasis. However, severe hypoglycemia did not inhibit mTORC1 in $RagA^{GTP/GTP}$ newborn mice (62). We hypothesize that the Rag pathway may signal the availability of glucose and amino acids to mTORC1, inhibiting mTORC1 (63). In $RagA^{GTP/GTP}$ fibroblasts, mTORC1 is resistant to glucose deprivation, and glucose, like amino acids, controls its recruitment on the lysosomal surface, where mTORC1 is activated (64). Therefore, Rag GTPases transmit glucose and amino acid concentration signals to mTORC1, playing a key role in autophagy induction, nutritional homeostasis and the survival ability of newborn mice (62).

Metformin directly affects glucose metabolism and inhibits tumor growth. Glucose metabolism in lung cancer mainly includes the glycolysis, aerobic oxidation and pentose phosphate pathways. The glycolysis pathway produces less energy (ATP) per mole of glucose than the aerobic oxidation pathway, but glycolysis pathway can provide energy more quickly. Under aerobic conditions, tumor cells also preferentially utilize glucose glycolysis capacity as their primary energy source (Warburg effect) (55). In NSCLC, adenocarcinoma uses glycolysis for energy under normal oxygen conditions. Squamous cell carcinoma is more likely to have a high rate of anaerobic glycolysis due to hypoxia in the tumor microenvironment, slow diffusion and metastasis. Metformin can promote glycolysis by changing the activity of certain glucose metabolism enzymes (including fructose-2,6-biphosphatase) and can promote the conversion of glucose metabolism to glycolysis in NSCLC cells (65). This may stimulate the growth of the NSCLC cells. However, due to less energy per unit provided by glycolysis, reduced ATP production leads to increased AMP levels, which results in an increased intracellular ratio of AMP to ATP and an imbalance of energy metabolism, thereby realizing the antitumor effect of metformin (66).

A study has revealed that squamous cell carcinoma has a higher uptake rate of 18F-fluorodeoxyglucose (FDG) than adenocarcinoma, but adenocarcinoma has a higher metastasis potential and poorer disease-free survival time (67). Another phase II study in advanced NSCLC cancer randomized patients to receive metformin (1,000 mg twice daily) combined with platinum-containing chemotherapy in a controlled diet with or without metformin. It discovered that the uptake of 18F-FDG on baseline positron emission tomography (PET) images was significantly higher in squamous cell carcinoma than that in non-squamous NSCLC. Metformin significantly reduced the risk of tumor progression and death in lung squamous cell carcinoma with high uptake of 18F-FDG, indicating that the antitumor effect of metformin is highly dependent on glucose metabolism (68). In a recent single-blind phase II trial (69), metformin was used to treat inoperable early-stage NSCLC. PET scans were performed at the beginning of treatment, in the middle of treatment (after 2 weeks of metformin or placebo administration) and after 6 months. The results revealed that most metformin-treated subjects had PET Response Criteria in Solid Tumors (PERCIST) (70) metabolic responses on PET imaging, leading to increased glucose metabolic activity in most tumors, demonstrating that the effect of metformin on tumor growth may be influenced by glucose metabolism in the tumor environment. In addition, insulin is a growth hormone that

promotes division, while metformin can directly or indirectly inhibit tumor growth by reducing serum insulin levels and improving glucose metabolism in hyperinsulinemia (71,72).

Inhibiting complex I of the mitochondrial respiratory chain. Studies have found that metformin can cross the plasma (1,55) and mitochondrial (73) membranes to affect tumor metabolism by reaching the mitochondria. Metformin gets positively charged at physiological pH, and organic cation transporters mediate the movement of metformin across the cell membrane in NSCLC. Metformin targets mitochondrial complex I, inhibits mitochondrial complex I and attenuates the oxidation of nicotinamide adenine dinucleotide, reducing the proton gradient across the mitochondrial intima and proton-driven ATP synthesis. Metformin directly inhibits adenosine deaminase to increase AMP, leading to an increase in the ratio of AMP to ATP in cells, catalyzing the conversion of ATP to cyclic AMP (cAMP), resulting in imbalanced cell energy metabolism and inhibiting tumor cell growth (74). Therefore, metformin increases the cAMP level, which activates 5'-AMPK and its downstream signaling pathway, inhibiting tumor growth and proliferation (55). Simultaneously, metformin reduces reactive oxygen species production, oxidative stress and DNA damage by inhibiting mitochondrial complex I, thus reducing the risk of mutation (75). In addition, interactions between biguanides and mitochondrial copper ions are critical for metformin metabolism, with copper chelators inhibiting metformin-activated 5'-AMPK-dependent signaling and S6 protein phosphorylation (76). Extensive P-electron delocalization can stabilize the binding of the biguanides to mitochondrial copper, enabling the biguanides to regulate AMPK, glucose production, gluconeogenesis gene expression and mitochondrial respiration (77).

Regulating lung miRNA. An animal study revealed that metformin regulated 42 out of 1,281 pulmonary miRNAs in smoke-free mice through multiple mechanisms, including AMPK, stress response, inflammation, NF-κB, Tlr9, TGF, p53, cell cycle, apoptosis and antioxidant pathways, Ras, Myc, Dicer, angiogenesis, stem cell recruitment and angiogenesis. In smoke-exposed mice, metformin significantly reduced DNA adduct levels and oxidative DNA damage, normalized the expression of certain miRNAs, and thus protected mouse lungs from smoke-induced changes in DNA and miRNA, thereby inhibiting pretumor lesions of the lungs and kidneys (78). Among the miRNAs involved, miR-148b and miR-30b are known miRNA families that can regulate AMPK (79) and are associated with the activation of AMPK by metformin. In addition, metformin regulates the expression of a number of miRNAs involved in cell cycle regulation, such as let-7f, miR-30b, miR-362, miR-376c, miR-466h, miR-490 and miR-574. They are also important mediators of the antitumor activity of metformin through the AMPK pathway (80). miR-137 targeting SLC22A18 has been revealed to significantly inhibit the proliferation, invasion and migration of NSCLC cells (81). miR-7 regulates the occurrence and development of lung cancer through PI3K regulatory subunit γ/AKT, Bcl-2, IGF-1R and other signaling pathways. In NSCLC, metformin regulation of the AMPK pathway is also associated with the upregulation of miR-7 (82,83). Dong *et al* (84) also found that metformin significantly upregulates miR-7 in a time and dose-dependent manner through the AMPK pathway, and that the upregulation of miR-7 reduces growth, migration and invasion of NSCLC cells. Recently, Jin *et al* (85) found that metformin reduced the growth, migration, invasion and epithelial-mesenchymal transition (EMT) of NSCLC cells by regulating miR-381/yes-associated protein (YAP) activity.

Affecting the tumor and its microenvironment. Peripheral immune cells and angiogenesis are two major components of the interaction of a tumor with its microenvironment. Altering the tumor microenvironment can significantly affect tumor growth and the therapeutic effect. A study on tumor cell lines, including those for NSCLC, found that metformin enhanced $CD8^+$ T cell memory by altering fatty acid metabolism, and promoted rejection of solid tumors in control mice, but did not exert this effect on T cell-deficient mice (86). Metformin treatment of tumor tissue significantly increased the number and activity of $CD8^+$ tumor-infiltrating lymphocytes (TILs), and protected them from apoptosis and exhaustion. Metformin-mediated effects were significantly reduced when AMPK was knocked out (87). Furthermore, metformin treatment reduced the expression of Ki67 (a proliferation signal) and caspase-3 (an apoptosis signal), but this effect was attenuated when $CD8^+$ T cells were deficient. These results suggested that metformin reduces Ki67 and caspase-3 expression through $CD8^+$ TILs in the tumor microenvironment (80). Similarly, when $CD4^+$ T cells were depleted in the tumor microenvironment, the antitumor effect of metformin was significantly weakened (88).

In addition, the antitumor effect of metformin is closely associated with the adaptive immune response to the tumor microenvironment. Studies on animals have confirmed that metformin treatment can reduce lung cancer-associated $Foxp3^+$ Tregs by 65% and tumor-associated Tregs by 50% (88). $Foxp3^+$ Tregs are necessary for KRAS-mediated lung tumorigenesis in the tumor microenvironment (58). In addition, due to changes in blood supply and the energy imbalance of tumor cells, the concentration of glucose and other metabolites in the tumor microenvironment is low, resulting in an acidic interstitium and low oxygen content (89), and a lack of energy supply for locally infiltrating tumor T cells. Recent study has revealed that metformin inhibits tumor cell oxidative metabolism and oxygen levels in the tumor microenvironment. Metformin increases oxygen supply to TILs, rescues T cells from an anoxic environment and enhances their role, and may have potential for the immune treatment of patients (90).

Downregulation of silent information regulator T1 (SIRT1) can enhance the antitumor effect of cells. SIRT1 is involved in the development of a variety of tumors through the deacetylation of histones and non-histones. A study found that 62% of NSCLC tissues overexpressed SIRT1, significantly reducing the OS rate of affected patients (91). In NSCLC cell lines with different LKB1 expression states, metformin combined with SIRT1 inhibitor Tenovin 6 could synergically inhibit SIRT1 expression in NSCLC cells regardless of LKB1 status. Even in LKB1-deficient A549 cells, the combination of metformin and Tenovin 6 significantly reduced SIRT1 expression, increased the acetylation of p53 at lysine 382 and enhanced the stability

of p53 (91). Metformin inhibited SIRT1 promoter activity by upregulating the hypermethylation binding of hypermethylated in cancer 1 protein on the SIRT1 promoter and synergistically induced caspase 3-dependent apoptosis. The research has confirmed that metformin combined with Tenovin 6 enhances the antitumor effect by downregulating SIRT1 expression independently of LKB1 (91).

In a previous study, the activation of protein phosphatase 2 (PP2A) by metformin inhibited the growth, invasion and activity of A549 and H1651 tumor cells and promoted apoptosis (92). PP2A is a tumor suppressor in a number of cancer types; it inhibits the carcinogenic activity of AKT and Myc by catalyzing serine dephosphorylation. PP2A inhibitor α4 is often overexpressed in cancer cells (92). Metformin activates PP2A by preventing PP2A inhibitors (PP2A regulatory subunit α4 and E3 ubiquitin ligase midline 1) from interacting with their catalytic subunits, resulting in increased BAX expression, decreased Myc expression and AKT inactivation (92).

Inhibiting YAP expression in lung cancer cells. YAP is a carcinogenic protein whose overexpression and activation are associated with lung, liver, colon, ovarian and breast cancer; it has been linked to a poor prognosis, metastasis and progression of lung cancer due to its ability to promote cell cycle progression and inhibit apoptosis (93). Jin *et al* (94) found that YAP mRNA and protein levels in NSCLC tissues were higher than those in normal lung tissues. Metformin treatment significantly reduced YAP mRNA and protein levels and their downstream targets. Metformin was shown to interfere with the binding of the transcription factor interferon regulatory factor-1 to YAP promoter. Thus, YAP expression in lung cancer cells was decreased. Inhibition of YAP promoter activity reduced cell proliferation, migration, invasion and EMT, and increased cell senescence and apoptosis. In mice with lung cancer, 250 mg/kg/day metformin reduced tumor volume, increased survival rate and decreased YAP expression level in transplanted tumors (94).

Metformin promotes survivin degradation, induces apoptosis and inhibits NSCLC cell proliferation through the AMPK-dependent protein kinase A (PKA)/glycogen synthase kinase 3β (GSK-3β) pathway. Survivin is an anti-apoptotic protein that is often overexpressed in malignant cells (95). Luo *et al* (95) found that metformin downregulated survivin level, without changing its mRNA level, enhancing its proteasome degradation by inhibiting PKA activity through downstream GSK-3β activation. PKA activator (8-Br-camp and forskolin) and GSK-3β inhibitor (LiCl and small interfering RNA) can increase survivin activity and enhance lung cancer cell proliferation (94).

Salani *et al* (96) demonstrated that microcystin 1 in NSCLC can inhibit the effect of metformin on the IGF-1 pathway and that microcystin is necessary for tumor inhibition by metformin. The study also proposed that metformin can significantly upregulate the transcription levels of intracellular matrix metalloproteinase 2 (MMP2) and MMP9, enhancing the migratory rate and invasive ability of human lung adenocarcinoma A549 cells *in vitro* (96).

Others. Ataxia telangiectasia mutated (ATM) encodes a tumor suppressor protein, a key component of the DNA damage response network system, and is required for DNA repair and cell cycle control. As a cellular stressor, metformin participates in ATM-mediated repair through AMPK-dependent and AMPK-independent mechanisms and activates the cell repair process, which may have a protective effect on the malignant transformation of cells (97).

Metformin promotes apoptosis through the MAPK signaling pathway and upregulation of GADD153. MAPKs, serine/threonine proteases, regulate various cell physiological processes and play an important role in apoptosis. Metformin can induce lung cancer cell cycle arrest through the MAPK signal transduction pathway, thus playing an anti-proliferative and pro-apoptotic role in lung cancer cells (98).

In summary, the antitumor mechanisms of metformin are not completely clear. However, certain pathways have been established as aforementioned. One of these pathways acts to block protein synthesis by inhibiting mTORC1. This effect can be achieved through AMPK-dependent and AMPK-independent pathways. Anabolic events in the plasma membrane, cytoplasm and mitochondria of tumor cells are tightly regulated by the LKB1-AMPK pathway. AMPK is indicated to regulate the PI3K/AKT/mTOR pathway, which stimulates gene expression, cellular growth and survival. Activation of the LKB1-AMPK pathway by metformin leads to downregulation of the downstream target mTOR. Inhibition of mTORC1 blocks protein synthesis. The inhibition of mTOR can also be achieved by inhibiting IGFs and their downstream targets. In addition, metformin can inhibit the mTORC1 signal by inhibiting and regulating the activity of the Rag GTPases complex. This process is mediated by regulating glucose and amino acid concentration. Metformin also inhibits tumor growth by affecting tumor energy metabolism; it suppresses tumor progression by inhibiting glycolysis and the mitochondrial respiratory chain. The immune microenvironment is critical for tumor growth. The effects of metformin on the tumor microenvironment are closely associated with the decrease in $Foxp3^+$ Tregs and the increase in $CD8^+$ T cells. Metformin regulates pulmonary miRNAs associated with DNA damage or the cell cycle. The mechanisms of metformin in the treatment of lung cancer are summarized in Fig. 2.

3. Application of metformin in lung cancer treatment

Metformin and chemotherapy in lung cancer. Chemotherapy is one of the main treatments for lung cancer, but most patients will develop drug resistance as the treatment progresses, resulting in tumor recurrence and progression. Several metformin trials have proved that metformin can increase chemotherapy sensitivity, reverse the resistance of chemotherapy drugs and improve the therapeutic effect of tumor chemotherapy.

Metformin increases the sensitivity of chemotherapy in lung cancer. The interaction between metformin and chemotherapy drugs has been studied using a mouse lung adenocarcinoma transplanted tumor model. Metformin and doxorubicin were combined to treat lung adenocarcinoma in mice. Compared with the doxorubicin alone group, the metformin treatment group did not exhibit an increased tumor recurrence rate, even in the lower doxorubicin dose group, suggesting that

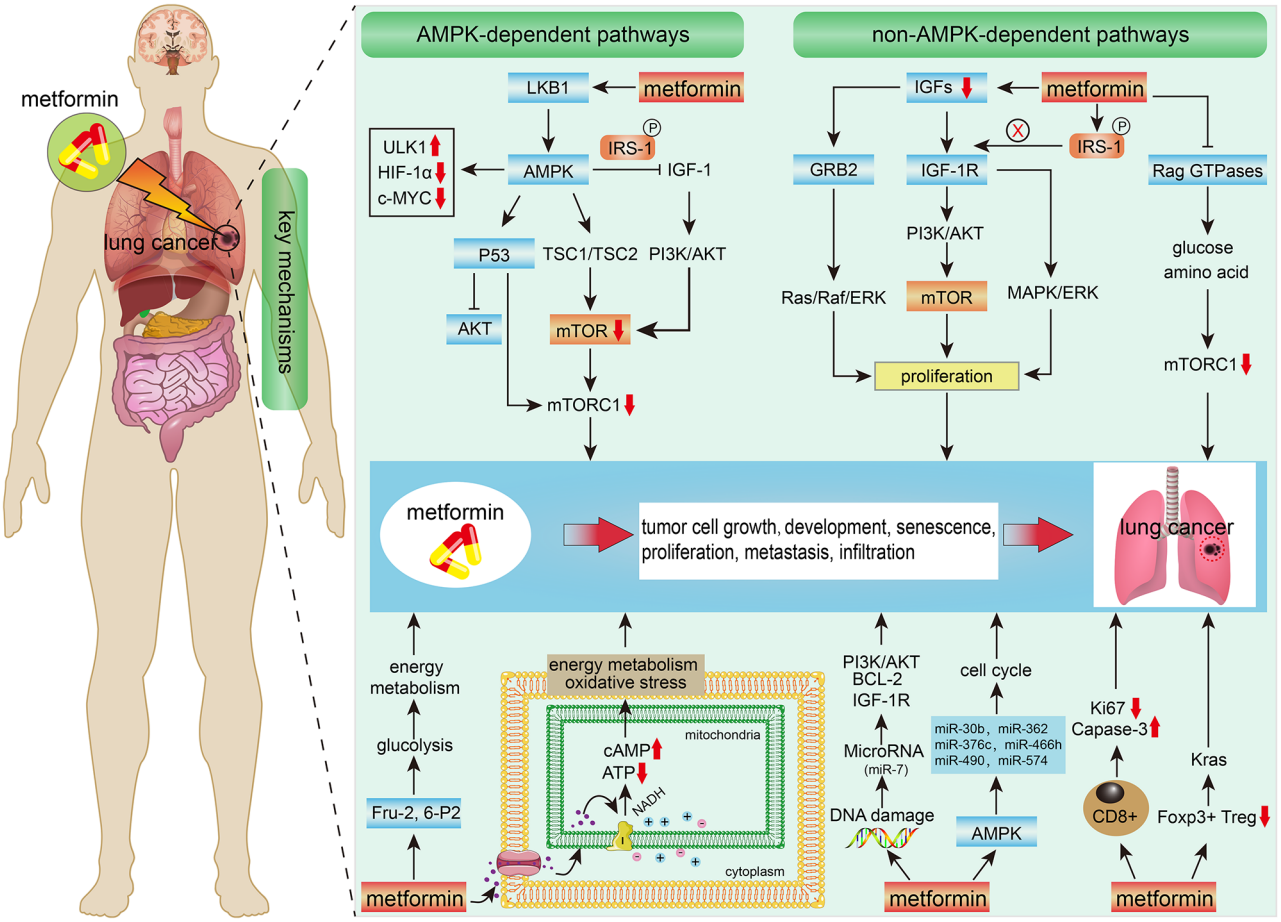

Figure 2. Mechanisms of metformin in the treatment of lung cancer. AMPK, adenosine monophosphate-activated protein kinase; LKB1, liver kinase B1; ULK1, UNC-51-like kinase 1; HIF-1α, hypoxia-inducible factor 1α; IRS-1, insulin receptor substrate 1; IGF-1, insulin-like growth factor 1; TSC, tumor suppressor gene binding sclerosis complex; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; IGF-1R, type 1 IGF receptor; GRB2, growth factor receptor-bound protein 2; IRS-1, insulin receptor substrate 1; PI3K, phosphatidylinositol 3-kinase; AKT, serine/threonine kinase; c-MYC, myelocytomatosis oncogene; RAS, renin-angiotensin system; MAPK, mitogen-activated protein kinase; Raf, v-raf-leukemia oncogene; ERK, extracellular signal-regulated kinase; Bcl-2, B-cell lymphoma 2; miR, microRNA; KRAS, Kirsten rat sarcoma viral oncogene homolog; Foxp3, forkhead box P3; ATP, adenosine triphosphate; cAMP, adenosine 3'5'-cyclic monophosphate; NADH, nicotinamide adenine dinucleotide phosphate; Treg, regulatory T cell; Rag, recombination activation gene; GTPases, guanosine triphosphatases.

metformin can increase the chemotherapy sensitivity of doxorubicin to lung adenocarcinoma (99). Iliopoulos *et al* (99) used mice injected with A549 lung cancer cells in the right flank as a research model to explore the antitumor effect of metformin combined with chemotherapy drugs and found that the tumor volume decreased more significantly in the metformin + chemotherapy group than that in the chemotherapy group alone, and no tumor recurrence was found in the metformin group. However, tumor recurrence occurred in the group with chemotherapy alone (99). Tseng *et al* (52) found that metformin at 0.1 mmol/l combined with paclitaxel had a stronger cytotoxic effect on lung cancer cell lines than paclitaxel chemotherapy alone, suggesting that metformin could improve the therapeutic effect of paclitaxel on lung cancer (52). One RCT included 99 patients with stage IV NSCLC who received platinum-based chemotherapy (without radiotherapy) from five hospitals (including 19 patients whose lung cancer recurred after treatment), although there was no statistical difference in OS time between the groups. However, the metformin chemotherapy group (n=39) was superior to the insulin (n=35) and other hypoglycemic drug (n=25) groups in terms of PFS time (17). In addition, another study has revealed that metformin can increase the antitumor effect of cisplatin or etoposide on large cell lung cancer (15).

To date, two clinical trials of metformin combined with chemotherapy in NSCLC have been conducted, including a phase II trial in which all patients received metformin in combination with chemotherapy [carboplatin (area under the curve (AUC)=5) + pemetrexed (500 mg/m^2) intravenously every 21 days for 4 cycles]. Subjects maintained pemetrexed treatment until disease progression or until they could no longer tolerate treatment. Oral metformin (500 mg), administered twice daily for 1 week, starting from the 1st day of chemotherapy cycle 1 (C1D1), and increased by 500 mg/day at C1D8 and C1D15, eventually reaching 1,000 mg twice daily, was continued as oral metformin treatment until disease progression or intolerance (100). In another open-label phase II trial, patients were randomized 3:1 to receive chemotherapy with or without metformin. The chemotherapy regimen in this study was carboplatin (AUC 6) + paclitaxel (200 mg/m^2) + bevacizumab (15 mg/kg), in an intravenous infusion every 21 days for 1 day, for a total of 46 cycles (101). However, the

two trials remain in progress, and the results are expected to guide the therapeutic dose and course of metformin in lung cancer, and evaluate the adverse reactions and therapeutic effects of metformin combined with chemotherapy. A pooled analysis of individualized data from two phase II trials evaluated metformin in combination with platinum-based chemotherapy with or without bevacizumab in untreated non-diabetic patients with advanced NSCLC (102). A total of 33 patients were included in the pooled analysis, and the combined median PFS and combined median OS times for all patients were 6.0 and 14.8 months, respectively. PFS and combined median OS were 6.6 and 13.3 months, respectively, in patients with EGFR mutation, and 17.5 and 13.3 months, respectively, in patients with KRAS mutation. This study confirmed the efficacy and tolerability of metformin combined with chemotherapy, suggesting that KRAS or EGFR mutations may be key molecules affecting the difference in efficacy of metformin combined with standard chemotherapy (102).

An open-label randomized controlled study of gemcitabine plus cisplatin + metformin in patients with stage IV NSCLC showed no improvement in objective response rate (ORR) or OS compared with gemcitabine plus cisplatin (P=0.109 and P=0.119) (103). Another prospective study of the pemetrexed + carboplatin + metformin regimen in the treatment of advanced NSCLC also yielded negative results (100). In summary, most current studies suggest that metformin can enhance the efficacy of chemotherapy drugs for lung cancer. However, some clinical trials have shown no further benefit, so more *in vitro* experiments and clinical studies are needed to verify the efficacy of metformin combined with chemotherapy for lung cancer. Metformin can reduce the chemotherapeutic drug resistance in lung cancer. At present, platinum-based chemotherapy is the first-line treatment for advanced NSCLC, but drug resistance is inevitable at the late stage of treatment. A study has found that cisplatin resistance is related to signal transducer and activator of transcription 3 (STAT3) phosphorylation, reactive oxygen species (ROS) production and IL-6 secretion, while metformin can inhibit cisplatin-induced ROS generation, STAT3 phosphorylation and autocrine IL-6 secretion, thus improving the chemical sensitivity of NSCLC to cisplatin (104). STAT3 promotes tumor proliferation, tumor cell survival and angiogenesis through overexpression of anti-apoptotic proteins (Bcl-2-like protein 1 and myeloid cell leukemia 1), cell cycle regulating proteins (cyclin D1 and c-Myc) and VEGF in NSCLC (105). The STAT3 signaling pathway activates various cytokines and growth factors, which are critical in tumor cell growth and apoptosis. Metformin improves the cisplatin resistance of lung cancer cells by inhibiting STAT3 activity through the LKB1-AMPK pathway and mTOR pathway-dependent mechanisms (106). Studies have revealed that metformin can improve cisplatin cytotoxicity and improve the cisplatin resistance of tumor cells (107,108).

Metformin and radiotherapy for lung cancer. Radiotherapy is another important treatment for lung cancer, especially for patients who lose the opportunity for surgery at a later stage or cannot tolerate chemotherapy. However, radiotherapy may cause radiation-related side effects, such as insensitivity to bone marrow suppression pneumonia secondary to lung infection. Koritzinsky suggested that radiosensitivity was related to the efficiency of the insulin receptor, including the repair of tumor cell DNA damage, cell redistribution in the cell cycle, re-replication (tumor cell replication) and the reoxygenation ability (the degree of hypoxia in the tumor) (109). The study by Storozhuk *et al* (30) confirmed that metformin combined with radiotherapy could continuously activate the ATM/AMPK/p53/$p21^{cip1}$ signaling pathway and inhibit the AKT/mTOR/eukaryotic initiation factor (eIF) 4E-binding protein 1 (4EBP1) signaling pathway, thus improving the sensitivity of radiotherapy. EIF4E and 4EBP1 have been found to be overexpressed in cancer tissues, such as lung cancer, breast cancer and colorectal cancer (110). This leads to a significant increase in the activity of the eIF4F complex, which further promotes the translation initiation process of various proteins, such as c-myc, cyclin D1, VEGF and ODC, and induces tumor resistance to radiation. Inhibiting the 4EBP1 pathway would enhance sensitivity to radiotherapy (111). DNA damage after irradiation (mainly DNA double-strand breaks) can activate serine/threonine-protein kinase Chk2 through ATM to block the cell cycle for DNA repair, thereby activating AMPK and p53, and p53-mediated apoptosis is one of the main mechanisms of cell death after irradiation (112). ATM inhibition has been revealed to enhance the sensitivity of radiotherapy combined with cisplatin in NSCLC cell lines (112). Notably, a study found that metformin can enhance the effect of radiotherapy in the absence of AMPK, suggesting that there are other mechanisms to enhance the sensitivity of radiotherapy (113). The interaction between the tumor and its microenvironment (including immune cells) is also considered to impact radiotherapy response significantly (114,115). Irradiation increases the number of TILs, induces upregulation of programmed death ligand 1 (PD-L1) on tumor cells and diversifies T-cell receptor libraries (116,117). In a retrospective analysis of 74 patients with NSCLC who received concurrent chemoradiotherapy, $CD8^+$ TIL density was associated with good survival (118). The effect of irradiation and metformin on TILs is one of the mechanisms of increasing radiotherapy sensitivity. In addition, metformin has been shown to be a good radiosensitization agent by inhibiting the G_1 phase of the cell cycle, angiogenesis and the AMPK/AKT/mTOR/4EBP1 pathways in different NSCLC cell lines (30). Storozhuk *et al* (30) studied NCI-H1299, A549, SK-MES and other lung cancer cells, and found that metformin combined with radiotherapy could significantly reduce the tumor proliferation capacity and survival coefficient of cells. In an A549 transplanted mouse tumor model, the tumor inhibition in the metformin combined with radiotherapy group was more prominent than that in the radiotherapy group. Metformin activated the ATM/AMPK/p53/$p21^{cip1}$ pathway, inhibited the AKT/mTOR/4EBP1 pathway, induced G_1 phase cell cycle arrest and enhanced apoptosis. Metformin or irradiation inhibited xenograft growth, while the combination treatment enhanced it more than each treatment used alone. Ionising radiation and metformin induced sustained activation of the ATM/AMPK/p53/$p21^{cip1}$ pathway and inhibition of the AKT/mTOR/4EBP1 pathway in the tumors, reduced expression of angiogenesis and enhanced expression of apoptotic markers (30). The cytotoxicity of metformin in cancer stem cells, a rare cell pool, could theoretically also partially improve the efficacy of irradiation, but further studies are needed to confirm this (119,120).

Metformin synchronous chemoradiotherapy for lung cancer. Since 2013, three studies have involved metformin combined with radiotherapy for NSCLC. In a retrospective multicenter study of all patients with stage III NSCLC plus type 2 diabetes who received platinum-based chemotherapy and chest irradiation (mean total dose, 66.1 Gy), metformin improved radiotherapy response in reoxygenated tumors. The results suggested that metformin could improve PFS during concurrent chemoradiotherapy in diabetic patients with locally advanced (LA)-NSCLC (121). Simultaneously, metformin combined with concurrent radiotherapy and chemotherapy for treating LA-NSCLC was studied, and it was found that this type of treatment in LA-NSCLC could effectively improve the short-term efficacy and prolong the survival time of patients, without increasing the adverse reactions. Although metformin has enhanced radiotherapeutic effects on NSCLC *in vitro*, these effects have not been proven in the clinic. The combination of metformin and radiotherapy in NSCLC treatment shows an antagonistic effect. Therefore, the design of future clinical studies of metformin and radiotherapy in NSCLC treatment should be cautious.

Effect of metformin on targeted drug therapy for lung cancer. Metformin combined with targeted drug therapy exhibits a synergistic effect. Targeted drugs for lung cancer have been widely used in clinical practice. The main medications used are monoclonal antibodies and small-molecule TKIs. In human lung squamous cell carcinoma cells, gefitinib downregulated the expression of DNA mismatch repair protein MSH2 through the p38/MAPK pathway, and enhanced the cytotoxic and growth inhibitory effects of gefitinib on lung cancer cells (122). Retrospective analysis showed that metformin and EGFR-TKIs have a synergistic therapeutic effect on patients with NSCLC plus type 2 diabetes with EGFR mutation (123,124). In addition, metformin combined with EGFR-TKIs has been reported to significantly improve the clinical efficacy in patients with NSCLC plus type 2 diabetes (125). In a study targeting the treatment of LKB1 wild-type NSCLC cells, the addition of gefitinib to metformin inhibited EGFR phosphorylation and its downstream signaling. Increased c-Raf/B-Raf isomerization induced MAPK activation, thereby inducing significant apoptosis *in vitro* and *in vivo*, which suggests a synergic effect of metformin combined with EGFR-TKIs on LKB1 wild-type NSCLC cells (126).

The main studies on metformin combined with TKIs in lung cancer treatment include the trial NCT03071705 (127), which evaluated the efficacy and safety of various TKIs (erlitinib, afatinib or gefitinib) ± metformin as a second-line treatment for diabetic patients with advanced NSCLC and EGFR mutation. The results have been published. The median PFS time was significantly longer in the EGFR-TKI plus metformin group (13.1 months; 95% CI, 9.8-16.3) compared with the EGFR-TKI group (9.9 months; 95% CI, 7.5-12.2) (hazard ratio, 0.60; 95% CI, 0.40-0.94; P=0.03). The median OS time was also significantly longer for patients receiving the combination therapy (31.7 months; 95% CI, 20.5-42.8; vs. 17.5 months; 95% CI, 11.4-23.7; P=0.02) (127). Two ongoing studies to determine whether metformin and EGFR-TKIs have a synergistic effect in patients with non-diabetic lung cancer are the CGMT (NCT01864681) and METLUNG (NCT05445791) trials. The CGMT trial consists of >200 patients in a multicenter, phase II, randomized, double-blind, placebo-controlled study and aims to assess the safety and efficacy of treatment with metformin as first-line therapy for stage IIIb-IV NSCLC with EGFR mutation; the main purpose of this experiment is a comparative study of the 1-year PFS rate. The secondary objective of this trial was to compare the 2-year OS rate, the 2-year PFS rate, the ORR and the DCR between the two treatments and evaluate their relative therapeutic safety. The METLUNG trial was designed to evaluate the efficacy and safety of metformin + erlotinib as a treatment for patients with EGFR mutant-type stage IIIB-IV NSCLC.

Metformin combined with targeted drugs can overcome the resistance to targeted drugs. Applying targeted drugs in lung cancer has significantly improved the prognosis of patients with lung cancer, but almost all targeted drugs will cause resistance in the treatment process and affect the therapeutic effect. TKI drug resistance is a common and intractable problem in the clinical treatment of lung cancer, resulting in poor treatment effects and shortened survival times for patients. There are multiple mechanisms of drug resistance, which can be divided into primary resistance and acquired resistance. Currently, the identified acquired drug resistance of first-generation EGFR-TKIs is mainly caused by the mutation of EGFR-T790M and the gene amplification of c-Met, which account for ~50 and 20% of cases, respectively (128). Other possible drug resistance mechanisms include the occurrence of phenotypic EMT of tumor cells, the interaction between IGF-1R and the EGFR receptor signaling pathway, and the activation of P13K/AKT/mTOR signaling by the loss of the PTEN gene (129).

Metformin can reverse EGFR-TKI resistance by inhibiting the PI3K/AKT/mTOR signaling pathway (56). Li *et al* (130) reported that metformin combined with EGFR TKI blockers (gefitinib or erlotinib) *in vivo* and *in vitro* inhibited the IL-6/STAT3 signaling pathway, reversed EMT and overcame drug resistance in NSCLC cells. In a study of lung cancer cell lines with KRAS/LKB1 mutation, EGFR-TKIs induced apoptosis and drug resistance through the PI3K/AKT/mTOR signaling pathway. The addition of metformin and mTOR inhibitor MLN0128 induced a significant therapeutic response. The adenocarcinoma cells showed a higher therapeutic response than squamous cell carcinoma cells. Furthermore, the addition of an AKT inhibitor (MK2206) in squamous cell lung cancer cells also reversed the drug resistance of KRAS/LKB1 mutant cell lines and led to growth inhibition of lung squamous cell tumors (131).

Similarly, in combination with MEK inhibitors, metformin has shown anti-proliferative/pro-apoptotic effects and reduced EMT in LKB1 wild-type human NSCLC cell lines independent of KRAS mutation status (132). In addition, metformin can overcome IL-6-induced EGFR-TKI resistance in lung cancer cells by inhibiting STAT3 and AKT phosphorylation, and by enhancing AMPK activation (133). Pan *et al* (134) investigated whether metformin sensitized primary resistant NSCLC cells to gefitinib and found that primary resistance was more dependent on the IGF-1R pathway than acquired resistance. The IGF-1R pathway is more highly activated in primary EGFR-TKI resistant cells than in EGFR-TKI sensitive cells or those with acquired resistance. Compared with

gefitinib alone, combined metformin treatment can lead to growth inhibition, IGF-1R signaling pathway inhibition and increased apoptosis via the inhibition of AKT and the upregulation of Bcl2-like protein 11, resulting in increased sensitivity of primary drug-resistant cells to gefitinib (134). A study has shown that metformin can restore the sensitivity of drug-resistant NSCLC cells to the anaplastic lymphoma kinase (ALK) inhibitor crizotinib by inhibiting the IGF-1R pathway (135). However, a study comparing metformin alone with metformin in combination treatment with crizotinib in a xenograft mouse model of ALK-positive lung cancer found that metformin alone (100 mg/kg per day for 14 days) had a statistically significant effect on tumor growth inhibition. When combined with metformin, treatment with crizotinib (25 mg/kg) did not produce a stronger tumor-suppressive effect than crizotinib alone (136).

Furthermore, increased expression of hepatocyte growth factor (HGF) and its RTK c-Met has been observed in certain ALK-positive NSCLC tumor tissues, associated with acquired resistance to various TKIs (137,138). Alectinib, a second-generation ALK inhibitor, has become an important drug in the first-line treatment of advanced ALK-positive NSCLC. It was found that HGF level in the supernatant of ALK-positive cell lines increased over time. Neither exogenous nor endogenous HGF showed resistance to crizotinib, an ALK/MET dual-targeted small molecule inhibitor, but it was an important cause of alectinib resistance (137). GRB2-associated binding protein 1 (Gab1) is a key effector of the HGF/MET signal transduction pathway mediating alectinib resistance. Metformin combined with alectinib overcomes HGF by destroying the complex between MET and Gab1, inhibiting Gab1 phosphorylation and activating downstream signal transduction pathways, suggesting that metformin combined with alectinib may help overcome the alectinib resistance caused by the activation of the HGF/MET signaling pathway and improve the efficacy of alectinib (137).

Third-generation EGFR-TKIs, including lochitinib, have been used to treat patients with T790M mutations selectively, but resistance to third-generation EGFR-TKIs can still emerge during the treatment. Pan *et al* (139) investigated the effect of metformin on rociletinib sensitivity in drug-resistant NSCLC cells. The drug-resistant cells showed higher expression of p50/p65 heterodimer, phosphorylated (p)-AKT, IKK and IKBα, as well as higher phosphorylation levels of IKBα and IKK, compared with the parental control cells. Drug-resistant cells mediated NF-κB activation through the PI3K/AKT pathway leading to increased p-AKT level. Adding NF-κB inhibitor TPCA-1 to the rociletinib treatment decreased cell viability, increased proliferation inhibition and apoptosis, and significantly reduced p-AKT, p50/p65, p-IKK and p-IKBα levels. These results suggested that inhibition of NF-κB may sensitize the drug-resistant cells to rociletinib. A combination of metformin and rociletinib had a similar effect. Metformin inhibited NF-κB activity, resulting in increased sensitivity to rociletinib, decreased p-AKT, p-IKBα, p-IKK, p50 and p65 levels, and reduced nuclear translocation of p50/p65. Compared with treatment alone, combination therapy significantly reduced the proliferation, viability and invasion of NSCLC cells. Therefore, metformin and rociletinib synergistically inhibited the NF-κB signaling pathway and overcame EGFR-TKI resistance in T790M mutant NSCLC cells (139). These findings suggest that metformin may delay the emergence of EGFR-TKI resistance in patients with NSCLC.

Metformin and immunotherapy of lung cancer. Overexpression of PD-L1 often occurs in NSCLC, resulting in a poor prognosis for patients with lung cancer (140,141). Immunotherapy is currently approved as the standard first- and second-line treatment for advanced NSCLC and has achieved marked results in the treatment of NSCLC. Animal experiments found that LKB1 and PD-L1 expression in NSCLC tissues were significantly correlated (142). Downregulated LKB1 reduced the PD-L1 level in TC-1 cells cells, while overexpressed LKB1 increased the PD-L1 level in A549 cells, further confirming that AMPK mediates PD-L1 upregulation through LKB1. The inhibition of AMPK significantly reduced PD-L1 levels in NSCLC cells with intact LKB1. The combination of metformin and anti-programmed cell death protein 1 (PD-1) antibody effectively inhibited the growth of tumors expressing LKB1. LKB1 upregulated the expression of PD-L1 in NSCLC by activating the AMPK and KEAP1/NRF2 signaling pathways, and improved the therapeutic effect of PD-1 inhibitors on LKB1 wild-type NSCLC (142). In addition, to study the association between AMPK activation and NK cells in PD-1 therapy, metformin was used as an AMPK activator to induce AMPK activation. The results showed that metformin-induced AMPK activation combined with NK cell-mediated killing of tumor cells could significantly inhibit tumor growth in mice (143). Studies showed that NSCLC with KRAS mutation was more sensitive to PD-1/PD-L1 inhibitor therapy (144-147), and LKB1 loss was detected in one-third of KRAS-mutant NSCLC (148). However, NSCLC with this mutant subtype was more aggressive and resistant to immunotherapy (149,150). Another study showed that the efficacy of PD-1 inhibitors was reduced in patients with lung adenocarcinoma with LKB1 mutation, but not in patients with KRAS mutation (151). In addition, patients with lung adenocarcinoma without concurrent LKB1 or EGFR mutations and TP53 mutations had prolonged PFS times when treated with anti-PD-1 inhibitors, suggesting that LKB1 plays a key role in NSCLC response to PD-1/PD-L1 inhibitors (149). Afzal *et al* (152) studied the clinical efficacy of metformin in combination with immune checkpoint inhibitors (ICIs) in patients with NSCLC. A total of 50 patients with NSCLC received ICIs plus metformin or no metformin. The results revealed that the total response rate, DCR, median OS and PFS times were higher in the combined metformin group than those in the non-combined metformin group, and the same results were obtained in the subgroup analysis (second-line/third-line ICIs) (152). These results suggest that the prognosis of patients with NSCLC is better for those who receive both metformin and ICI therapy. A study reported a unique case of a patient with SCLC who received nivolumab monotherapy for 2 years until disease progression, and then metformin plus nivolumab, which resulted in a sustained partial response for >6 months. These results suggest that metformin may help overcome the acquired resistance to PD-1 inhibitors (153). However, further clinical studies are required to confirm this. At present, studies have indicated that metformin has anticancer effects, and numerous clinical studies have demonstrated that metformin significantly improves anticancer activity in patients with

Table I. Therapeutic efficacies of metformin in lung cancer.

First author, year	Number of subjects	Combination treatment	Effect of treatment/conclusions	(Refs.)
Tan *et al*, 2011	99	Chemotherapy	Improved PFS time	(17)
Marrone *et al*, 2018	25	Carboplatin, paclitaxel, bevacizumab	Improved PFS rate at 1 year	(101)
Parikh *et al*, 2019	33	Platinum-based chemotherapy	Effect of metformin on PFS and OS time is related to the mutation status of KRAS and EGFR.	(102)
Sayed *et al*, 2015	30	Gemcitabine and cisplatin	Metformin administration reduced occurrence of chemotherapy-induced nausea. Metformin had no effect on ORR, PFS time and OS time.	(103)
Wink *et al*, 2016	682	Concurrent chemoradiotherapy	Metformin use was associated with an improved distant metastasis-free survival (DMFS) rate at 2 years and PFS time.	(121)
Tsakiridis *et al*, 2021	96	Chemoradiotherapy	Metformin is not recommended in patients with locally advanced NSCLC who are candidates for. chemoradiotherapy	(155)
Arrieta *et al*, 2022	70	EGFR-TKI	Improved PFS and OS time of patients with a BMI of ≥24	(165)
Chen *et al*, 2015	90	EGFR-TKI	Improved PFS time, OS time, ORR and DCR	(123)
Arrieta *et al*, 2019	139	EGFR-TKI	Improved PFS and OS time.	(127)
Afzal *et al*, 2019	50	ICIs	Improved ORR, DCR, median OS time and PFS time	(152)
Yendamuri *et al*, 2019	434	ICIs	A tendency to improved OS in metformin users only in patients with a BMI >25 kg/m^2, and the strength of the association was higher in patients with a BMI >30 kg/m^2	(154)

OS, overall survival; PFS, progression-free survival; ORR, overall response rate; DCR, disease control rate; BMI, body mass index; NSCLC, non-small cell lung cancer; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; ICI, immune checkpoint inhibitor.

NSCLC. The therapeutic efficacies of metformin on lung cancer are summarized in Table I. Notably, most of the studies show that metformin provides better outcomes when used in addition to existing treatments, including chemotherapy, targeted therapy and immunotherapy. There were three studies investigating the efficacy of metformin combined with EGFR-TKI, and all three confirmed that EGFR-TKI combined with metformin could achieve longer PFS and OS times compared with EGFR-TKI treatment alone. Yendamuri *et al* (154) found that ICIs combined with metformin could result in better OS time. This effect was limited by body mass index. Notably, these results may provide a new strategy to strengthen the therapeutic effects of EGFR-TKI and ICIs. At present, most of the studies support the antitumor effects of metformin. However, some clinical trials show no further benefits on NSCLC. There may be several reasons for the different conclusions: i) A high degree of clinical heterogeneity among different trials, as studies were conducted in different ethnicities and different regions; ii) the doses of metformin and the combination treatments were different; and iii) some studies included individuals with diabetes, while others did not. This also affected the conclusions. Therefore, the effect of metformin on the treatment and prognosis of lung cancer remains controversial, requiring confirmation with further tests and studies. Further studies are needed to evaluate the effect of metformin on the outcome of patients with NSCLC. The ClinicalTrials.gov website indicates that a number of prospective clinical trials (Table II) are currently assessing the effects of metformin on lung cancer. Notably, the clinical trial NCT05445791 is committed to recruit 312 participants with NSCLC (stage IIIB-IV) to evaluate the PFS time in patients with NSCLC and EGFR mutations undergoing treatment with TKIs plus placebo vs. TKIs plus metformin. The clinical trial NCT01864681 also focuses on the effect of metformin on EGFR-TKI therapy. These trials may provide a new strategy for overcoming EGFR-TKI resistance. The clinical trial NCT02115464 showed that the addition of metformin to chemoradiotherapy was associated with worse treatment efficacy and increased toxic effects compared with combined modality therapy alone. Metformin was not recommended for patients with LA-NSCLC who are candidates for chemoradiotherapy (155). Despite low accrual rates, the clinical trial NCT02285855 showed that the majority of patients treated with metformin exhibited metabolic responses according to PERCIST criteria on PET imaging. In contrast to the effect of metformin on the majority of physiological tissues, most

Table II. Active clinical trials with metformin in lung cancer (www.ClinicalTrials.gov; accessed June 2022).

Clinical trial number	Trial phase	Title	Region/ institute	Metformin dose	Combination treatment	Stage	Investigation purpose	Status	Study type
NCT02115464	Phase 2	Advanced lung cancer treatment with metformin and chemoradiotherapy	Canada	2,000 mg, daily, for 12 months	Chemotherapy and radiotherapy	Stage IIIa or stage IIIb	To determine the effect of metformin on the proportion of patients free of disease progression at 12 months after initiation of drug treatment	Terminated	Interventional
NCT02285855	Phase 2	Metformin in non-small cell lung cancer (NSCLC)	United States	2,000 mg, daily, for 3 weeks	Radiotherapy	Stage I-II	To determine the effect of metformin on the response of patients with NSCLC treated with hypofractionated radiation therapy	Terminated	Interventional
NCT02019979	Phase 2	Metformin and carbohydrate restriction with platinum-based chemotherapy in stage IIIB/IV non-squamous non-small cell lung cancer (NS-NSCLC)	United States	1,000 mg, bid, for 3 weeks	Carbohydrate restricted diet, platinum based chemotherapy	Stage IIIB or IV	To determine the effect of metformin and carbohydrate restriction on the response of patients with non-squamous NSCLC treated with platinum-based chemotherapy	Terminated	Interventional
NCT04931017	Phase 2	Metformin for chemoprevention of lung cancer in overweight or obese individuals at high risk for lung cancer	United States, Canada	Unknown	-	-	To examine whether metformin extended release as a preventative treatment may lower the chance of developing lung cancer, and whether it may help the patients' immune system learn to lower a certain type of immune cell (regulatory T cells) that are linked to tumor development	Recruiting	Interventional
NCT03086733	Phase 2	Phase II lung metcore preoperative metformin for lung cancer	United States	850 mg, bid	Surgery	Stage I-IIIa	To determine the effect of metformin on Ki67 apoptosis in patients with NSCLC	Completed	Interventional

Table II. Continued.

Clinical trial number	Trial phase	Title	Region/ institute	Metformin dose	Combination treatment	Stage	Investigation purpose	Status	Study type
NCT01717482	Phase 2	Metformin as a chemoprevention agent in non-small cell lung cancer	United States	850 mg, bid	Standard of Care Observation	Stage Ia-IIIa	To investigate whether it is better to receive the drug metformin with standard of care for lung cancer or just standard of care	Terminated	Interventional
NCT01997775	Phase 2	Metformin in stage IV lung adenocarcinoma	Taiwan	500 mg, tid	Chemotherapy combining cisplatin and pemetrexed or targeted therapy	Stage IV	To determine whether metformin is effective in lowering plasma IL-6 level and improving the treatment response in patients with NSCLC	Terminated	Interventional
NCT03874000	Phase 2	Sintilimab combined with metformin in first-line chemotherapy refractory advanced NSCLC patients	China	500 mg, bid	Sintilimab	Stage IV	To determine the effect of metformin on objective response rate in patients with first-line chemotherapy refractory advanced NSCLC	Unknown	Interventional
NCT02109549	-	Influence of the use of the diabetic drug metformin on the OS and treatment-related toxicity in advanced stage non-small cell lung cancer patients	Netherlands	Unknown	Concurrent radiochemo-therapy	Advanced stage	To test whether patients suffering from non-insulin-dependent diabetes mellitus, treated with metformin, have improved local tumor control	Completed	Observational
NCT03048500	Phase 2	Nivolumab and metformin hydrochloride in treating patients with stage III-IV non-small cell lung cancer that cannot be removed by surgery	United States	Unknown	Nivolumab	Stage IV or non-resectable stage III	To assess antitumor activity of the combination treatment of metformin hydrochloride (metformin) with nivolumab in patients with NSCLC with and without prior exposure to PD-1/PD-L1 inhibitors	Active, not recruiting	Interventional
NCT03994744	Phase 2	Assessing safety and efficacy of sintilimab and metformin combination therapy in SCLC	China	1,000 mg, bid	Sintilimab	Extended disease stage of SCLC	To assess safety and efficacy of sintilimab and metformin combination therapy in SCLC	Recruiting	Interventional

Table II. Continued.

Clinical trial number	Trial phase	Title	Region/ institute	Metformin dose	Combination treatment	Stage	Investigation purpose	Status	Study type
NCT03071705	Not Applicable	Metformin plus TKI use in patients with non-small cell lung carcinoma	Instituto National de Cancerologia	500 mg, bid	EGFR-TKI	Unknown	To assess the PFS period in patients with advanced NSCLC in treatment with TKIs and metformin vs. TKI alone	Unknown	Interventional
NCT02186847	Phase 2	Chemotherapy and radiation therapy with or without metformin hydrochloride in treating patients with stage III non-small cell lung cancer	Multicenter	500 mg, bid (1-7 days); 500 mg, tid (8-14 days); 2,000 mg daily (15-126 days)	Radiation and chemotherapy	Stage III	To determine whether metformin hydrochloride added to chemoradiotherapy can improve PFS in patients with locally advanced NSCLC	Active, not recruiting	Interventional
NCT04170959	Phase 2	The addition of metformin to definitive radiotherapy in patients with stage III NSCLC (RADFORMIN)	Belgium	500 mg, daily (1-7 days); 500 mg bid (7-14 days)	Standard of care	Stage III	To identify subsets of patients who derive maximum benefit of adding metformin to radiotherapy using innovative biomarkers	Terminated	Interventional
NCT01864681	Phase 2	Combination of metformin with gefitinib to treat NSCLC	China	500 mg, bid	Gefitinib	Stage IV	To determine whether metformin in combination with gefitinib is effective in patients with previously untreated advanced or metastatic NSCLC with EGFR mutations	Completed	Interventional
NCT01578551	Phase 2	Study of metformin plus paclitaxel/ carboplatin/ bevacizumab in patients with adenocarcinoma	United States	500 mg, bid-1,000 mg, bid	Paclitaxel, carboplatin, bevacizumab	Stage IV	To determine the 1-year PFS rate of the combination of metformin and standard chemotherapy in patients with previously untreated advanced or metastatic pulmonary adenocarcinoma	Terminated	Interventional

Table II. Continued.

Clinical trial number	Trial phase	Title	Region/ institute	Metformin dose	Combination treatment	Stage	Investigation purpose	Status	Study type
NCT05445791	Phase 3	Metformin plus tyrosine kinase inhibitors for treatment of patients with non-small cell lung cancer with EGFR mutations (METLUNG)	Mexico	500 mg, bid	Tyrosine kinase inhibitors	IIIB-IV	To evaluate the PFS in patients with NSCLC with EGFR mutations under going treatment with TKIs plus placebo vs. TKIs plus metformin	Recruiting	Interventional

SCLC, small cell lung cancer; NSCLC, non-SCLC; bid, twice a day; tid, three times a day; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PFS, progression-free survival; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1.

tumors had increased metabolic activity in response to metformin (69). The clinical trial NCT01578551 showed that there was a significant benefit in terms of PFS with the use of metformin in advanced NSCLC (101). No published results are available for other completed or terminated trials.

4. Limitations and challenges of using metformin in lung cancer

Dose of metformin for lung cancer. Although several studies support the efficacy and feasibility of metformin in cancer treatment, metformin is mainly used in patients with diabetes. In clinical application, for non-diabetic patients, the feasibility of treating lung cancer with metformin alone or in combination with other anti-lung cancer treatment schemes may be limited due to its possible adverse reactions leading to intolerance in some patients (156). The most common toxicity of metformin is gastrointestinal toxicity, including mild anorexia, a metallic taste in the mouth, nausea, diarrhea and abdominal pain (157). Although the symptoms are typically mild, transient and reversible with dose reduction or withdrawal, the side effects of metformin may increase when combined with chemotherapy (especially platinum-based) and radiotherapy (158). However, a small prospective randomized phase II study that included 15 patients with stage IV NSCLC who received both metformin and chemotherapy, found that metformin combined with chemotherapy reduced gastrointestinal reactions to chemotherapy. The metformin group was found to have a lower incidence of nausea compared with the combined treatment group (26.7 vs. 66.7%; P=0.03) (103). Metformin can accumulate in the body and cause a rare but severe form of lactic acidosis. In addition, the main risks of metformin treatment are kidney damage, sepsis, dehydration, liver damage and acute congestive heart failure (157). Based on the experience of Wink *et al* (121), a conventional dose of metformin combined with concurrent chemoradiotherapy is safe and feasible. However, in this study, the dose of metformin was not reported, and only a cautious, gradual increase in the dose during the first few weeks of administration was recommended (121). The antitumor effects of metformin seem to increase with increasing dose. In addition, an important limitation of a number of experimental studies is that the metformin concentrations used in numerous experiments are greater than the conventional doses applied for diabetes treatment (72). However, these high doses are inappropriate for practical clinical use due to the potential drug toxicity. Most current retrospective studies and corresponding meta-analyses were conducted on diabetic patients, and metformin was used at a conventional treatment dose. In the *in vitro* tests on the effect of metformin on lung cancer, the concentration of metformin was significantly higher than the blood concentration of the treatment dose of diabetic patients and showed a concentration dependence (130). Therefore, whether the clinical metformin dose has such an effect on the tumor is debatable.

In addition, a number of factors affect the effectiveness and reactivity of metformin in tissues. For example, tissue expression of transporters that mediate metformin uptake differs between normal and tumor cells, and may be affected by various drugs, such as antibiotics and proton pump inhibitors (159). Malabsorption of metformin in target cells may

limit its potential to treat cancer. Overall, the metabolic state of the patient and the interactions between tumor molecules add to the complexity of the impact of metformin on the tumor (160). It remains to be further confirmed whether the anti-lung cancer effect of a conventional metformin dose can be achieved in *in vitro* trials.

Administration route of metformin for lung cancer. Metformin has been used orally in clinical studies of diabetes mellitus and lung cancer, but the study by Memmott *et al* (60) has shown that intraperitoneal injection of metformin can produce higher plasma metformin levels, resulting in tissue-specific regulation of the AMPK and mTOR pathways. Intraperitoneal injection of metformin inhibits the mTOR pathway in lung tissue by reducing the response to insulin or IGF-1, independent of AMPK. In this research, metformin administration through intraperitoneal injection was unexpectedly well tolerated throughout the study and did not significantly affect body weight in the mice (data were not shown). Metformin reduced tumor diversity by 66%, mean tumor volume by 50% and total tumor load by 72%. S6 phosphorylation in the tumor was reduced by 40%. Therefore, the intraperitoneal injection of metformin was more effective than oral injection in preventing NNK-induced lung tumorigenesis and inhibiting mTOR (60). A recent *in vitro* study evaluating metformin sterol liposomes as an inhaled treatment for lung cancer was conducted by mixing stearin and cholesterol. Metformin liposome showed a significant inhibitory effect on A549 cells ($P<0.05$), and it increased significantly with the increase of dose and exposure time. The feasibility of liposomes for aerosol delivery provides a new strategy for metformin inhalation administration, which may be an effective inhalation therapy for lung cancer (161). Therefore, the administration route of metformin in treating tumors needs to be further explored in clinical trials.

Population selection of combined metformin therapy for lung cancer. A number of clinical trials have evaluated the anticancer activity of metformin, but the results have been inconsistent. There are numerous factors influencing the trial results, such as the use of different subgroups of patients with lung cancer. Further studies that screen for metformin-sensitive subgroups of lung cancer to improve the efficacy and reduce adverse drug reactions in patients with lung cancer are awaited. A randomized phase II study reported that EGFR-TKI combined with metformin showed better PFS and OS times than EGFR-TKI alone in patients with EGFR-mutated lung adenocarcinoma (127). Some case-control studies have suggested that squamous cell carcinoma may have more metabolic characteristics than adenocarcinoma. There is a markedly elevated expression of the glucose transporter 1 (GLUT1) in lung squamous cell carcinoma, which augments glucose uptake and glycolytic flux. Elevated GLUT1-mediated glycolysis in lung squamous cell carcinoma strongly correlates with high ^{18}F-FDG uptake and poor prognosis (162). Compared with lung adenocarcinoma, lung squamous cell carcinoma has a higher level of glucose metabolism to maintain the metabolism required for rapid tumor growth. The expression of HK2 (the rate-limit enzyme and the first committed step in glucose metabolism) in lung squamous cell carcinoma was significantly higher than that in lung adenocarcinoma and normal tissues (13). A recent study showed that metformin significantly improved the prognosis of patients with squamous cell carcinoma and high FDG uptake (68). These results suggest that metformin may be more effective in patients with squamous cell lung cancer with high FDG uptake. The aforementioned research also found that within the subgroup with TP53 mutations (n=33), the metformin group exhibited better OS than the control group (HR, 0.23; 95% CI, 0.17-0.38; P=0.021). By contrast, within the subgroup with wild-type TP53 (n=80), the metformin group exhibited worse OS (HR, 1.61; 95% CI, 0.97-2.81; P=0.091). The results suggest that metformin may have a better antitumor effect on patients with lung cancer and TP53 mutation (39). However, all the aforementioned studies are small sample trials, and larger studies are required to explore and find a suitable population for lung cancer combined with metformin to improve the effective rate of lung cancer treatment and prolong the PFS and OS times of affected patients.

Recently, the effects of metformin on lung cancer have gained the attention of a number of researchers, and numerous reviews have already been previously published. Li *et al* (163) and Chen *et al* (164) reviewed the effects of metformin on lung cancer. While the association between metformin and lung cancer was complex, the effects of metformin on lung cancer remained controversial and the mechanisms were intricate. Therefore, new studies were instigated. The present review gives a more comprehensive overview of the mechanisms, in addition to the involvement of the AMPK signaling pathway and the AMPK-independent signaling pathway. In contrast to the review by Chen *et al* (164), the present review summarized other mechanisms. The mechanisms of metformin inhibiting complex I of the mitochondrial respiratory chain, regulating lung miRNAs, and affecting the tumor and its microenvironment were preliminarily reviewed. The present review described the administration route of metformin for lung cancer and the population selection of combined metformin therapy for lung cancer. This will help further research select an appropriate administration route and population. In contrast to the review by Li *et al* (163), the present review illustrated the fact that metformin can enhance the effect of immunotherapy, and more comprehensive and updated studies were included.

5. Conclusion

Numerous studies have revealed that metformin plays a direct or indirect antitumor role in regulating the lung cancer cell cycle, inhibiting cell proliferation and promoting apoptosis. Metformin alone or in combination with chemoradiotherapy, targeted drug therapy and immunotherapy has therapeutic effects on lung cancer and is expected to improve the prognosis of patients with lung cancer. However, more randomized, prospective, standardized and quantitative trial results are needed to further explore the selection of metformin, the effective treatment dose and the treatment approaches, to improve the survival time and prognosis of patients with lung cancer.

Acknowledgements

Not applicable.

Funding

This study was supported by the Wanzhou District Science and Technology Joint Medical Research Project, Chongqing (grant no. wzstc-kw2021011), the Chongqing Medical Scientific Research Project (grant no. 2021MSXM042) and the General Program of Chongqing Natural Science Foundation (grant no. cstc2020jcyj-msxmX0713).

Availability of data and materials

Not applicable.

Authors' contributions

PH, JZ, QL and KS were responsible for gathering the associated research and designing the review. PH was responsible for creating the figures. JX, QL and KS contributed to the study design, interpretation of the research articles, editing of the manuscript and critical revision of the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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