Progress in the application and mechanism of metformin in treating non-small cell lung cancer (Review)

CHAN LI*, YANG XUE*, YU-RONG XI* and KE XIE

Department of Oncology, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, Chengdu, Sichuan 610072, P.R. China

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Abstract. At present, the incidence and mortality of lung cancer demonstrate an increasing trend. Non-small cell lung cancer (NSCLC) accounts for ~80-85% of all lung cancer cases. Therefore, developing novel and more effective treatments is of great importance. The use of combination therapies, where several anticancer agents are used together, is a promising strategy. Recent studies demonstrate that metformin, which has been utilized for treating diabetes mellitus for >50 years, has antitumor effects in numerous types of cancer including NSCLC. Its antitumor effects can be direct and indirect, and it is able to synergize with other physical therapies including targeted anticancer therapy, chemotherapy and radiotherapy. The present review discusses how metformin affects cellular energy metabolism in NSCLC, the mechanism of its antitumor action and its synergy with other therapies. Information and analysis are provided in the present review to stimulate further studies on metformin as an adjunct anticancer treatment.

Contents

Correspondence to: Dr Ke Xie, Department of Oncology, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, 32 West Second Section First Ring Road, Chengdu, Sichuan 610072, P.R. China
E-mail: 840246753@qq.com

*Contributed equally

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1. Introduction

The importance of developing improved treatments for non-small cell lung cancer (NSCLC) cannot be overestimated. Lung cancer remains one of the major causes of mortality, with incidence on the increase in numerous parts of the world (1); NSCLC is the most common type, accounting for 80-85% of all lung cancer cases. Targeting the carcinoma with a combination of several drug types and physical methods, including radiotherapy and phototherapy, is proving to be an effective strategy (2).

Metformin is a biguanidine and a hypoglycemic agent. Since its introduction in Europe in 1957, metformin has been used as a drug for lowering elevated blood glucose levels in diabetes mellitus patients (3). In 2005, it was suggested that metformin could reduce the incidence of cancer, making it into the focus of tumor research (4) and potential applications for metformin in oncology were investigated. It was reported that metformin is able to perform its antitumor action by altering neoplastic cellular energy metabolism (5). Since 2013, to the best of our knowledge there have been >173 ongoing clinical trials on the use of metformin in cancer (6). Furthermore, in a recent study, Birsoy *et al* (7) showed a link between the glucose limitation activity of phenformin, another biguanide, and its effects on the metabolic determinants of cancer cell sensitivity. Due to the rapid growth of cancer cells and high consumption of nutrients, particularly glucose, it was observed that glucose concentration is lower in tumors than in normal tissues (7). However, the majority of cancer cells are able to develop and reproduce rapidly in spite of the low-glucose conditions (7). Biguanide drugs, e.g., phenformin and metformin, are inhibitors of mitochondrial oxidative phosphorylation, and this is thought to account for the observed antineoplastic activity of these diabetes drugs (7).

Since 2004, the present authors have been focusing on the antitumor effects of metformin in NSCLC. A multi-center clinical trial confirmed that metformin could improve chemotherapy survival outcomes for diabetes mellitus patients who have NSCLC (8). The present review discusses how metformin affects cellular energy metabolism in NSCLC, the mechanism

of its antitumor action and its synergy with other therapies. The goal is to investigate the feasibility of adjunct metformin for treating NSCLC and to stimulate further study.

2. Glucose metabolism in NSCLC and the effects of metformin

Glucose metabolism in normal cells includes glycolysis, aerobic oxidation and the pentose phosphate pathway. The first step of glycolysis is the enzymatic conversion of glucose to pyruvate, which is then reduced to lactate. In aerobic oxidation, pyruvate enters the tricarboxylic acid cycle, and it is eventually completely oxidized to water and CO_2 with the production of adenosine triphosphate (ATP) (Fig. 1) (9).

Compared with aerobic oxidation, glycolysis provides less energy per mole of glucose, but it operates at a faster rate. Even under aerobic conditions, cancer cells preferentially utilize glycolysis as their main energy source rather than oxidative phosphorylation and this is known as the 'Warburg effect' (10).

Under normoxic conditions, adenocarcinomas perform glycolysis, whilst squamous cell carcinomas, which are subjected to varying degrees of hypoxia, perform a high level of glycolysis even in anaerobic environments to obtain sufficient energy for survival (11). These findings prompted further studies into how this finding can be utilized in developing new treatments. In particular, the oral antidiabetic drug metformin is able to stimulate glycolysis by altering the activity of specific metabolic enzymes, including fructose-2, 6-bisphosphate (9); therefore, it may promote the switch to glycolysis in NSCLC cells as the main method of producing energy. On the surface, this may have a stimulatory effect on the growth of NSCLC cells, but in fact the switch in the energy pathway, in the presence of metformin, is associated with its primary antitumor mechanism (Fig. 1) (9). As glycolysis provides less energy per mole of glucose, a decrease in ATP generation results in an increased level of adenosine monophosphate (AMP), which leads to an increase in the ratio of intracellular AMP to ATP and an energy metabolism imbalance. This is one of the mechanisms by which metformin is able to achieve its antineoplastic activity. It is likely to involve the active 5'-AMP-activated protein kinase (AMPK) and its downstream signaling pathways (9).

3. Cellular transport of metformin in NSCLC

The antitumor effect of metformin on energy metabolism is dependent on whether metformin can be transported into the mitochondria inside NSCLC cells. It has been observed in tracking experiments that metformin is able to move across the cell plasma membrane (9,12-15) and the mitochondrial membranes (12,16,17).

Transport of metformin across the NSCLC cell membrane. At physiological pH, metformin is positively charged (12), meaning the movement of metformin across NSCLC cell membranes may be mediated by organic cation transporters (OCTs) (9). Organic cation transporter 1 (OCT1) is primarily responsible for metformin uptake in the liver (9). Solute carrier family 22 member 18 (SLC22A18) and OCT1 share certain homology (13). The *Homo sapiens* gene SLC22A18 is located on chromosome 11 at 11p15.5 (13). It has been demonstrated that microRNA-137 significantly inhibits NSCLC cell proliferation, invasion and migration, as it targets SLC22A18 (14). Provided that SLC22A18 is highly expressed in the NSCLC tissue, it may actively transport metformin into the NSCLC cells, particularly in squamous cell carcinoma and adenocarcinoma. An examination of NSCLC cells has observed that SLC22A18 is primarily expressed in the cell membranes and cytoplasm (13). Additionally, SLC22A18 is not expressed in normal lung tissues, and it is upregulated in squamous cell carcinoma and adenocarcinoma (15).

Transport of metformin across the mitochondrial membrane of NSCLC cells. Mitochondria have both an outer and an inner membrane. Compared with the inner membrane, the permeability of the outer membrane is relatively high (16). Molecules with a molecular mass ≤5,000 kDa or less can freely travel through the outer membrane of mitochondria, therefore the environment of the intermembrane space and cytoplasm is similar (16). As metformin is a simple molecule with a molecular mass of only 129 kDa, it is able to pass through the outer membrane and enter the intermembrane space freely (16). At physiological pH, metformin exists as a cation (>99.9%) (12). The mitochondrial membrane potential allows the metformin cation to accumulate in the matrix of the mitochondria (17).

4. Antitumor mechanism of metformin and its application in NSCLC

The mitochondrial electron transport chain is composed of complexes I, II, III and IV, all of which have the ability to transfer electrons (18). The protons along the concentration gradient move back across the inner membrane through the enzyme ATP synthase, with the aid of the mitochondrial membrane potential (18). The flow of protons back into the matrix of the mitochondrion via ATP synthase provides sufficient energy for adenosine diphosphate to combine with inorganic phosphate to form ATP (18).

Metformin is thought to target complex I, enabling its antidiabetic and antitumor activity (9). Metformin limits respiration and citric acid cycle activity in the mitochondria and alters cellular bioenergetics (19). A reduction in ATP generation results in increased levels of AMP, which in turn leads to an increase in the ratio of intracellular AMP to ATP, as well as an energy metabolism imbalance (9). This is likely to drive two major signaling pathways: The inhibition of glucagon-induced cyclic AMP synthesis in the liver, and the activation of 5'-AMPK and downstream signaling pathways (9). The antitumour activity of metformin may be direct or indirect (systemic), and its direct effects involve the AMPK signaling pathway (Fig. 2) (9).

The systemic influence is secondary and results in several consequences, including a decrease in body weight, anti-inflammatory actions, improvements in insulin-resistance, and a reduction in systemic levels of glucose and insulin. The direct effects include AMPK-dependent and AMPK-independent signaling pathways (9).

Indirect antitumor effects of metformin in NSCLC: Decreasing body weight. The primary mode of action of metformin in causing body weight loss is via decreasing appetite (20). The

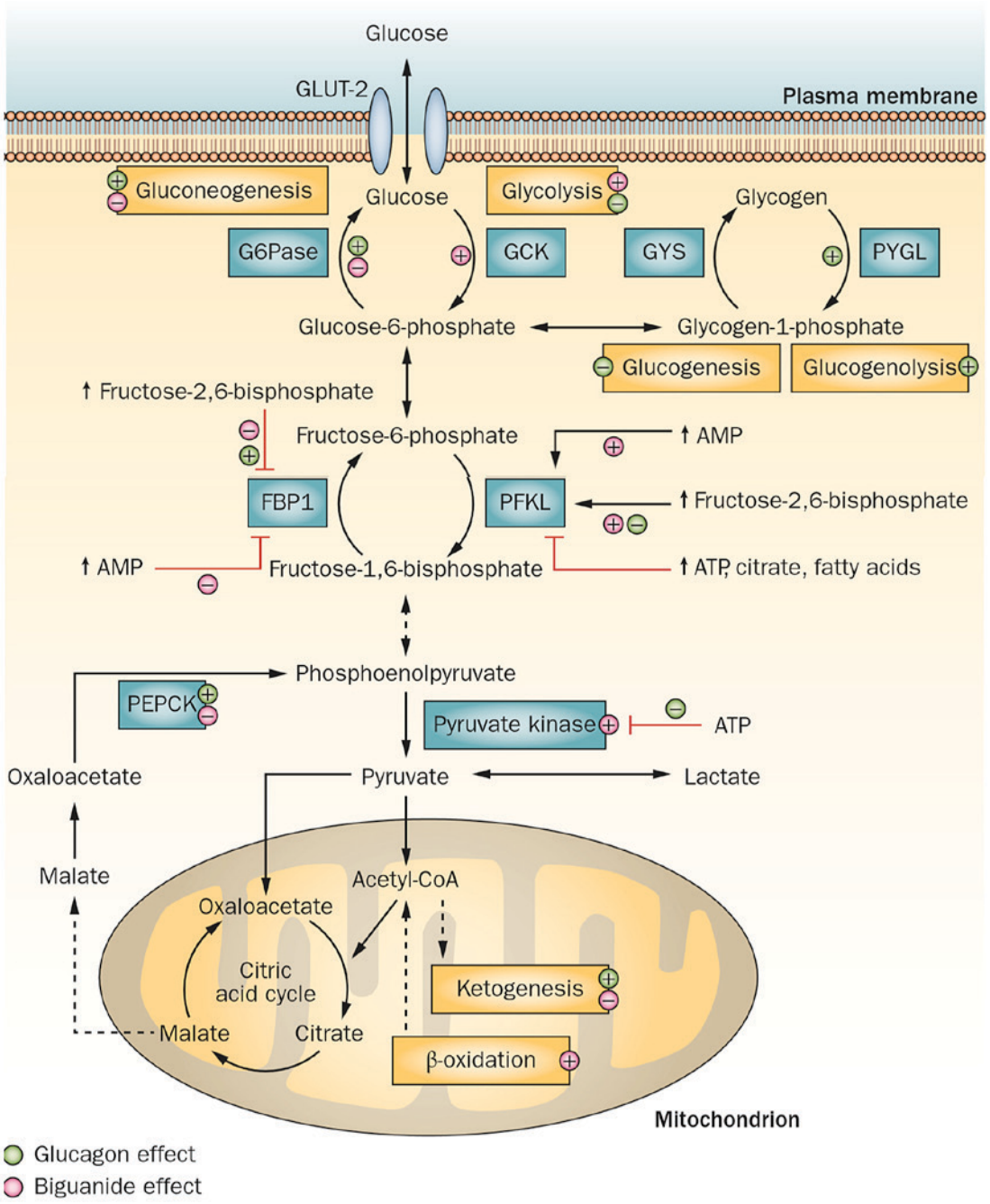

Figure 1. Glucose metabolism (glycolysis and aerobic oxidation) and the hypoglycemic mechanism of biguanides. AMP, adenosine monophosphate; ATP, adenosine triphosphate; FBP1, fructose-bisphosphatase 1; GLUT-2, solute carrier family 2 member 2; G6Pase, glucose-6-phosphatase; GCK, glucokinase; GYS, glycogen synthase 1; PYGL, phosphorylase, glycogen, liver; PFKL, phosphofructokinase, Liver Type; PEPCK, phosphoenolpyruvate carboxykinase 2, mitochondrial. Reprinted by permission from Macmillan Publishers Ltd.: Nature Reviews Endocrinology (9), copyright (2014).

secondary mechanisms include improvements in gastrointestinal physiology and circadian rhythms, and regulation of fat oxidation and storage of fat in liver, skeletal muscle or adipose tissue (20). Dahlberg *et al* (21) evaluated the association between body-mass index and clinical outcomes for 2,585 NSCLC patients. It was reported that obese patients had improved outcomes earlier in the study compared to normal or overweight patients, but subsequently obesity was demonstrated to increase risk (21). Therefore, for obese NSCLC patients, reducing weight can improve prognosis and metformin may aid in accomplishing this.

Indirect antitumor effects of metformin in NSCLC: Inflammation, tumor progression and immunity. There is increasing evidence that certain tumor-associated inflammatory markers (C-reactive protein, Toll-like receptors 2 and 4, and tumor necrosis factor-α) are associated with poorer prognosis of certain types of cancer, including NSCLC (22-24). Antitumor immune responses involve CD8+ T lymphocytes and mature dendritic cells (mDCs) (22-28). Alifano *et al* (25) reported on the role of systemic inflammation, nutritional status and tumor immune microenvironment in determining the outcome

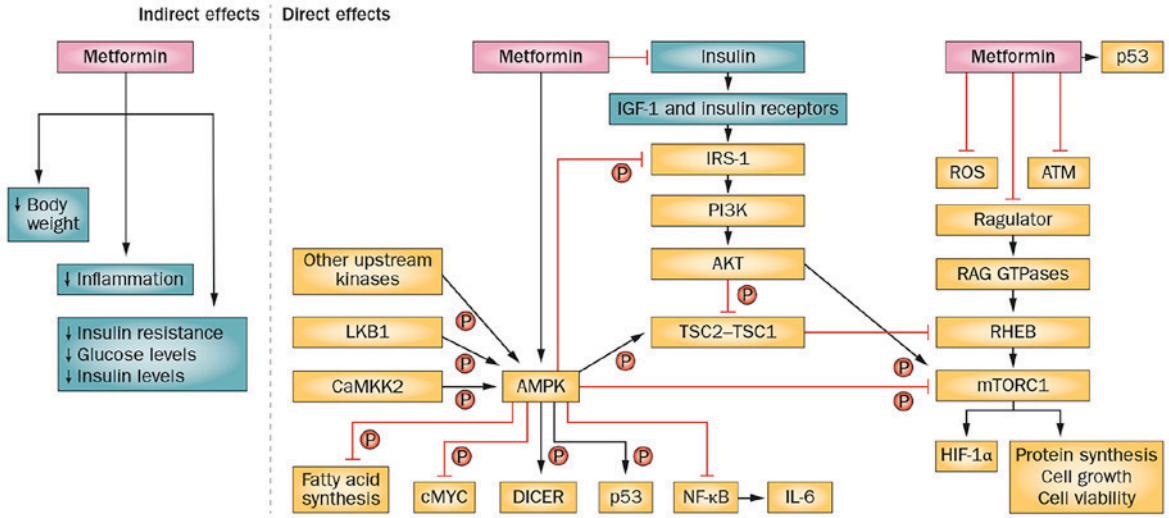

Figure 2. Antitumor mechanisms of metformin. AMPK, adenosine monophosphate-activated protein kinase; ATM, ataxia-telangiectasia mutated; CaMKK2, calcium/calmodulin dependent protein kinase kinase 2; DICER, dicer 1, ribonuclease III; HIF-1α, hypoxia inducible factor 1 alpha subunit; IGF-1, insulin like growth factor 1; IL-6, interleukin-6; IRS-1, insulin receptor substrate 1; LKB1, serine/threonine kinase 11; mTORC1, mechanistic target of rapamycin complex1; NF-κB, nuclear factor-κB; PI3K, phosphoinositide 3-kinase; RHEB, ras homolog enriched in brain; ROS, reactive oxygen species; TSC2-TSC1, tuberous sclerosis 1-tuberous sclerosis 2. Reprinted by permission from Macmillan Publishers Ltd.: Nature Reviews Endocrinology (9), copyright (2014).

of resection in NSCLC patients. It was suggested that the tumoral immune microenvironment is associated with long-term outcome in primary and metastatic tumors in resected NSCLC patients (25).

In NSCLC, a high intratumoral concentration of mDCs and low intratumoral numbers of CD8+ T lymphocytes are associated with an improved prognosis (25). The intratumoral concentration of mDCs is inversely correlated with age. It is lower in males, smokers, and patients with squamous cell carcinoma or chronic obstructive pulmonary disease (25). Therefore, inhibiting inflammation may improve the prognosis of patients with NSCLC. As well as glycemic control, metformin reduces a number of inflammatory markers (C-reactive protein, tumor necrosis factor-α, Toll-like receptors 2 and 4) and causes oxidative stress in obese type 2 diabetic patients (24). Furthermore, metformin is able to reduce the production of tumor necrosis factor-α by inhibiting the extracellular signal-regulated kinase-1/2-early-growth response-1 (ERK1/2-Egr-1) signaling pathway in human monocytes (26). Consequently, metformin is able to improve the prognosis of NSCLC.

Indirect antitumor effects of metformin in NSCLC: Improvements in insulin-resistance, and reductions in insulin and glucose levels. An examination of Kirsten rat sarcoma viral oncogene homolog (K-RAS)-induced effects revealed that hyperglycemia is able to promote the expansion of tumor-initiating lung bronchoalveolar stem cells (BASCs) in bronchoalveolar duct junctions (29). The active *K-RAS* oncogene is able to increase the expression of glucose transporter 1, thereby promoting glucose uptake and glycolysis in BASCs (29). Hyperglycemia is also able to increase autonomous hyperplasia of BASCs, which leads to oxidative stress and the production of reactive oxygen species (ROS), as well as a reduction in mitochondrial function and inhibition of oxidative phosphorylation (29). Therefore, lung cancer patients may benefit from metformin treatment via the reduction of blood glucose levels.

The ability of metformin to reduce circulating glucose levels may be explained by multiple mechanisms including: i) Reduction in glucose output by inhibiting gluconeogenesis in the liver (9); ii) increase in insulin-mediated glucose uptake in the skeletal muscle by elevating circulating glucagon-like peptide-1 (GLP-1) (30); and iii) increase in expression of GLP-1 receptors in the pancreas (31,32). Metformin indirectly inhibits dipeptidyl peptidase-4 activity, which reduces the breakdown of GLP-1, thereby increasing levels of circulating GLP-1 (9). Metformin has limited effects on glucose absorption in the digestive tract, and marginally delays the absorption process (9). Metformin also improves insulin resistance by reducing circulating insulin levels (9).

Direct antitumor effects of metformin in NSCLC. Metformin is able to inhibit the mammalian target of rapamycin complex 1 (mTORC1) through either AMP kinase-dependent or independent signaling pathways to achieve antitumor effects (Fig. 2) (9). Metformin may also suppress the phosphorylation of cytokines, including translation initiation factor 4E-binding protein 1 and S6 kinase-1, by inhibiting mTOR. Metformin is able to decrease protein synthesis, tumor cell proliferation and survival (33). Solid tumors often exhibit a hypoxic microenvironment state (34). Hypoxia-inducible factor-1 (HIF-1) is a nuclear transcription factor produced by cells under hypoxic conditions. HIF-1α, a subunit of HIF-1, is responsible for HIF-1 activity and is highly expressed in NSCLC cells. HIF-1α serves an important role in NSCLC tumorigenesis, local invasion and distant metastasis, by upregulating the expression of the nuclear proliferation protein antigen Ki-67 and vascular endothelial growth factor (VEGF) (34). By inhibiting mTORC1, metformin inhibits HIF-1α and is able to reduce local invasiveness and metastasis in NSCLC (33).

Metformin improves the prognosis of NSCLC via the AMPK signaling pathway. Metformin may cause phosphorylation of the tumor suppressor gene tuberous sclerosis 2 by phosphorylating AMPK, which in turn results in the inhibition of the mTORC-1 activator, GTPase and Ras homolog enriched in brain. The liver kinase B-1 (LKB1) gene in *Homo sapiens* encodes a tumor suppressor that phosphorylates the AMPK α subunit at Thr-172 to activate AMPK (33). Activated AMPK inhibits tumorigenesis by inhibiting mTOR (Fig. 2) (9). The phosphorylation of AMPK may also result in direct phosphorylation and inhibition of the positive regulatory-associated protein of mTOR (Fig. 3) (33). Ultimately, metformin may achieve its primary antitumor effect by inhibiting mTORC1. The incidences of LKB1 mutations in adenocarcinomas and squamous cell carcinomas are reported to be 13 and 5%, respectively (35). With certain LKB1 gene mutations, the LKB1-AMPK axis remains mostly functional and can be stimulated by metformin in NSCLC cells (33). Metformin could serve an effective role in the treatment of NSCLC via the LKB1-AMPK-mTOR signaling pathway (33,35-37). NSCLC patients with a high level of phosphorylated (p)-AMPK have higher overall survival (OS) and recurrence free survival (RFS) rates, particularly in adenocarcinoma. However, in squamous cell lung cancer, the level of pAMPK does not affect OS and RFS. Taken together, these data support the conclusion that NSCLC patients may benefit from metformin adjunct therapy through its inhibition of mTOR by activating the LKB1/AMPK signaling pathway (38).

Metformin improves NSCLC prognosis via an AMPK-independent signaling pathway. Metformin was reported to target liver tumor-initiating cells through the phosphoinositide 3-kinase (PI3K)/AKT/mTOR survival pathway both *in vivo* and *in vitro* (39). The PI3K/AKT/mTOR signaling pathway was aberrantly activated in squamous cell lung carcinoma (40), and the aberrant activation of this signaling pathway is more common in squamous cell lung carcinoma than in adenocarcinoma (41). In addition, in patients with adenocarcinoma and epidermal growth factor receptor (EGFR)-activating mutations, the aberrant activation of the PI3K/AKT/mTOR signaling pathway is one of the mechanisms of acquired resistance to EGFR-tyrosine kinase inhibitors (TKIs) (41). A further study demonstrated that activation of the PI3K/AKT/mTOR signaling pathway in NSCLC leads to a more aggressive form of the disease and a poorer prognosis (42). Inhibition of the PI3K/AKT/mTOR signaling pathway may overcome radioresistance, chemoresistance and immune evasion in NSCLC (42). Metformin can block the insulin-like growth factor-1-insulin signaling pathway via phosphorylation of insulin receptor substrate 1 (IRS-1), which inhibits the IRS-1/PI3K/AKT signaling pathway to prevent mTOR activation (Fig. 2) (9). Therefore, metformin may provide benefit to NSCLC patients through its inhibition of the PI3K/AKT/mTOR signaling pathway (9).

5. Use of metformin in the treatment of NSCLC

Metformin and targeted therapy. The development of targeted therapies for lung cancer has been centered on pharmaceutical interventions to block the EGFR-TKI axis. A number of EGFR-TKI inhibitors are currently in use, including gefitinib (Iressa) and erlotinib (Tarceva), which are able to block EGFR-mediated proliferation and anti-apoptosis signaling pathways. However, following ~10 months of treatment, these agents may lose their effectiveness due to drug resistance (43). The primary resistance of TKI is associated with *K-RAS* mutations. The causes of secondary resistance of TKI include: Second-site mutation of the EGFR kinase domain (T790M), other kinase amplifications (such as MET), NSCLC conversion into small cell lung cancer and epithelial-mesenchymal transition (EMT).

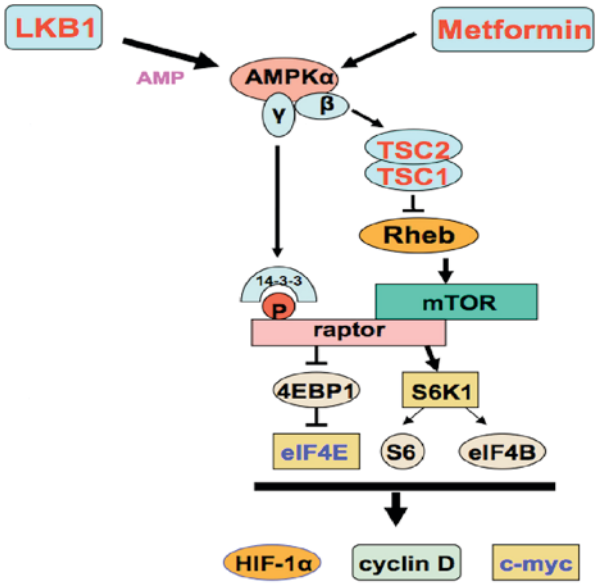

Figure 3. AMPK and its downstream signaling pathways. AMP, adenosine monophosphate; AMPK, adenosine monophosphate-activated protein kinase; eIF4E, E74 like ETS transcription factor 4; RAPTOR, regulatory associated protein of MTOR complex 1; RHEB, Ras homolog enriched in brain; S6K1, ribosomal protein S6 kinase B1; TSC1, tuberous sclerosis 1; TSC2, tuberous sclerosis 2; 4EBP1, eukaryotic translation initiation factor 4E binding protein 1. Reproduced with permission (33).

As metformin is able to inhibit the PI3K/AKT/mTOR signaling pathway, using metformin in combination with an EGFR-TKI blocker could produce a synergistic, antiproliferative effect on the tumor. As mentioned previously, the aberrant activation of the PI3K/AKT/mTOR signaling pathway is one of the mechanisms by which patients with adenocarcinoma and EGFR-activating mutations are able to gain resistance to EGFR-TKI blockers. Metformin adjunct therapy could reverse EGFR-TKI resistance by inhibiting the PI3K/AKT/mTOR signaling pathway (41). Li *et al* (43) reported that when metformin was combined with an EGFR-TKI blocker (gefitinib or erlotinib) *in vivo* and *in vitro*, the interleukin-6/signal transducer and activator of transcription 3 (IL-6/STAT3) signaling pathway was inhibited, which reversed EMT and eventually overcame resistance in NSCLC cells. In 2013, one clinical trial involving combined metformin and gefitinib to treat NSCLC patients resulted in one-year progression-free survival (6).

Sorafenib is a novel, multi-target anticancer drug that inhibits a number of kinases, including AMPK (44). Sorafenib has dual antitumor effects. When it is used in combination with metformin to treat NSCLC, the antitumor AMPK

signaling pathway is activated through either calcium/calmodulin-dependent-kinase-kinase-2 or LKB1. Sorafenib can also cause cytosolic calcium mobilization and mitochondrial calcium overload. Therefore, it can promote mitochondrial ROS production and lead to tumor cell death (44). Notably, the average tumor volume and growth rate were lower in patients treated with the combination therapy vs. patients treated with sorafenib alone (44).

Metformin and radiotherapy. Radiotherapy is a method of destroying tumors via the biological effects of ionizing radiation (45). The ataxia-telangiectasia mutated (ATM) gene product is part of a signal transduction cascade that is important for repairing damaged DNA and, as a consequence when mutated may lead to sensitivity to ionizing radiation. Therefore, the degree of radiosensitivity or radioresistance of NSCLC cells is dependent on ATM. Lung cancer radiotherapy can achieve antitumor effects by causing G2-M arrest and cytotoxicity, by activating the ATM-AMPK-tumor protein p53 (p53)/cyclin dependent kinase inhibitor 1A ($p21^{cip1}$) signaling pathway, reducing the phosphorylation of AKT and inhibiting AKT-mTOR-eukaryotic translation initiation factor 4e binding protein 1 (4EBP1) (46). Resistance to radiation is associated with the AMPK and AKT-mTOR signaling pathways. Metformin is able to activate AMPK and inhibit AKT-mTOR to sensitize lung tumors to ionizing radiation (9).

Storozhuk *et al* (47) tested the hypothesis that metformin can inhibit growth and enhance radiosensitivity of NSCLC through ATM and AMPK. It was reported that combined treatment with metformin and radiotherapy consistently activated the ATM-AMPK-p53/$p21^{cip1}$ signaling pathway and inhibited the AKT-mTOR-4EBP1 signaling pathway (47). A constant concentration of 7.8 μM metformin was achieved in patients who took a daily dosage of 850-1,700 mg. As a daily dosage of 2.5-3.0 g of metformin has no notable toxicity, a recommended dosage of 850-1,700 mg metformin is well within the safe range (47). Since 2013, to the best of our knowledge there have been three studies involving the use of metformin in combination with radiotherapy to treat NSCLC (48-50). These trials led to progression-free survival according to Response Evaluation Criteria in Solid Tumors (51).

Metformin and chemotherapy. Cisplatin is the most commonly used chemotherapy drug for the treatment of NSCLC, and it has been used as the first-line treatment for NSCLC in patients without EGFR mutations (52). Chemoresistance to cisplatin is associated with ROS production, IL-6 secretion and STAT3 phosphorylation. In NSCLC, STAT3 is active and may facilitate tumor proliferation, survival and angiogenesis by overexpressing anti-apoptotic proteins (Bcl-2-like protein 1 and myeloid cell leukemia 1), cell cycle regulators (cyclin D1 and c-Myc), and VEGF (52). Cisplatin is able to promote the generation of ROS and the phosphorylation of STAT3 (52).

Metformin is able to inhibit cisplatin-induced ROS generation, STAT3 phosphorylation and autocrine IL-6 secretion, thereby enhancing the chemosensitivity of NSCLC to cisplatin (52). Furthermore, metformin is able to improve the effect of cisplatin in A549/CDDP cells (53). Metformin also has a synergistic effect with cisplatin or etoposide in large cell lung carcinoma cells (NCI-H460) by increasing the antitumor effectiveness of the chemotherapeutics (54). There are several clinical trials underway to examine the effectiveness of the combination of cisplatin or carboplatin with metformin. Using a combination of cisplatin or carboplatin with metformin may be a promising treatment for NSCLC patients (49,50,55,56).

6. Safety of metformin as an antitumor adjuvant

The safety of metformin has been previously investigated for the treatment of type 2 diabetes mellitus. A number of major adverse effects have been reported concerning gastrointestinal reactions, including nausea, abdominal discomfort and diarrhea. These symptoms are usually mild and can be treated (57). At present, large-scale clinical trials and meta-analyses have yet to show evidence that metformin increases the risk of lactic acidosis (57). Metformin has no reported renal toxicity, but it is excreted by the kidneys (57). This means metformin may accumulate in patients with renal impairments, thus increasing the risk of lactic acidosis. However, metformin-induced liver toxicity is rare (57). Considering that metformin has been in use for >50 years and its side-effects (including low incidence of hypoglycemia and transient gastrointestinal reactions) have been thoroughly investigated and that the benefits outweigh the side effects, the conclusion is that it is safe for use in clinical trials for the treatment of cancer (57).

7. Future prospects

NSCLC is the most commonly occurring solid tumor worldwide (1). Although progress in improving chemotherapy, radiotherapy and targeted therapies has increased patient survival, the five-year overall survival time remains low (1). Cisplatin one of the most commonly used first-line chemotherapeutic agents for NSCLC (52), has several toxic side effects. The aim is to find novel adjuvant drugs, in order to enhance the antitumor effect of cisplatin, but without increasing the dose and toxicity. There is compelling evidence from a number of preclinical studies to suggest that the antidiabetic drug metformin is a prime candidate as an adjunct to chemotherapy and radiotherapy in the treatment of NSCLC (45-47,52-54,58). It has been demonstrated that metformin is able to improve survival time among diabetes mellitus patients with stage IV NSCLC (59). Furthermore, numerous NSCLC patients in advanced stages are too weak to undergo chemotherapy, radiotherapy or surgery, thus reducing their chance of survival. Combining metformin with such treatments, may be a promising strategy to improve patient survival.

Squamous cell lung carcinoma is insensitive to chemotherapy, radiotherapy and targeted therapy (40). For the majority of patients, it is too late to undergo surgical resection by the time they are diagnosed. The US Food and Drug Administration has approved nivolumab and ramucirumab for the treatment of NSCLC, but improvements in overall survival and recurrence rates have yet to be reported. As described previously, the PI3K/AKT/mTOR signaling pathway is aberrantly activated in squamous cell lung carcinoma (40), which has been correlated with poor prognosis for these patients (42). As metformin is able to inhibit the PI3K/AKT/mTOR signaling pathway (42), it may improve survival outcomes when used

in combination with everolimus or gemcitabine and cisplatin chemotherapy.

At present, to the best of our knowledge there are nine clinical trials involving the use of metformin in treating NSCLC. The potential for the long-term use of metformin in improving survival and recurrence is yet to be evaluated.

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