Advances in metformin-based metabolic therapy for non-small cell lung cancer (Review)

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Abstract. Therapeutic approaches that target the metabolism of tumor cells have been a popular research topic in recent years. Previous studies have demonstrated that glycolysis inhibitors reduce the proliferation of non-small cell lung cancer (NSCLC) cells by interfering with the aerobic glycolytic pathway. However, the mitochondrial oxidative phosphorylation (OXPHOS) pathway in tumor cells has also been implicated in lung cancer metabolism. Metformin, a known inhibitor of mitochondrial OXPHOS, has been indicated to reduce NSCLC morbidity and mortality in clinical studies. The present article reviewed the therapeutic effects of metformin against NSCLC, both as a single agent and combined with other anticancer treatments, in order to provide a theoretical basis for its clinical use in adjuvant therapy for NSCLC.

Contents

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1. Introduction

Lung cancer is the most common cause of cancer-related death worldwide (1). According to the latest statistical report from the American Cancer Center from 2021, lung cancer has the second-highest incidence and the highest mortality rate among all malignancies (2). There are primarily two types of lung cancer: Non-small cell lung cancer (NSCLC) and SCLC, and the former accounts for ~85% of all lung cancer cases (3). Although valuable progress has been made in the treatment of NSCLC in previous years, the high metastasis rate, post-operative recurrence rate and resistance to chemotherapeutic drugs in lung cancer have led to unsatisfactory outcomes (4,5). The rates of successful treatment and survival remain low and the 5-year survival rate is 21% (2), which may be attributed to the fact that NSCLC is usually diagnosed at an advanced stage, with no surgical options (6,7). Therefore, it is particularly important to explore new treatments and develop novel drugs.

Certain metabolic alterations, also referred to as metabolic reprogramming, are commonly observed in tumor cells and are proposed to be hallmarks of cancer (8). Given the vast differences in metabolism between healthy and tumor cells, there is hope that selective targeting of tumor metabolism may be achieved while limiting toxicity to healthy tissue. The most striking and characteristic metabolic alteration in cancer cells is anomalous glucose metabolism and cancer cells tend to utilize glycolysis to obtain energy even under aerobic conditions via a process called 'aerobic glycolysis' (9). The implications of this finding overshadowed the importance of mitochondria for tumor growth for a long time. However, in recent years, there has been increasing evidence that metformin exerts its anticancer effects through the inhibition of oxidative phosphorylation (OXPHOS) of tumor cell mitochondria, and metabolic pathways based on metformin targeting have only recently become the focus of intensive research. In order to establish a systematic literature review, the online search engine PubMed was used for the present study. Studies published within the last 10 years were retrieved using the key terms 'Metformin' and 'Lung Cancer'. In the present review, NSCLC metabolism was discussed with a focus on the potential of metformin-based targeting of NSCLC metabolism and the associated mechanisms, and the available preclinical and clinical evidence was assessed.

2. Aerobic glycolytic pathways and targeted therapy in NSCLC

Glucose is the most abundant and important energy source in organisms and it is metabolized in cells via two major pathways (Fig. 1): Glycolysis, which takes place under anaerobic conditions, and complete oxidation, which occurs under aerobic conditions (10). In the 1920s, Otto Heinrich Warburg discovered that cancer cells, unlike normal cells, use the glycolytic pathway to obtain energy for growth even in the presence of oxygen, which is a phenomenon known as 'aerobic glycolysis' or the Warburg effect (9), and aerobic glycolysis is a common metabolic phenotype in NSCLC (11). In positron emission tomography (PET)/CT, the high rate of glycolysis in NSCLC is reflected by the high uptake of 18F-fluorodeoxyglucose at the corresponding tumor sites (12). It has been reported that lung cancer cells exhibit upregulated expression of all key glycolytic enzymes [hexokinase 2 (HK2), phosphofructokinase and pyruvate kinase (PK)] (13), suggesting that the essential enzymes of the aerobic glycolytic pathway have a critical role in the development of lung carcinoma. Therefore, various drugs that interfere with glycolytic glucose transport proteins and key enzymes are being studied for their potential as anticancer agents (14).

Glucose transporter 1 (GLUT1), which drives the intracellular transport of glucose, is the first rate-limiting factor in glycolysis (15). Lung cancer cells have a high rate of glycolysis and high GLUT1 expression (16), and research focusing on GLUT1 may be important for lung cancer treatment. WZB117 (WZB) is a synthetic small molecule that inhibits glucose transport by downregulating GLUT1 expression (17). A study suggested that WZB may enhance toxic effects on the NSCLC cell line H460 by limiting glycolysis (18). In a nude mouse tumor transplantation model of lung cancer, WZB was indicated to inhibit tumor growth by inhibiting GLUT1 and limiting the glycolytic flow (19,20). 2-Deoxy-D-glucose (2-DG), another glycolysis inhibitor, restricts tumor growth by binding to HK and preventing glucose from accessing the enzyme (5,18). In recent years, research on microRNAs (miRNAs/miRs) has expanded and their association with NSCLC has been explored. For instance, Jia *et al* (21) demonstrated that miR-206 levels were reduced in NSCLC cells and tissues and overexpression of miR-206 was able to inhibit glycolysis and cell proliferation by targeting the 3'-untranslated region of HK2 and downregulating HK2 expression. PKM2 is essential for tumorigenesis and An *et al* (22) demonstrated that small ubiquitin-related modifier 1 (SUMO1) overexpression increased glycolysis and promoted the growth of A549 cells *in vitro* by modifying PKM2 at Lys-336. Knockdown of SUMO1 in A549 cells resulted in a marked decrease in the protein expression of PKM2, suggesting that SUMO1-modified PKM2 may be a potential therapeutic target for NSCLC.

The study of aerobic glycolytic pathways and the functions of key enzymes in tumor cells is vital for the treatment of NSCLC. Since the Warburg effect was described, there has been an increase in research focusing on aerobic glycolysis (14) and there have been several attempts to limit the growth of lung cancer by cutting off its energy supply. Although a reversal of the Warburg effect may be a broad anticancer strategy, therapeutic approaches to limit aerobic glycolysis in NSCLC have been only partially successful (23). The expression of PKM2 was previously indicated to be required for aerobic glycolysis and it was proposed that PKM2 provides a growth advantage to tumors. However, Israelsen *et al* (24) excised PKM exon 10 to terminate PKM2 protein synthesis while still allowing the splicing and protein expression of PKM1, which demonstrated that the loss of PKM2 accelerated tumor formation in a nude mouse xenograft tumor model. Similarly, Cortés-Cros *et al* (25) knocked down PKM2/M1 in established tumors and observed no significant difference in the growth of A549 lung cancer xenografts *in vivo*. These studies suggest the presence of other alternative metabolic pathways.

Mitochondria are the main sites of ATP release during oxidative phosphorylation and the original hypothesis of the Warburg effect was that cancer cells have a defective mitochondrial function, resulting in impaired aerobic respiration, necessitating the reliance on glycolysis for ATP supply (26). However, later studies have indicated that mitochondrial function is not impaired in most cancer cells and that mitochondria have an important role in cancer metabolism (27-29). In addition, although the ratio of glycolysis to OXPHOS increases, in absolute terms, both glycolysis and oxidative phosphorylation are more active in cancer cells than in normal cells and the two processes coexist (30). Given that mitochondria are essential for tumorigenesis and cancer cell proliferation (31-33), targeting the mitochondrial OXPHOS metabolic pathway may be a viable approach for inhibiting the growth of cancer cells (34).

3. Mechanisms underlying the effects of metformin in lung cancer treatment

Metformin has been the safest and most widely prescribed drug for type 2 diabetes (T2D) (35). It downregulates cytosolic OXPHOS by inhibiting mitochondrial electron transport chain complex I (ETC I), thereby hampering the oxidative phosphorylation required for tumor cell growth (36-38). Initial interest in the use of metformin for preventing and treating lung cancer arose from a number of clinical studies suggesting that metformin reduces the risk of lung cancer in individuals with diabetes (39-41).

Studies have gradually revealed the mechanism of action of metformin in the treatment of cancer (Fig. 1). Metformin has indirect (insulin-dependent) and direct (insulin-independent) anticancer effects (42). The indirect anticancer effect of metformin results from the attenuation of the stimulatory effect of hyperinsulinemia on lung cancer growth via an increase in insulin sensitivity and decrease in circulating insulin levels (43). By contrast, the direct effect of metformin is caused by the activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK). Metformin indirectly activates AMPK by disrupting mitochondrial ETC I, leading to reduced ATP synthesis and an increased cellular AMP/ATP ratio (44). It is generally speculated that the AMPK activation-mediated anticancer activity of metformin may be dependent on liver kinase B1 (LKB1). Metformin exerts its antitumor effects mainly through the AMPK/LKB1/mammalian target of rapamycin (mTOR) complex 1 (mTORC1) signaling pathway, causing apoptosis of cancer cells (45-48). LKB1 is a classical tumor suppressor (49) and mutations in

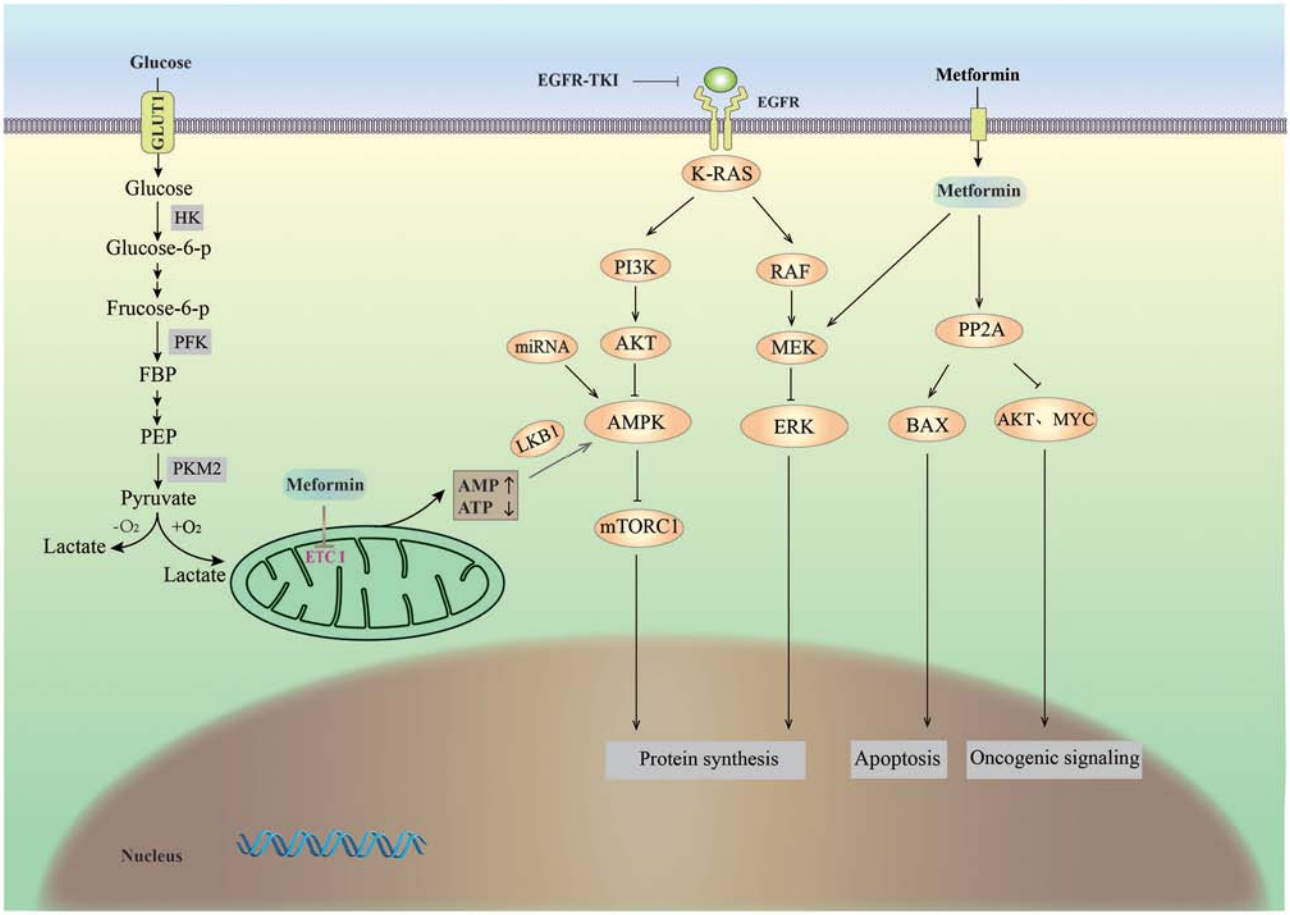

Figure 1. Glucose metabolism and possible molecular actions of metformin in non-small cell lung cancer. GLUT1, glucose transporter 1; HK, hexokinase; PFK, phosphofructokinase; PKM2, pyruvate kinase M2; ETC I, electron transport chain complex I; K-RAS, Kirsten rat sarcoma viral oncogene homolog; PI3K, phosphoinositide 3-kinase; miRNA, microRNA; LKB1, liver kinase B1; AMPK, adenosine monophosphate-activated protein kinase; mTORC1, mechanistic target of rapamycin complex 1; RAF, Raf oncogene; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; PP2A, protein phosphatase 2A; BAX, B-cell lymphoma 2-associated X protein; MYC, myelocytomatosis oncogene; ↑, promoting effect; T, inhibiting effect.

this gene are associated with Peutz-Jeghers cancer susceptibility syndrome (50,51). Genetic mutations in LKB1 are also observed in certain sporadic cancers, particularly squamous cell carcinoma and lung adenocarcinoma cells (52,53). AMPK is the direct substrate of LKB1. Metformin interferes with cellular energy metabolism by disrupting ETC I; the low energy states induce LKB1-mediated AMPK activation and indirectly inhibit mTORC1 to regulate cell growth (54-56). In addition, metformin may also have anticancer activity via AMPK activation independent of LKB1 (57-59). Guo *et al* (57) evaluated the effects of metformin on human NSCLC H1299 (LKB1-positive) and H460 (LKB1-deficient) cells. They indicated that metformin inhibits NSCLC proliferation in a time- and dose-dependent manner, induces cell cycle arrest in G0/G1 phase and increases apoptosis independent of LKB1 protein levels. They also observed that knockdown of LKB1 using short hairpin RNA does not affect the anti-proliferative effect of metformin on H1299 cells.

mTORC1, a serine/threonine protein kinase belonging to the PI3K-related kinase family, acts as a regulator of cell growth and metabolism (60). Activated AMPK inhibits tumor growth by inhibiting mTORC1, which blocks protein synthesis and proliferation in cancer cells (61,62). As mTORC1 is frequently mutated in cancers and functions downstream of several oncogenic pathways, various tumors, including lung cancer tumors, exhibit elevated mTORC1 activity (63). The RAS/PI3K/AKT/mTOR signaling pathway is an important cellular signaling cascade in which RAS activates PI3K and AKT and indirectly regulates mTORC1 (9,64,65). Among the three RAS genes (H-, K- and N-RAS), the highest mutation frequency was observed for the K-RAS gene in lung cancer (6.5% frequency for squamous cell carcinoma and 26% for adenocarcinoma in Western populations) (66). Metformin induces apoptosis via the downregulation of the downstream targets of K-RAS in human A549 lung adenocarcinoma cells with K-RAS mutations (67). It has also been reported that metformin inhibits mTORC1 signaling in an AMPK-independent manner and that this inhibition of mTORC1 activation and signaling may be Rag GTPase-dependent (68).

The anticancer effects of metformin are also speculated to be related to miRNAs in NSCLC. A recent study by Dong *et al* (69) indicated that metformin inhibits the growth, migration and invasion of A549 cells by upregulating AMPK-mediated miR-7 expression and regulating the AKT/mTOR and MAPK/ERK pathways. Recently, it has been

reported that high Yes-associated protein (YAP) may induce the growth and metastasis of NSCLC. Metformin also disrupts the growth and metastasis of NSCLC by inhibiting the activity of the miR-381-YAP-Snail axis (70).

However, the gene knockout of AMPK does not completely block the effects of metformin against cancer development, suggesting the presence of alternative mechanisms (57,59). Protein phosphatase 2 (PP2A) is considered a tumor inhibitor in a variety of tumors (71) and the PP2A inhibitor α4 is usually overexpressed in tumor cells. Zhou *et al* (72) determined that metformin increases the apoptotic rate of A549 and H1651 lung cancer cells by disrupting the interaction of PP2A inhibitors (α4 and MID1) with the catalytic subunit and activating PP2A. This effect is associated with inhibited oncogenic activity of AKT and MYC, as well as Bax phosphorylation, suggesting that PP2A may also be a potential metformin target in lung cancer therapy.

4. Metformin monotherapy for NSCLC

Preclinical studies. Metformin has been used as a single agent in *in vivo* and *ex vivo* studies and the data obtained in preclinical studies suggested that it had certain anticancer activity (Table I). *In vitro* studies indicated that metformin inhibited the proliferation of lung cancer cells in a time- and concentration-dependent manner and increased phosphorylation of AMPK (73-75). Lee *et al* (76) exposed A549, H460, H1299, H1650 and H226 cells to 0-10 mM metformin and observed decreased cell proliferation and colony-forming capacity, and increased protein levels of p53, p21 and growth arrest and DNA damage protein 45A. In a study by Ko *et al* (77), treatment of H2087 cells with 0.25-4 mM metformin revealed a decrease in cell colonization and invasion, and upregulated expression of phosphorylated (p)-ERK. Wang *et al* (78) treated A549, H1975 and HCC827 cells with 0.2 mM metformin and observed that not only did they inhibit cell proliferation, but they also induced cell cycle arrest in the S phase and increased apoptosis.

Progress has also been made in research on the effects of metformin in animal models of lung cancer. Nicotine-derived nitrosamides, also known as 4-(methylnitrosamino)1-(3-pyri dyl)-1-butanone (NNK), have been identified as inducers of lung cancer (79). In a study by Memmott *et al* (80), in which A/J mice were exposed to NNK and then received intraperitoneal injections of metformin, metformin was observed to reduce tumorigenesis by 72%. This study demonstrated that metformin prevents the tobacco carcinogen-induced development of lung tumors via inhibition of Akt, upstream of mTOR, and indirect inhibition of mTOR. In three other xenogeneic models of A549 cell origin, treatment with metformin significantly reduced tumor growth and metastatic capacity *in vivo*, and reduced the expression of proteins such as Ki-67, proliferating cell nuclear antigen (PCNA), Akt and Myc (72,78,81). In a study by Moro *et al* (73) on patient-derived xenografts (PDXs), metformin (100 mg/kg/day) partially inhibited the tumor growth of PDXs with wild-type LKB1 (maximum inhibition rate, 50.5±14.8%), but had no significant inhibitory effect on LKB1-mutant PDXs, and with increasing doses of metformin, p-AMPK expression was increased and Ki67 expression was decreased, indicating that LKB1-deficient tumors have an impaired ability to adapt to metabolic stress induced by metformin treatment. More recently, in another study on wild-type LKB1 PDXs, metformin only induced apoptosis in wild-type LKB1 PDXs with high expression of miR-17, suggesting that high miR-17 expression increased sensitivity to metformin treatment (82).

Retrospective clinical studies. Preclinical studies have indicated that metformin has anticancer effects and numerous retrospective clinical studies have demonstrated that metformin significantly improved anticancer activity in patients with NSCLC compared to those not taking metformin (Table II). Several retrospective studies suggested that metformin use is associated with a decreased risk of lung cancer (41,83,84). Metformin use was also significantly associated with a favorable prognosis of patients with NSCLC (85,86). In a retrospective study assessing overall survival (OS) of patients with T2D and metastatic lung cancer, patients treated with metformin had 20% higher survival rates than those who did not take metformin (87). A comprehensive systematic evaluation and meta-analysis of 10 published retrospective studies by Cao *et al* (88) determined that treatment with metformin significantly improved survival, with corresponding increases in OS and progression-free survival (PFS) of 23 and 47%, respectively. In addition, analyses stratified by tissue type indicated a significant improvement in OS and PFS in NSCLC, suggesting that metformin may be an effective treatment option for patients with diabetes combined with lung cancer. However, Kim *et al* (89) performed a retrospective study of 336,168 individuals regarding lung cancer incidence with a median study duration of 12.86 years and observed that metformin treatment did not reduce lung cancer incidence in the diabetic population. The potential use of metformin in lung cancer prevention should be reconsidered and requires to be further validated in randomized controlled trials.

5. Metformin combined with glycolysis inhibitor

Metformin exerts toxic effects on NSCLC cells as an OXPHOS inhibitor (90). However, under standard high-glucose conditions, metformin treatment primarily causes cell cycle arrest without any signs of cell death (91). A study by Elgendy *et al* (92) indicated that glucose consumption and lactate production increased in a time- and dose-dependent manner after HCT116 cells were treated with metformin, indicating that the rates of glycolysis increased. Conversely, under low-glucose conditions, these cells exhibited a rapid increase in oxygen consumption and a consequent increase in OXPHOS. These findings are consistent with those of certain studies reporting that inhibition of glycolysis is associated with increased activity of OXPHOS and *vice versa* (93-96). Preclinical evidence suggested that, similar to other anticancer drugs, the effectiveness of metformin was limited in *in vitro* studies and it is feasible to combine drugs to simultaneously target multiple metabolic pathways in NSCLC to improve treatment efficacy. In a study by Hou *et al* (97) on a combination of metformin and the glycolysis inhibitor 2-DG in NSCLC treatment, enhanced DNA damage, DNA adduct formation, intracellular reactive oxygen species levels and

Table I. Preclinical studies on metformin in non-small cell lung cancer.

A, No treatment

Author (year)	Cell/animal model	Metformin dose	Combination treatment	Finding/effect of treatment	(Refs.)
Moro *et al* (2018)	A549, H1299	50-250 mM	-	↓ Cell proliferation ↑ G1 cell cycle arrest ↓ MMP	(73)
Luo *et al* (2019)	A549, H460	0-80 mM	-	↓ Cell proliferation ↑ Apoptosis ↓ c-FLIPL, PKA ↑ GSK-3β	(74)
Riaz *et al* (2019)	A549, H460	2 μM-8 mM	-	↓ Cell proliferation ↓ Colony formation ↓ ERCC1 ↑ p-MAPK	(75)
Lee *et al* (2019)	A549, H460, H1299, H1650, H226	0-10 mM	-	↓ Cell proliferation ↓ Colony formation ↓ SIRT1 ↑ p53, p21 ↑ GADD45A	(76)
Ko *et al* (2020)	H2087	0.25-4 mM	-	↓ Cell viability ↓ Colony formation ↑ p-ERK	(77)
Wang *et al* (2021)	A549, H1975, HCC827	0.2 mM	-	↓ Cell proliferation ↑ Apoptosis ↓ Colony formation ↑ Gs cell cycle arrest	(78)
Zhou *et al* (2019)	A549 xenograft Nu/J nude mice	5 mg/ml	-	↓ Lung cancer metastases ↓ Tumor growth	(72)
Wang *et al* (2021)	A549 xenograft male nude mice	250 mg/d	-	↓ Tumor growth	(78)
De Bruycker *et al* (2019)	A549 xenograft female nude mice	100 mg/kg	-	↓ Tumor growth ↓ Ki-67	(81)
Moro *et al* (2018)	PDXs SCID mice	100, 800 mg/kg	-	↓ Tumor growth ↓ Ki-67 ↑ p-AMPK	(73)
Borzi *et al* (2021)	PDXs	400 mg/kg	-	↑ Apoptosis ↓ Tumor volume	(82)

B, Treatment

Type/author (year)	Cell/animal model	Metformin dose	Combination treatment	Finding/effect of treatment	(Refs.)
Glycolysis inhibitor					
Hou *et al* (2016)	A549	0-10 mM	2-dDG	↓ Cell proliferation ↑ DNA damage ↑ ROS level ↑ Apoptosis ↑ AMPK	(97)
Yakisich *et al* (2019)	H460	0-10 mM	2-DG/WZB	↓ Cell proliferation ↓ Number of colonies	(18)
Radiation					
Wang *et al* (2017)	A549, H460	5 mM	2 Gy	↓ Colony formation	(104)

Table I. Continued.

B, Treatment

Type/author (year)	Cell/animal model	Metformin dose	Combination treatment	Finding/effect of treatment	(Refs.)
Chemotherapy					
Huang *et al* (2020)	A549, H838	3-12 mM	Cisplatin	↓ Cell proliferation ↓ Nrf2 ↑ Apoptosis	(105)
EGFR-TKI					
Wang *et al* (2017)	A549, HCC827, H332M	5 mM	Erlotinib	↓ Cell proliferation	(106)

MMP, mitochondrial membrane potential; c-FLIPL, cellular F ADD-like IL-1β-converting enzyme-inhibitory protein; PKA, protein kinase A; GSK-3β, glycogen synthase kinase 3β; ERCC1, excision repair cross complement-1; p-MAPK, phospho-mitogen-activated protein kinase; SIRT1, sirtuin 1; GADD45A, growth arrest and DNA damage protein 45A; p-ERK, phospho-ERK; PDXs, patient-derived xenografts; p-AMPK, phospho-AMP-activated protein kinase; ROS, reactive oxygen species; AMPK, AMP-activated protein kinase; Nrf2, nuclear factor erythroid 2-related factor-2; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor.

Table II. Retrospective clinical trials on metformin in non-small cell lung cancer.

A, Prevention

Author (year)	Stage	Number of subjects	Finding/effect of treatment	(Refs.)
Wang *et al* (2021)	NS	295573	↓ Risk of lung cancer	(78)
Xiao *et al* (2020)	NS	-	↓ Risk of lung cancer	(84)
Kang *et al* (2021)	NS	732199	↓ Risk of lung cancer	(41)

B, Treatment

Author (year)	Stage	Number of subjects	Finding/effect of treatment	(Refs.)
Arrieta *et al* (2016)	IV	1106	↑ OS	(85)
Xu *et al* (2018)	NS	255	↑ OST ↑ DFST	(86)
Cao *et al* (2017)	NS	-	↑ OS ↑ PFS	(88)

NS, not specified; OS, overall survival; OST, overall survival time; DFST, disease-free survival time; PFS, progression-free survival; ↑, increase; ↓, decrease.

mitochondrial membrane potential alteration, as well as increased apoptosis, caspase-3 activity, p-p38 and p-AMPK levels, were observed, indicating that combined treatment was more effective against NSCLC than either drug alone. Similarly, in a study by Yakisich *et al* (18), who studied the effect of metformin alone or in combination with 2-DG and WZB on H460 cell viability, a strong synergistic effect was discovered. The combination of metformin and glycolytic inhibitors led to a marked reduction in intracellular ATP and increased cell death by inhibiting both metabolic pathways in lung cancer.

Another strategy to influence the aerobic glycolytic pathway in cancer cells includes the inhibition of glucose concentrations in culture media *in vitro* and diet restrictions to lower blood glucose levels *in vivo* (98). Several studies have indicated that cancer cells cultured under low glucose concentrations or in sugar-free media are more susceptible to the cytotoxic effects of metformin (55,91,99). Restricted diets exhibited a strong synergistic effect on anticancer activity in preclinical models of lung adenocarcinoma. Elgendy *et al* (92) treated mice undergoing a 24-h feeding/fasting cycle with metformin and they observed impaired tumor growth only when the drug was administered during fasting-induced hypoglycemia. This indicated that metformin combined with fasting-induced hypoglycemia synergistically inhibited the growth of

Table III. Clinical trials on metformin in non-small cell lung cancer.

Author (year)	Clinical trial number	Status	Trial phase	Stage	Metformin dose (mg/day)	Combination treatment	Primary purpose	(Refs.)
-	NCT03086733	Completed	2	I-IIIA	850	-	Treatment	-
-	NCT01717482	Terminated	2	IA-IIIA	1700	-	Prevention	-
-	NCT04931017	Not yet recruiting	2	NS	-	-	Prevention	-
-	NCT02109549	Completed	2	Advanced stage	-	-	Treatment	-
-	NCT04170959	Terminated	2	III	500	-	Treatment	-
-	NCT02019979	Terminated	2	IIIB-IV	1000	Restricted diet	Treatment	-
-	NCT02285855	Terminated	2	NS	2000	Radiation	Treatment	-
Skinner *et al* (2021)	NCT02186847	Active, not recruiting	2	III	1000, 1500, 2000	Chemo-radiotherapy	Treatment	(109)
Tsakiridis *et al* (2021)	NCT02115464	Terminated	2	IIIA-IIIB	2000	Chemo-radiotherapy	Treatment	(110)
Kubo *et al* (2018)	NCT03874000	Recruiting	2	IIIB, IIIC, IV	1000	ICIs	Treatment	(115)
-	NCT03048500	Active, not recruiting	2	III-IV	-	ICIs	Treatment	-
Arrieta *et al* (2016)	NCT03071705	Unknown	-	NS	1000	TKI	Other	(85)
-	NCT01864681	Completed	2	IIIB-IV	1000	EGFR-TKI	-	-
-	NCT03709147	Recruiting	2	IIIB-IV	1500	Chemo-immunotherapy + FMD	Treatment	-

NS, not specified; ICIs, immune checkpoint inhibitors; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; FMD, fasting-mimicking diet.

transplanted tumors in nude mice. In addition, an ongoing clinical trial aims to determine whether the combination of metformin and fasting improves PFS in patients with advanced lung adenocarcinoma compared with historical data on metformin alone (100). In another clinical trial, the investigators will assess for the first time the efficacy of combining standard-of-care platinum-based chemoimmunotherapy with metformin plus/minus a fasting-mimicking diet in patients with LKB1-inactive, advanced lung adenocarcinoma (ClinicalTrials.gov identifier no. NCT03709147).

6. Clinical progress of metformin combined with standard anticancer drugs

In recent years, under single treatment regimens [chemotherapy, immune checkpoint inhibitors (ICIs) and targeted therapies] patients have exhibited relapses due to the development of acquired drug resistance (101-103). There is growing evidence that metformin exerts its anticancer effects by inhibiting tumor metabolism and that metformin may be a potential candidate for combination therapy in NSCLC. A number of preclinical studies have reported good results of metformin acting concurrently with radiotherapy, tyrosine kinase inhibitors (TKIs) and ICIs in NSCLC (104-107), which has encouraged the use of combination therapies. In a meta-analysis of 14 clinical studies comprising 3,856 patients, the combination of metformin with standard antineoplastic drugs significantly improved OS in patients with lung cancer (108). These results suggest that metformin combined with radiotherapy may be an effective regimen for the treatment of patients with NSCLC. However, in two recent randomized clinical trials, Skinner *et al* (109) and Tsakiridis *et al* (110) reported poorer outcomes for patients with NSCLC treated with metformin in combination with radiotherapy, suggesting that the addition of metformin to radiotherapy did not improve OS in patients with NSCLC and increased toxicities, which contrasts the results of previous studies. Promising results have also been reported by two recent studies of metformin in combination with TKIs and ICIs, respectively, which suggested that metformin was able to significantly improve PFS and OS in patients with NSCLC by overcoming acquired resistance to TKIs and enhancing PD-1 blockade by anti-PD-1 antibodies, respectively (101,102). Other studies (111-114) suggested that metformin may increase tumor response to ICI through a variety of mechanisms, including upregulation of $CD8^+$ tumor-infiltrating lymphocytes and their function, downregulation of myeloid suppressor cells with immunosuppressive effects, reduction of tumor hypoxia, anti-angiogenic effects and shifting the composition of the patient's gut flora to bacterial strains that may respond better to immunotherapy (107). Although it has been suggested that metformin treatment may exert a synergistic antitumor effect with ICIs, the study by Jacobi *et al* (107) did not obtain any positive association between metformin and ICIs in the treatment of patients with diabetes combined with NSCLC. More prospective studies are required to further evaluate the effect of metformin in combination with radiotherapy, TKIs and ICIs on the outcome of patients with NSCLC. A search on https://clinicaltrials.gov indicated that a number of prospective clinical trials (Table III) are currently evaluating the preventive and therapeutic effects of metformin alone or in combination with other treatment options for NSCLC. One of these is an ongoing open, single-arm, phase II clinical trial (ClinicalTrials.gov identifier no. NCT03874000) to evaluate the safety, efficacy and pharmacokinetics of the metformin-sintilimab combination in the treatment of NSCLC (115).

7. Prospects and conclusions

Therapeutic methods that target the metabolic differences between tumor cells and normal cells have potential in cancer treatment and the restriction of aerobic glycolysis in tumors has been somewhat effective in inducing lung cancer cell apoptosis. There are also increasing reports confirming the important role of mitochondria in the development and growth of cancer. In recent years, there has been increasing evidence of the antitumor effects of metformin as an OXPHOS inhibitor and in a number of retrospective clinical trials, metformin has produced beneficial effects on survival outcomes in patients with NSCLC. The theory of the antitumor effects of metformin involves its action on several major signaling pathways, including indirect (insulin-dependent) and direct (activation of AMPK pathways) and corresponding targets, such as PI3K, K-RAS, mTORC, PP2A and miRNA. However, as with aerobic glycolysis inhibitors, metformin alone exhibited limitations in its effectiveness in *in vitro* trials. Drugs that target enzymes or metabolites of key metabolic pathways may be highly specific and effective but must be matched to responsive tumors that are likely to adapt rapidly. Preclinical evidence in recent years has demonstrated synergistic effects of metformin in combination with glycolysis inhibitors, radiotherapy, EGFR-TKIs and ICIs in NSCLC, but it is not consistent with the results of certain retrospective studies and clinical trials, and more prospective studies are required to further evaluate the influence of metformin combination effects on the outcomes for patients with NSCLC. However, metformin inhibits mitochondria in a dose-dependent manner and at high doses, although it is able to impair tumor growth, it may also lead to lactic acidosis (116). The clinical application of experimental doses of metformin may be challenging. Of note, metformin accumulates in tissues at concentrations several times higher than those in the blood and the positive charge on metformin has been indicated to promote its accumulation in the mitochondrial matrix <1,000-fold (>20 mmol/l). Hence, metformin concentrations of 1-10 mmol/l, which have been used in preclinical models, may also be effective during cancer treatment in clinical settings (117). In a recent study, Reinfeld *et al* (118) used PET tracers to measure glucose uptake in specific cellular subpopulations in the tumor microenvironment and determined that in a range of cancer models, myeloid cells have the greatest glucose uptake capacity within the tumor, followed by T cells and cancer cells. Furthermore, they observed that cancer cells had higher uptake of glutamine than of glucose. In the future, more in-depth basic research on target metabolic pathways in lung cancer is required to provide an improved theoretical basis for adjuvant lung cancer therapy.

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Authors' contributions

NC and JBH designed the review and edited the manuscript. NC and JBH wrote the manuscript. NC, YSZ and LCW collected and analyzed data. 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

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Competing interests

The authors declare that they have no competing interests.

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