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Review Article

Mechanisms of metformin inhibiting cancer invasion and migration

Yong Chang Chen[1,2], He Li[1*], Jing Wang[1*]

[1]Gynecologic Oncology Clinical Research Center, Hunan Cancer Hospital, Central South University, Changsha 410013, Hunan, China; [2]University of South China, Hengyang 421001, Hunan, China. [*]Equal contributors.

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Abstract: Cancer currently ranks among the leading causes of death globally. Cancer invasion and metastasis transform locally grown cancers to a systemic and life-threatening disease, which accounts for the most significant challenge in cancer treatment. Recent studies showed that Metformin, the most commonly used first-line oral drug for the treatment of type 2 diabetes (T2DM), could prevent and treat various cancers. Moreover, multiple evidence suggested that metformin inhibited cancer invasion and metastasis, which could improve the prognosis of cancer patients administrated with metformin. To better understand the anti-cancer role of metformin, the present review summarized the potential mechanisms of inhibiting cancer invasion and metastasis by metformin, including AMPK signaling pathway, EMT signaling pathway, epigenetic modification and so on. However, multiple problems remain unresolved and more clinical trials are needed to prove the inhibition of cancer invasion and metastasis by metformin.

Keywords: Metformin, cancer, invasion, migration

Introduction

Cancer invasion and migration

It is projected that cancer will be the leading cause of death and the most significant impediment to a long-life expectancy in the 21st century. With a rapidly increasing global cancer morbidity and mortality, it was estimated that approximately 18.1 million new cancer cases and 9.6 million cancer-related deaths would occur in 2018 [1]. At present, the clinical challenges facing cancer management include drug resistance, unavoidable metastasis and the spread of cancer, among other factors. Cancer cell migration and invasion are the basis of cancer metastasis and spread [2]. As a hallmark event in cancer, cancer metastasis transforms locally grown cancers into systemic, metastatic, and life-threatening diseases. Cancer invasion is a heterogeneous adaptation process that involves changes in cell morphology and the production of cell polarity, leading to cell body translocation. The initial step in local tissue invasion involves activation of signal transduction pathways that control cytoskeletal dynamics of cancer cells, then the turnover of cell matrix and cell connections, and finally, cancer cells actively migrate to adjacent tissues [3, 4]. Metastases occur when invading cancer cells enter the blood and lymphatic vessels, penetrate the basement membrane and endothelial wall and diffuse through the vascular cavity to colonize distant organs [5]. Similar to cells in primary cancer, metastatic cells also proliferate, invade, and enter blood vessels, leading to secondary metastases [6, 7]. Cancer cells have excellent adaptability to different environmental conditions and the ability to have more migration strategies [8, 9]. Acquiring an invasive behavior involves the activation of signaling pathways related to cytoskeleton dynamics, as well as the transformation of cell matrix and cell-to-cell adhesion [9]. Depending on the cell type and tissue environment, migration of cells can occur in two main ways: cancer cells migrate alone without a cell-cell connection, or they could co-migrate after retaining cell-cell adhesion [9]. In both migration processes, cytoskeletons act as engines, whereas

Table 1. Clinical trials of metformin in different cancers

Cancer type	No. of cases	Country	Ending point	Ref.
Pan-cancer	1,353	Netherlands	Cancer-specific mortality	[13]
Pan-cancer	10,309	Canada	Cancer-specific mortality	[14]
Pan-cancer	112,408	U.K.	Cancer-specific mortality	[15]
Pan-cancer	21,595	Systematic Review	Cancer-specific mortality	[16]
Breast cancer	5,634	China	The 5-year survival rates	[17]
Breast cancer	8,381	Multiple countries	DFS, DDFS and OS	[18]
Liver cancer	162	China	The 5-year survival rate	[19]
NSCLC	750	SEER-Medicare Database	Survival rate	[20]
CRC	47,597	China	Survival rate	[21]
Prostate cancer	2901	United States	PSA-RFA, DMFS, PCSM and OS	[22]
Endometrial cancer	4132	meta-analysis	Cancer-specific mortality	[23]

DFS: Disease-free survival; DDFS: Distant disease-free survival; OS: Overall survival; NSCLC: Non-small cell lung cancer; CRC: Colorectal cancer; PSA-RFS: Prostate-specific antigen-recurrence-free survival; DMFS: Distant metastases-free survival; PCSM: Prostate cancer-specific mortality.

cell surface receptors function as transmission channels. The dynamic coupling and interaction with the surrounding tissue structure constitute the basic migration process [10]. Although many strategies for the treatment of cancer have been exploited, clinical use will be restricted due to the aggressiveness of various forms of cancer. Therefore, cancer invasion and metastasis pose the most significant challenge in cancer eradication.

Mechanisms of metformin to inhibit cancer invasion and migration: a review

Metformin was synthesized based on the structure of guanidine in 1920. While metformin retained the hypoglycemic effect of its parent compound, it has reduced levels of toxicity. First, it was used in Europe and was subsequently approved by the US food and drug administration (FDA) in 1994 for the treatment of T2DM in the United States. Due to its definite treatment effect, safety, and lower cost of use, metformin has become the most commonly used first-line oral drug for the treatment of T2DM [11]. It reduces gluconeogenesis in the liver and improves insulin sensitivity by increasing uptake of peripheral glucose and reducing the concentrations of basal and postprandial plasma glucose [12].

Until now, some cohort studies have shown a significant association between metformin administration and improved survival in cancer patients. For instance, a prospective cohort study of 1,353 patients with T2DM was conducted in the Netherlands. Here the use of metformin significantly reduced cancer-specific mortality by 57% [13]. Also, in Canada, a large retrospective study was conducted, and the results indicated a 20% reduction of cancer-specific mortality among metformin users compared to its non-users [14]. Elsewhere, meta-analysis results suggested that administration of metformin reduced cancer-specific mortality by 35% [15]. Besides, patients treated with metformin showed a 15% reduction in overall cancer mortality [16]. In addition, more clinical studies indicated that metformin improved the outcome of patients with various types of cancer, patients, including breast [17, 18], liver [19], non-small cell lung [20], colorectal [21], prostate [22] and endometrial cancers [23] (**Table 1**). Recently, metformin has been shown to be an effective adjuvant therapy for cancer patients. Subsequently, patients with colorectal and prostate cancers undergoing radical radiation therapy could benefit from metformin use [24]. In a nutshell, the prognosis of patients with cancer can be significantly improved by metformin administration. Lately, multiple researches showed that metformin had inhibitory effects on cancer invasion and migration, which could account for the improvement of prognosis in cancer patients. For example, in a study by the Safar Kheder team, metformin was shown to inhibit the migration of thyroid cancer cells [25]. In addition, metformin reduced the invasion and migration of pancreatic ductal carcinoma [26]. The present review summarizes the researches on the mechanism of

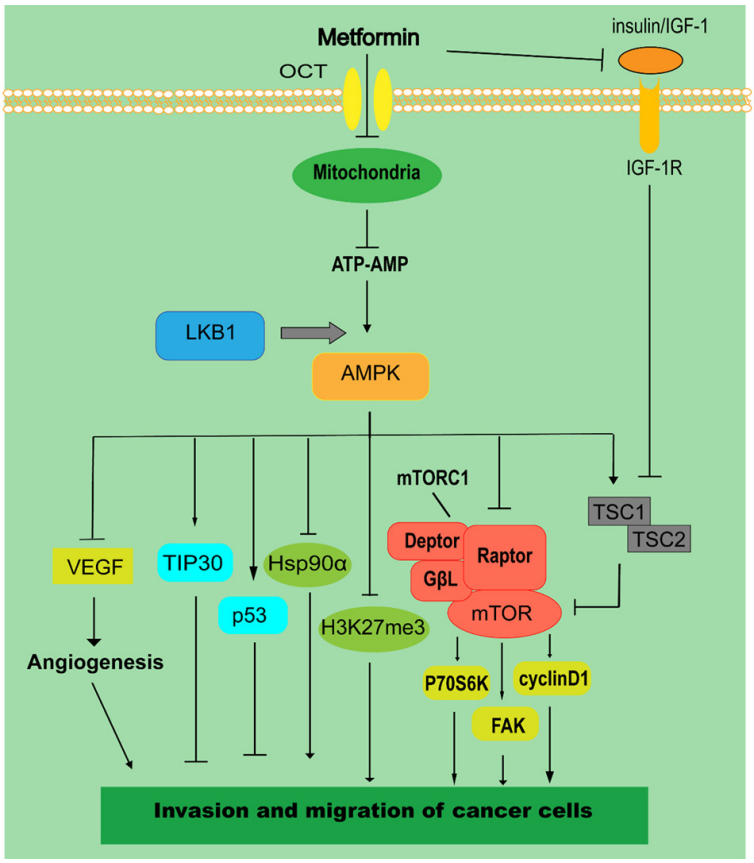

Figure 1. Metformin inhibits cancer invasion and migration *via* AMPK signaling pathway. After uptake by the organic cation transporter (OCT), metformin causes a reduction in ATP *via* inhibition of the mitochondrial respiratory chain complex 1, leading to activation of AMPK. Similarly, metformin can inhibit mTORC1 by inhibiting IGF-1R. Activated AMPK may suppress mTORC1 and change the expression of proteins such as P70S6K, FAK and cyclin D1. Also, it can directly inhibit the expression of Hsp90α and H3K27me3 protein or inhibit the expression of TIP30 and p53 genes. At the same time, metformin-induced AMPK activation can inhibit VEGF and reduce angiogenesis. P70S6K, p70 S6 Kinase; FAK, Focal Adhesion Kinase; IGF-1R, insulin-like growth factor 1 receptor; Hsp90α, heat shock protein 90α; H3K27me3, histone H3 lysine 27 trimethylation; VEGF, vascular endothelial growth factor.

metformin in inhibiting cancer invasion and migration, to obtain a better understanding of its effect on cancer invasion and migration.

Metformin inhibits invasion and migration through the AMPK signaling pathway: a review

Eukaryotes have evolved a very complex system that can detect low levels of cellular ATP to regulate metabolism based on nutrient utilization, through the serine/threonine kinase AMP-activated protein kinase (AMPK) complex. This energy switch controls the growth of cells and other biological processes, including lipid and glucose metabolism and autophagy [27]. Presently, AMP-activated protein kinase (AMPK) has attracted much attention because it's a potential target for disease treatment caused by metabolic disorders, including diabetes, obesity, fatty liver diseases, and cancer [27]. Metformin, the most regularly used drug for the treatment of T2-DM, inhibits mitochondrial glycerophosphate dehydrogenase, and thereby mitochondrial respiration. Besides, metformin affects the functioning of lysosomes through AMP-activated protein kinase (AMPK) signaling [28]. Several recent studies have indicated that metformin inhibited the invasion and migration of cancer cells by activating the AMPK signaling pathway (**Figure 1**).

Liver kinase B1 (LKB1), a tumor suppressor gene, is inactivated in various tumor types, especially in lung adenocarcinoma (about 30% of cases). Its function is majorly mediated by downstream AMPK [29]. Actually, numerous recent studies have indicated that metformin activated LKB1/AMPK signaling, which resulted in aerobic glycolysis inhibition in cells containing a functional LKB1/AMPK pathway. On the other hand, it induced cancer cell death in cells that lacked the functional LKB1/AMPK pathway by reducing levels of ATP. Hence, making susceptible cells lack the ability to cope with energy stress [30]. Of note, AMPK forms a complex by activating TSC2 and TSC1, which reduces the activity of mTOR complex 1 (mTORC1), hence inhibiting cell growth after translation. Additionally, this result confirmed that loss of AMPK activity promoted the development of lymphomas in mice models [31].

Recently, it was proposed that invasion and migration of cancer were inhibited by metformin through the AMPK/mTOR signaling pathway in glioma cells [32]. Also, it was shown that

it played a similar role as a low glucose environment to activate AMPK, which controls the migration of liver cancer cells [33]. Likewise, it enhanced the inhibitory effect of cisplatin on bile duct cancer cells *via* the AMPK/mTOR pathway and inhibited invasion of cancer cells through regulating the expression of FAK [34]. In Esophageal Carcinogenesis, *in vivo* and *in vitro* treatments with metformin enhanced the activation of AMP-activated protein kinase (AMPK), and weakened signals from downstream molecules (such as p-mTOR, p-p70S6K, and cyclin D1 expression), thereby inhibiting cancer cell metastasis in the long run [35].

Additionally, metformin was also found to inhibit the migration of ovarian cancer by the AMPK pathway, which reduced histone H3 lysine 27 trimethylation (H3K27me3) [36]. Recently, it was shown that cancer metastasis was inhibited by metformin that resulted in the secretion of heat shock protein 90α (Hsp90α) in an AMPKα1-dependent manner [37]. In addition, metformin, activated TIP30, a cancer suppressor *via* AMPK in liver cancer cells, promoting metastasis inhibition and sorafenib effects [38]. Consequently, it was reported that metformin also blocked melanoma invasion and metastasis in an AMPK/p53-dependent manner [39]. *In vivo* experiments on ovarian cancer, they have shown that metformin induced activation of the AMPK/mTOR signaling pathway to inhibit micro-vessel density and expression of the vascular endothelial growth factor, thus inhibiting metastatic nodules growth in the lung [40]. Moreover, it was suggested that metformin inhibited invasion of cells of the cholangiocarcinoma through activating AMPK signaling to inhibit mTOR and by blocking inhibitory effects of insulin-like growth factor 1 receptor (IGF-1R)/insulin receptor substrate 1 (IRS-1)/Akt pathway on tuberous sclerosis complex 2 (TSC2) [41]. During combined treatment with ursolic acid (UA), the invasion and metastasis of breast cancer cells can be inhibited. These effects were accompanied by down-regulation expression of CXCR4, uPA, vimentin, E-cadherin, N-cadherin, and MMP-2/9 proteins and AMPK/m-TOR signaling pathways regulation [42].

Metformin inhibits invasion and migration through EMT signaling pathways: a review

The process in which epithelial cells transdifferentiate into motile mesenchymal cells is called epithelial-mesenchymal transition (EMT). It is essential for development, healing of wounds, and stem cell behavior. Additionally, it is pathologically helpful during progression on fibrosis and cancer [43-45]. During EMT, there is loss in connection and apical-basal polarity in epithelial cells. Moreover, they reorganize their cytoskeleton and change the signaling pathways that define the shape of a cell and reprogram expression of a gene leading to an increase in mobility of individual cells and of invasive phenotype development [43, 46]. Hallmarks linked to EMT signal pathways that strengthen instability of adhesive connection include; E-cadherin down-regulation and N-cadherin up-regulation. Therefore, EMT is considered to be closely related to invasion and metastasis of cancer. In the recent past, several studies have explored the mechanisms by which cancer invasion and metastasis are inhibited by metformin as a result of inhibiting EMT signaling pathways (**Figure 2**).

Reduced expression of transcription factors driving EMT signaling: Aberrant gene expression in EMT signaling, particularly transcription factors such as SNAIL, TWIST, and zinc finger E-box-binding (ZEB), have contributed immensely to epithelial phenotype suppression and mesenchymal phenotype activation [47]. Metformin regulates the expression of transcription factors driving EMT signaling, except directly regulating the expression of E-cadherin and N-cadherin. In rectal cancer cells, metformin and phenformin were found to inhibit transforming growth factor-beta receptor 2-mediated Snail and Twist expression, which played significant roles in EMT and cancer invasion and migration [48]. By using microarray analysis and protein imprinting, it showed that combining 2-deoxyglucose with metformin down-regulated the expression of SNAI2 (TWIST) and ZEB1 and also inhibited glioblastoma cells invasion and migration [49]. Moreover, metformin inhibited the migration and invasion of pancreatic cancer cells by reducing Snail protein expression through activating LKB1 [50]. Subsequently, metformin also reduced the expression of SNAIL1 and ZEB1 in colorectal cancer, which promoted EMT inhibition and invasion, and migration of cancer cells [51].

Regulation of miRNAs in EMT signaling: Besides transcription factors, non-coding RNAs also regulate EMT signaling. These miRNAs are

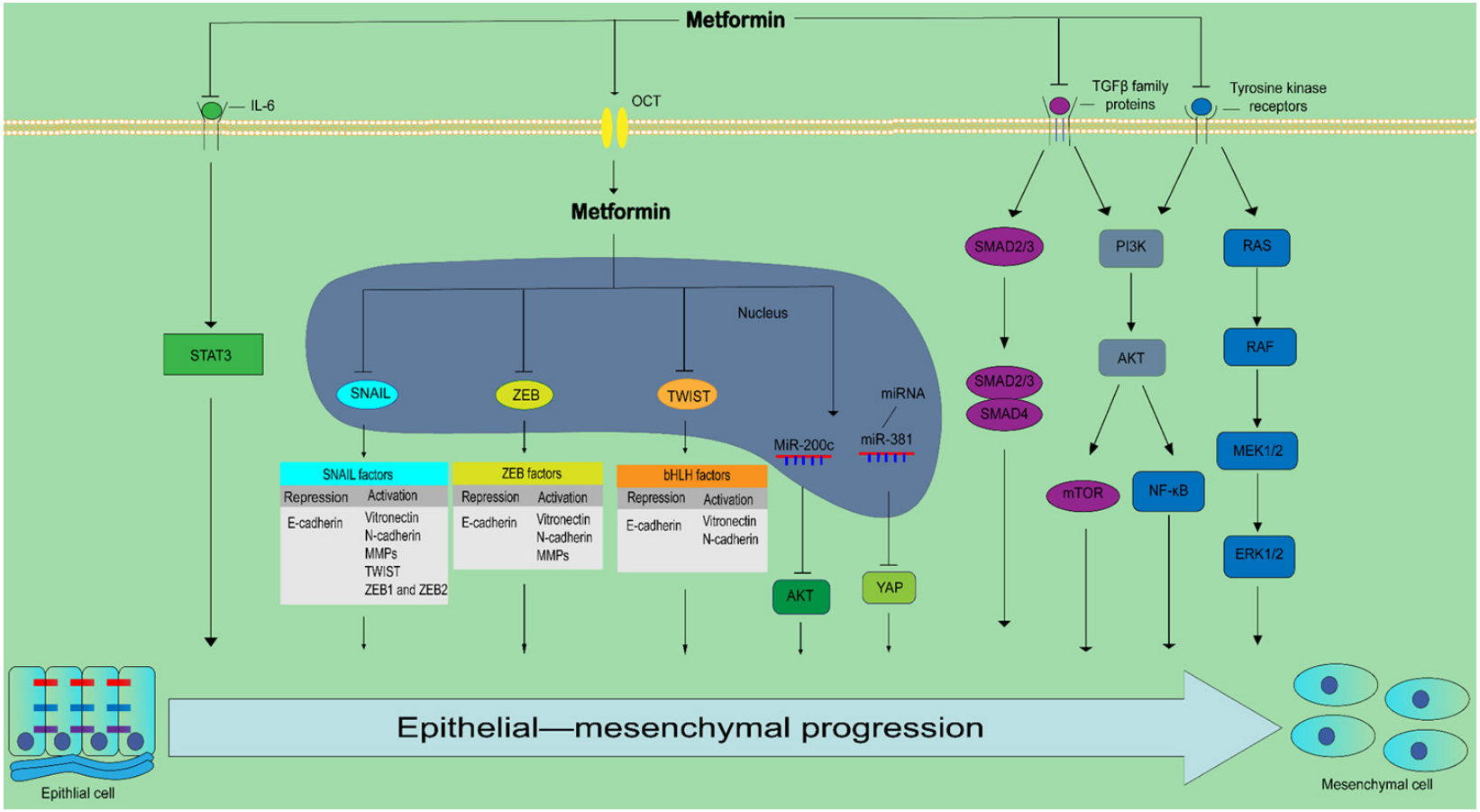

Figure 2. Metformin inhibits cancer invasion and migration by blocking EMT signaling pathway. Metformin suppress the process of EMT by driving down the expression of transcription factors such as SNAIL, TWIST, and ZEB. It can also regulate miRNAs in EMT signaling, including miR-200c and miR-381. In addition to the above mechanisms, metformin also inhibits EMT by antagonizing SMAD2 and/or SMAD3 combine with SMAD4 to form trimeric SMAD complexes activated by TGFβ family proteins; or inhibit PI3K/AKT/mTOR signaling by regulating TGFβ family proteins. Metformin also inhibited tyrosine kinases receptors and the main downstream pathways are PI3K/AKT/NF-κB and RAS-RAF-MEK-ERK MAPK signaling. Metformin also reduces STAT3 activation by inhibiting IL-6. ZEB, E-box-binding; TGFβ, transforming growth factor-β; NF-κB, nuclear factor kappa-B; STAT3, transcription 3; IL-6, interleukin-6.

short RNAs with no ability to encode proteins. They were first intended to be "noise". However, miRNAs have been shown to participate in various biological processes, such as playing vital roles in EMT and invasion and migration of cancer. Earlier, it was suggested that metformin inhibited EMT by regulating the expression of miRNAs, thereby inhibiting cancer invasion and migration. It was also shown that metformin incubation increased miR-200c expression in breast cancer cells and inhibited EMT and cancer invasion and migration [52]. Moreover, in non-small lung cancer cells, metformin repressed the activity of the miR-381-YAP axis and disrupted cell migration and invasion [53].

Inhibition of TGFβ family proteins: The TGFβ family consists of three TGFβs, including two activins, many bone morphogenetic proteins (BMPs), and other homodimers and heterodimers of ligands. They all act through binary combinations of transmembrane dual-specificity kinase receptors (that is, receptors that act as Ser/Thr kinases, as well as Tyr kinases) [47]. EMT is induced through SMAD-mediated and non-SMAD signaling by TGFβ [41]. Usually, SMAD2 and SMAD3 are activated by TGFβ, which then combines with SMAD4 hence formulating a trimeric SMAD complex [54]. Once this complex is translocated into the nucleus, the expression of target genes in EMT signaling is regulated by cooperating with transcription regulators. Notably, it was established that cotreatment of metformin and cisplatin inhibited ovarian cancer cell metastasis as a result of inhibiting TGFβ1 expression and phosphorylation of both Smad2 and Smad3 [55]. Besides, Nakayama A *et al.* discovered that EMT was induced by ionizing radiation (IR) in esophageal squamous cell carcinoma. Also, this induction was disrupted by metformin through the TGF-β-Smad phosphorylation pathway and the non-Smad pathway inhibition [56]. In addition, TGFβ can activate the PI3K/AKT/mTOR signal pathway [57]. Liver cancer cell proliferation and invasion are inhibited by metformin through blockade of the TGFβ/AMPK/PTEN/AKT pathway, and thereby inadequate radiofrequency ablation [58]. Here, it was also discovered that concurrent treatment of aloe protein and metformin-induced liver cancer cell apoptosis and autophagy, which inhibited cell growth and invasion through the PI3K/AKT/mTOR pathway [59]. Moreover, in glioblastoma cells, metformin was found to inactivate the AKT/PI3K signaling pathway and inhibit the invasion and migration of cancer cells [60]. Consequently, a combination of metformin and nelfinavir also inhibited migration and invasion of cervical cancer cells through the PI3K/AKT/mTOR signaling pathway [61]. Furthermore, TGF-β1-induced EMT in cervical carcinoma cells is abolished by metformin through inhibiting the mTOR/p70s6k signaling pathway to down-regulate PKM2 expression [62]. The invasive ability of pancreatic cancer cells is suppressed by metformin by blocking signaling in the autocrine TGF-β1 pathway [63]. In breast cancer, it was shown that TGF-β elicited a significant increase in cellular proliferation, migration, invasion, and motility. However, these effects can be abolished by a specific inhibitor against TGF-β receptor I and metformin, when used singly or in combination [64].

Blockade of EMT by other signal pathways: The RAS-RAF-MEK-ERK-MAPK signaling cascade represents a major pathway that is activated in response to growth factors by receptor tyrosine kinases (RTKs). Once activated, ERK1, ERK2, and MAPK can facilitate EMT by increasing the expression of its transcription factors and cell motility and invasion regulators [47]. Notably, ERK was inhibited by a combination of metformin and binemetinib through activation of AMPK to limit melanoma invasion and metastasis [65]. In endometrial cancer, it was discovered that the Akt and Erk (1/2) pathway caused the anti-invasive migration effect of metformin [66]. Additionally, metformin inhibited the expression of RAD51 through the ERK pathway to enhance cisplatin-mediated migration and inhibition of metastasis in triple-negative breast cancer (TNBC) cells [67].

Moreover, activation of AKT induced by RTK- or integrin promotes EMT by inducing expression of SNAIL through nuclear factor kappa-B (NF-κB) [47]. The migration and invasion of esophageal squamous carcinoma cells are prevented by metformin through AKT/NF-κB pathway inhibition [68]. A study on the relationship between chronic inflammation and cancers showed that the expression of interleukin-8 (IL-8) was inhibited by metformin as a result of inhibiting the nuclear translocation of NF-κB, which ultimately hindered cancer cell invasion and migration [69].

EMT is induced by insulin-like growth factor 1 (IGF1) in specific cell culture models [70]. By using iTRAQ-based quantitative proteome, metformin was shown to mainly regulate the insulin signaling pathway, which interfered with cell proliferation and invasion in cervical cancer [71].

Additionally, an inducer of angiogenesis called vascular endothelial growth factor (VEGF), also induces EMT. Brain metastasis of advanced gastric cancer was caused by the expression of VEGF and therapy on metformin, which suppressed cancer metastasis by reducing the expression of VEGF and causing EMT blockade [72].

During cancer-induced inflammation, interleukin-6 (IL-6) promotes EMT through Janus kinase (JAK)-signal transducer and activates transcription 3 (STAT3)-induced expression of SNAIL1 [73]. Metformin was found to block the invasion of cancer cells in lung cancer by inhibiting IL-6 signaling, which reversed EMT [74]. Similarly, metformin also inhibits EMT and metastasis in prostate cancer by repressing the COX2/PGE2/STAT3 axis [75]. Subsequently, Qi Pan *et al*. found that the invasion and metastasis of bladder cancer cells were blocked by metformin, which inhibited STAT3-mediated signaling [76].

Inhibiting cancer invasion and migration through other mechanisms: a review

In addition to the above pathways, metformin also affects the invasion and migration of cancer *via* other mechanisms. It was discovered that metformin works by alleviating oxidative stress and inflammatory signaling through the COX2 pathway, thereby inhibiting migration and invasion breast cancer [77]. Moreover, metformin was seen to inhibit cell proliferation, migration, and invasion by reducing the regulation of miRNA-mediated cancer stem cells (CSC) functioning in pancreatic cancer cells [78]. Xia C *et al.* found that metformin also inhibited metastasis-related lung adenocarcinoma transcript 1 (MALAT1)/miR-142-3p, to lessen invasion and migration of cervical cancer cells [79]. Long non-coding RNA H19 is involved in the pathogenesis of many human cancers [80]. It promotes cancer cell migration and invasion by inhibiting let-7. However, Yan L *et al*. showed that metformin-induced methylation in DNA antagonized the role of H19/let-7 axis in cancer cell migration and invasion [81]. Similarly, it was found that metformin had a profound antitumor effect on gastric cancer cells. Here, H19 was the key component of metformin inhibiting cell invasion in gastric cancer [82]. Consequently, metformin was proved to reduce H3 Lys9 histone methyltransferase (SUV39H1) by inhibiting integrin-FAK signaling, which inhibited cell migration in prostate cancer [83]. Recently, metformin was found to inhibit cell migration in colorectal cancer, which was associated with rebuilt adherent junctions and downregulation of FAK [84]. Additionally, the oncogene YAP participates in proliferation, apoptosis, migration, invasion and EMT of lung cancer cells. Here, the YAP promoter is inhibited by metformin by competing with IRF-1 in lung cancer cells and ultimately suppressing the progression of non-small lung cancer cells [85]. Again, metformin suppresses the proliferation and invasion of drug-resistant breast cancer cells by increasing the expression and localization of the cell membrane of Scribble (SCRIB, a cell polarity protein). This suppression subsequently enhances the interaction of SCRIB with MST1 and LATS1 and inhibits nuclear localization and transcriptional activity of YAP [86]. Human cervical squamous carcinoma cells are stimulated by TGF-β1. It is observed that metformin decreased Vimentin expression and caused downregulation of CAIX, an enzyme involved in metastasis of aggressive malignant cells, as a result of hypoxia master regulator HIF-1α suppression [87]. Subsequently, it was discovered that gastric cancer was inhibited by metformin via the inhibition of HIF1α/PKM2 signaling [88]. Also, research on hepatocellular carcinoma (HCC) suggested that metformin enhanced the effect of sorafenib to inhibited recurrence and metastasis after liver resection regulation of HIF-2α and TIP30 expression [89]. Dirat B *et al*. showed that the GTPase Rac1 inhibition mediated the anti-migration effect of metformin in prostate cancer cells [90]. In human fibrosarcoma cells, invasion and migration induced by phorbol-12-myristate-13-acetate (PMA) through Ca^{2+} dependent PKCα/ERK and JNK/AP-1 signaling pathways was inhibited by metformin [91]. A study conducted for the first time showed that a combination of metformin with 2-DG inhibited growth, migration and invasion and induced cell cycle arrest of ovarian cancer cells *in vitro*. This is achieved through activation of p38 MAPK and JNK pathways [92]. The

formation of sphingosine-1-phosphate (S1P) can be catalyzed by Sphingosine kinase (SPHK) to enhance cell proliferation, motility, and tumor progression. Also, metformin blocked proliferation and migration of ovarian cancer cells by inhibiting SPHK1 to target the metabolism of sphingolipid [93]. According to research by Buchu Wu *et al*., development and metastasis of ovarian cancer are inhibited by metformin through a reduction in cellular-ECM interactions [94].

Conclusions

Recently, it has been shown that metformin has anti-cancer effects, which could play specific roles in cancer prevention and treatment [95]. Due to its long-term use in the treatment of T2DM, its definite treatment effect, safety and lower cost, metformin becomes an attractive candidate for the prevention and treatment of cancer. Analysis of clinical trials registered on http://ClinicalTrials.gov in June 2020 has revealed 247 studies that use metformin in the treatment of cancer. Out of these 247 studies, 20 have results and results of 13 studies completed, published, and are available on http://ClinicalTrials.gov. And there are many researches for tumors on the treatment, prevention, and combination usage of chemotherapy with metformin. Presently, metformin has been shown to have significant anti-cancer effects on the cell lines, animal models and clinical studies. The primary mechanisms involve blockade of the cell cycle [96], inducing apoptosis [97, 98], and improving the cancer cell microenvironment (by lowering blood glucose and improving hyperinsulinemia) [99]. It was found that metformin may be used as adjuvant therapy in cancer treatment [24] since it increased the sensitivity of chemotherapy drugs to different types of cancer [100-102]. In addition, several clinical studies have confirmed that it could improve patient survival rate and prognosis, which may be achieved by inhibiting cancer invasion and migration.

This review explored and summarized the mechanisms and signaling pathways by which metformin inhibits cancer cell invasion and migration (**Table 2**). The main mechanisms outlined here were as follows: Firstly, it inhibits invasion and metastasis of cancer through the AMPK/mTOR signaling pathway, which could be one of the most common pathways by which metformin exerts its effects. Secondly, metformin inhibits the invasion and metastasis of cancer cells by inhibiting EMT-related signals. Besides, metformin could have hindered the invasion and metastasis of cancer cells through other methods other than the above two pathways. These include effects on oxidative stress and signaling of inflammatory molecules or genetically affecting lncRNAs, DNA methylation, and cancer-related genes, or even through Ca^{2+}-dependent pathways (**Figure 3**). All results from the above pathways indicated that metformin effectively inhibited cancer invasion and metastasis, and could provide a novel treatment strategy for cancer.

Although many current evidences show that metformin has effective cancer suppressive effects, the available information is highly limited. Generally, it is accepted that not all *in vitro* and *in vivo* work using animal models translates into clinical outcomes in humans. First of all, in most instances, metformin concentration used in *in vitro* studies is much higher than the therapeutic level accepted for use in humans [103]. Currently, laboratory and animal studies on metformin show that drug concentration is much higher than which can be used in the human body. Although some studies have found that low metformin concentrations (that is, the blood concentration that can be achieved in the human body) can suppress cancer [25, 104-106], few studies have explored whether different drug concentrations have different mechanisms of action.

Also, many clinical trials have shown that metformin has a useful antitumor effect [14, 16], but this is not obvious for its anti-cancer effect in some types of cancers [107, 108]. Recently, results obtained from a Cox regression analysis of 320,000 diabetics, did not support the association of metformin treatment with the incidence of major cancers (except prostate and pancreatic cancers) [109]. Therefore, there is a need for large-scale, randomized, double-blind, and placebo-controlled studies to conclusively examine the efficacy of metformin in different forms of cancers.

Recently, one of the cancer hallmarks that plays a significant role in the progression of cancer is high cellular glucose metabolism [110, 111]. Moreover, most previous clinical tri-

Table 2. Different mechanisms by which metformin inhibits invasion and metastasis in different cancers

Cancer type	Pathways	Cell line	Animal experiment	Ref.
Breast cancer	COX2 signaling	MDA-MB-231	NO	[77]
	MiR-200c/AKT signaling	MDA-MB-231, MCF-7, T-47-D and BT549	YES	[52]
	Scribble/MST1, LATS1/YAP signaling	MCF7	YES	[86]
	AMPK signaling	MDA-MB-231 and MCF-7	YES	[42]
TNBC	TGF-β signaling	MCF7, MDA-MB-468, BT-549, SUM159PT, HS578T, MDA-MB-436 and MDA-MB-231	NO	[64]
	ERK/RAD51 signaling	MDA-MB-231 and Hs578T	YES	[67]
Glioblastoma	AKT/PI3K signaling	SF268 and U87	NO	[60]
	AMPK/mTOR signaling	A172	NO	[32]
	EMT signaling	GBM-TS	YES	[49]
NSCLC	miR-381/YAP signaling	A549, H1299, Calu6, H520 and 95-D	YES	[53]
	IRF-1/YAP signaling	A549, H1299, Calu6 and H520	YES	[85]
Cervical cancer	MALAT1/miR-142-3p signaling	SiHa and HeLa	YES	[79]
	TGF-β/mTOR/p70S6K	HeLa and SiHa	NO	[62]
	HIF-1α/CAIX signaling	C-4I (CRL-1954) and HTB-35 (SiHa)	NO	[87]
	PI3K/AKT/mTOR signaling	CaSki, SiHa and HeLa	YES	[61]
	insulin signaling	SiHa and HeLa	YES	[71]
ESCC	EMT signaling	TE-9	YES	[56]
	AKT/NF-κB signaling	TE1, TE2, TE4, TE5, TE6, TE8, TE10, TE11, TE14, TE15 and T.Tn	YES	[68]
Esophageal cancer	AMPK/mTOR signaling	KYSE150 and KYSE410	YES	[35]
Rectal cancer	EMT signaling	SW837, SW1463, HCT116 and LS513	YES	[48]
Liver cancer	AMPK/TIP30 signaling	MHCC97H	YES	[38]
	AMPK signaling	Hep3B, C3A and HuH-7	NO	[33]
	HIF-1α signaling	MHCC97H	YES	[89]
	AMPK/PTEN/AKT signaling	HepG2 and SMMC7721	YES	[58]
	PI3K/AKT/mTOR signaling	HepG2 and Bel-7402	YES	[59]
Pancreatic cancer	LKB1/Snail signaling	Capan-1, Capan-2, PANC-1, Mia paca-2, CFPAN-1, BxPC-3, HPAC, SW1990, ASPC-1 and HEK-293T	NO	[50]
	miRNA signaling	AsPC-1, AsPC-1-GTR, MiaPaCa-2, and MiaPaCa-2-GTR	YES	[78]
	TGF-β1 signaling	Panc-1 and BxPC-3	YES	[63]
	MAPK signaling	SKOV3 and hey	NO	[92]
	ECM signaling	SKOV3 and HO8910-PM	YES	[94]
	AMPK/H3K27me3 signaling	SKOV3, ES2 and A2780	NO	[36]
	AMPK/VEGF signaling	A2780	YES	[40]
	SPHK1 signaling	TYKnu, CAOV3, Kuramochi and OVCAR5	YES	[93]
Lung cancer	IL-6 signaling	H1650 and PC-9	YES	[74]
Melanoma	AMPK/ERK signaling	A375, Mel Z, Mel IL, Mel MTP and Mel Me	NO	[65]
	AMPK/p53 signaling	A375, WM9, SKMel28, 1205Lu, Mel501 and Mewo	YES	[39]
Gastric cancer	VEGF signaling and EMT signaling	Clinical data	NO	[72]
	HIF-1α/PKM2 signaling	SGC7901 and BGC-823	NO	[88]
	non-coding RNA H19 signaling	AGS and SGC7901	NO	[82]
Prostate cancer	COX2/PGE2/STAT3 signaling	PC-3 and 22RV1	YES	[75]
	GTPase Rac1 signaling	PC3 and DU145	NO	[90]
	FAK signaling	PC-3 and C4-2B	NO	[83]
Human fibroblastoma	Ca^{2+}/PKCα/ERK/JNK/AP-1 signaling	HT-1080	NO	[91]
Bladder Cancer	STAT3 signaling	T24 and J82	YES	[76]
Colorectal cancer	EMT signaling	SW480 and HCT116	NO	[51]
Endometrial cancer	AKT/ERK (1/2) signaling	ECC-1	NO	[66]
Cholangiocarcinoma	AMPK/mTOR/FAK signaling	KKU-100 and KKU-452	NO	[34]
	AMPK/IGF-1R/IRS/TSC2 signaling	SNU-245 and SNU-1196	NO	[41]
Colorectal cancer	FAK signaling	SW-480 and HT-29	NO	[84]

TNBC: Triple-negative breast cancer; NSCLC: Non-small cell lung cancer; ESCC: Esophageal squamous cell carcinoma.

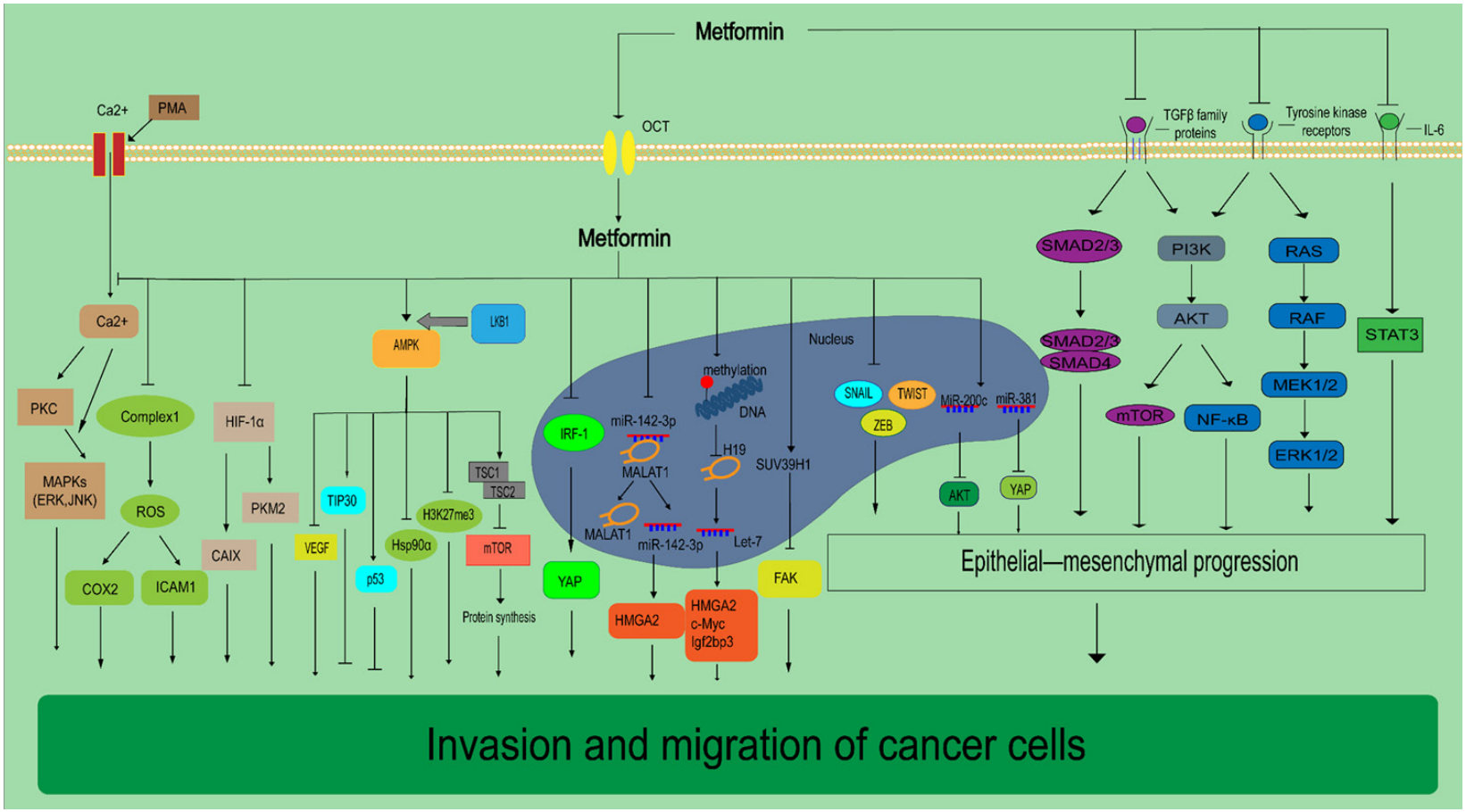

Figure 3. Mechanisms of metformin inhibiting cancer invasion and migration. Metformin inhibits cancer invasion and migration by activation of AMPK and inhibition of EMT processes. In addition, metformin also inhibits invasion and migration through these pathways. Such as, metformin works by inhibiting MALAT1/miR-142-3p or by inducing DNA methylation to antagonizes H19/let-7 axis; and the oncogene YAP promoter is inhibited by metformin by competing with IRF-1. It also reduced SUV39H1 by inhibiting integrin-FAK signaling. By inhibiting HIF-1α, metformin affects CAIX and PKM2 signals to achieve the effect of inhibiting invasion and migration. Metformin works by alleviating oxidative stress and inflammatory signaling through the COX2 pathway. Moreover, Ca^{2+} dependent PKCα/ERK and JNK/AP-1 signaling pathways are inhibited by metformin. MALAT1, metastasis-related lung adenocarcinoma transcript 1; H19, long non-coding RNA H19; IRF-1, interferon regulatory factor-1; SUV39H1, H3 Lys9 histone methyltransferase; HIF-1α, hypoxia inducible factor-1.

als on the efficiency of metformin focused on its anti-diabetic property, which is positively related to the development of cancers [112-114]. It was also suggested that diabetic patients diagnosed with cancer frequently have worse prognosis [115]. In addition, high glucose microenvironment accelerates tumor growth, and the anti-proliferative and pro-apoptotic effect of metformin is dependent on glucose concentration [116-118]. Therefore, it cannot be ruled out whether metformin decreases cancer risk and delays the progression of cancers by reducing glucose concentration and improving diabetes [115, 119]. Therefore excluding the effect of diabetes on the anticancer effect of metformin could be necessary, along with an evaluation of the effects of metformin on cancer patients without diabetes.

Lastly, since metformin has anti-proliferation, pro-apoptotic [120-123], and anti-invasion effects. More importantly, it was reported that metformin could improve chemosensitivity and reverse chemoresistance to various chemotherapeutics, including paclitaxel [124], cisplatin [67, 100], enzalutamide [125], EGF receptor tyrosine kinase inhibitors [74], and sofaenib [89]. Thus, it is not clear whether the anticancer effects of metformin are due to its anti-proliferation and pro-apoptotic effects, or its anti-invasion impacts, or even its chemosensitizing effects.

In summary, metformin inhibits cancer cell invasion and migration in some specific cancers. The present study has summarized the potential mechanisms of its anti-invasion and migration effects. There is no doubt that metformin is one of the most effective drugs that can inhibit cancer progression and has the potential for use in clinical practice. However, more clinical trials and basic research on this subject is needed.

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Disclosure of conflict of interest

None.

Address correspondence to: Drs. Jing Wang and He Li, Department of Gynecologic Oncology Clinical Research Center, Hunan Cancer Hospital, Central South University, 582 Xianjiahu Road, Yuelu District, Changsha 410013, Hunan Province, China. Tel: +86-731-8962821; E-mail: wangjing0081@hnca.org.cn (JW); lh0530@csu.edu.cn (HL)

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