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REVIEW

Research Progress on the Molecular Mechanism of Metformin Regulating AMPK Signaling Pathway in Inhibiting Liver Cancer

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Abstract: Liver cancer is one of the common malignant tumors worldwide and ranks among the top in cancer mortality statistics. In recent years, metformin, as a first-line drug with significant efficacy and low cost, has been widely used in clinical practice for the treatment of diabetes. Now, due to its potential inhibitory effect on cancer cells, it has received extensive attention. In view of this, a large number of studies have focused on the mechanisms by which metformin affects Liver carcinoma, with the AMPK pathway being particularly favored. This is because the AMPK pathway plays a significant role in autophagy, cell cycle arrest, inhibition of tumor angiogenesis, and suppression of epithelial-mesenchymal transition. To this end, we reviewed relevant research literature and systematically explored the mechanism of metformin's treatment of liver cancer through the AMPK pathway and its potential clinical applications. These studies provide an important basis for understanding the potential role of metformin in the treatment of liver cancer and provide important reference for future research on its new mechanisms and its clinical application.

Keywords: liver cancer, AMPK, cell cycle arrest, drug synergy, autophagy

Introduction

Despite the widely reported antitumor effects of metformin, its specific molecular mechanisms in liver cancer are complex and not yet fully elucidated. However, liver cancer is a common malignant tumor worldwide, including hepatocellular carcinoma, mixed cell carcinoma, and intrahepatic cholangiocarcinoma.[1–3] It is the sixth most common cancer globally and ranks third in cancer-related human deaths.[4,5] In China, the incidence of liver cancer is high, making it the third leading cause of cancer-related death.[6] The main triggering factors for liver cancer include: 1) biological factors: hepatitisB, hepatitisC, liver cirrhosis, aflatoxin, etc; 2) non-biological factors: alcohol, obesity, diabetes, hyperlipidemia, metabolic syndrome, etc.[7]

Diabetes is one of the most concerning factors among the causes of liver cancer mentioned above. Diabetes is an endocrine disease characterized by reduced insulin production, insulin resistance, or both. In recent decades, the incidence of type 2 diabetes has increased year by year and has become an epidemic worldwide.[8–10] Studies in different populations have found a significant correlation between diabetes and the incidence of liver cancer.[11]

Nowadays, there are various treatment options for liver cancer.[12] Although short-term prognosis can be improved, the long-term prognosis of liver cancer remains bleak, making new treatment methods an important focus of clinical research.[13,14] Metformin is an oral biguanide drug that has become a common medication for type 2 diabetes due to its significant efficacy and relatively few side effects (only gastrointestinal adverse reactions and rare lactic acidosis).[15–20] The mechanism of metformin in lowering blood sugar mainly involves reducing the production of liver glycogen, as well as reducing the absorption of glucose by the small intestine or reducing insulin resistance.[15,21–23] Many published studies have found that metformin can be used to treat or assist in the treatment of various cancers, such as: prostate cancer,[24]

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breast cancer,[25,26] colorectal cancer,[27] gastric cancer,[28] esophageal cancer,[29] glioma,[30] pancreatic cancer,[31] and liver cancer.[32,33] The latest retrospective studies also indicate the inhibitory effect of metformin on liver cancer.[34,35]

There are many pathways involved in the mechanism of metformin in the treatment of liver cancer. Among them, AMPK-related pathways have attracted special attention. It has been reported that the antitumor effect of metformin is mainly achieved by AMP activated protein kinase (AMPK).[36,37] Recent related studies have found that through the AMPK-mTOR pathway, autophagy of liver cancer cells can be induced and lipid accumulation in liver cancer cells can be reduced. Additionally,[38,39] the AMPK-P53 pathway can reduce epithelial-mesenchymal transition, thereby lowering liver cancer migration and invasion.[40] In models simulating glucose deficiency, the AMPK pathway can increase the lifespan of mice with liver cancer, allowing them to live to normal mature age.[41] It can promote oxidative phosphorylation through the AMPK-HIF1α axis, thereby disrupting glycolysis, not only inhibiting tumor cell growth but also causing DNA damage and apoptosis in hepatocellular carcinoma cells.[42] Given this, this article aims to systematically review how the AMPK pathway, as a central hub, integrates various downstream effects, and to focus on the challenges and opportunities in its clinical translation.

Mechanism of Metformin Acting on Liver Cancer Through AMPK

Metformin and AMPK

AMPK is an AMP-activated protein kinase and is a highly conserved trimer complex,[43] including an α subunit, a β subunit, and a γ subunit. Each subunit is encoded by 2–3 different genes,[44] the α subunit has catalytic activity, and the β and γ subunits help maintain protein structure.[45,46] Metformin activates AMPK by affecting mitochondrial respiratory complex I and AMP/ATP ratio, as well as upstream phosphorylation of Thr-172 of the catalytic α subunit.[47–50] Phosphorylation of Thr-172 in the α subunit is achieved through the activation of LKB1 (serine/threonine kinase).[51] It has also been reported in the literature that metformin can indirectly activate AMPK by inhibiting PKA (Protein Kinase A).[52]

AMPK acts as an energy sensor in eukaryotes[43] and plays an important role in the occurrence and development of metabolism and cancer.[53] In terms of energy and metabolism, AMPK can regulate the energy metabolism balance of cells, promote energy production, and limit energy use to maintain cell survival.[45,46] Its main functions include: enhancing the oxidation of fatty acids and glucose, inhibiting protein biosynthesis, thus achieving a dynamic balance of ATP levels.[54] In terms of cancer treatment, AMPK mediates antitumor effects through various mechanisms,[55] and the activation of AMPK may be involved in the tumor cell cycle arrest induced by metformin.[56] In addition, AMPK not only participates in inhibiting cancer cell proliferation, but also further enhances its anti-tumor activity by regulating p53 mediated tumor suppression (p53- tumor suppressor gene).[57]

Among the many AMPK-activated pathways, the mTOR pathway has been the most extensively studied. After AMPK activation, it participates in translation initiation and protein synthesis control by inhibiting the mammalian rapamycin target (mTOR), thereby regulating the growth and proliferation of tumor cells.[58] mTOR is a serine/threonine kinase, which is at least two multi-protein complexes, these complexes are called mTOR complexes 1 and 2, which play a key role in cell growth and differentiation.[58,59] mTOR is abnormally activated in HCC,[60] while metformin can inhibit the mTOR pathway[33,61,62] and inhibit the growth and metastasis of liver tumors.[63] In addition, a number of clinical and preclinical studies have shown that inhibiting mTOR can be used to treat many types of solid tumors, including esophageal squamous cell carcinoma, lung cancer, renal cell carcinoma and prostate cancer. Therefore, mTOR may be a potential target for HCC treatment.[64–67]

In short, as a potential metabolic tumor suppressor, AMPK has shown great potential in the prevention and treatment of cancer and has become a promising therapeutic target.[68–72] It is worth noting that in the following text, liver cancer refers to hepatocellular carcinoma, and cases of non-hepatocellular carcinoma are specially noted.

Autophagy

Autophagy refers to the process by which the cytoplasm is dissolved by lysosomes. Autophagy exists in three primary forms: microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA).[73] At present, there are two views on autophagy of tumor cells: 1) It is believed that autophagy can increase the resistance of tumors and help remove

necrotic cells, thereby promoting the progress of tumors;[74,75] 2) It is believed that autophagy is beneficial to cells. It can be used as a new way of treating tumors.[76,77] Studies have shown that autophagy can promote the apoptosis of cervical cancer,[78] breast cancer,[79] leukemia,[80] melanoma and lymphoma.[81,82] Although the role of autophagy remains controversial, studies have shown that autophagy plays a crucial role in liver diseases.[83] It is well known that metformin is an activator of AMPK and can also activate cellular autophagy.[81,84,85] Literature reports indicate that metformin activates AMPK to promote autophagy in pancreatic β-cells,[86] cerebral arteries,[87] breast cancer cells,[88] colorectal cancer cells,[89] and endometrial cancer cells.[90] Similarly, a large amount of literature confirms that metformin can also mediate autophagy in liver cancer.

Autophagy is a complex network woven by many factors such as TFEB,[91] E2F1,[92] FoxO1,[93] ATF4,[94] CEBPβ,[95] CHOP,[96] etc.[97] Among them, CEBPD is considered to be a tumor suppressor because its overexpression can cause cancer cells to die.[98] Metformin can induce increased expression of CEBPD through the AMPK pathway, thereby promoting tumor autophagy.[97] Src is a non-receptor tyrosine kinase that is highly expressed in a variety of tumors and is believed to play an important role in the occurrence and development of tumors.[99] Metformin can maintain the stability of CEBPD by inhibiting the expression of Src.[97] In addition, the viability of MHCC97H hepatocellular carcinoma cells significantly decreased after treatment with metformin (10mM), and the effect became more pronounced over time. Western blot analysis showed increased levels of autophagy-related protein LC3-II (a marker of autophagy initiation) and decreased levels of p62, which is caused by reduced phosphorylation of mTOR and p70 S6 kinase under AMPK activation.[97,100,101] Metformin can also be combined with sorafenib to increase the expression of microtubule-associated protein light chain 3 (LC3), leading to autophagy.[100] However, a few studies suggest that metformin inhibits autophagy. The article found that metformin (0.016–2 mM) treatment of serum-starved H4IIE cells dose-dependently reduced the expression of six autophagy-related proteins (Atg3, Atg5, Atg7, Atg12, LC3B, beclin-1), thereby inhibiting autophagy, and this effect could be blocked by an AMPK inhibitor. This phenomenon only occurs under glucose deprivation conditions (in glucose-free dishes), but not in dishes lacking only amino acids (with 5.5 mM glucose).[102] We believe this is related to the nutritional status of the cells, and the study may have been limited to macroautophagy while neglecting chaperone-mediated autophagy(CMA). The mechanism of metformin-mediated autophagy is shown in Figure 1.

Recent studies have confirmed this view, showing that the dual role of autophagy is related to the cellular environment and associated regulatory pathways. It acts as a tumor suppressor in the early stages of cancer cells but promotes cell survival and progression in later stages.[103] Under conditions of nutrient deprivation, AMPK activates

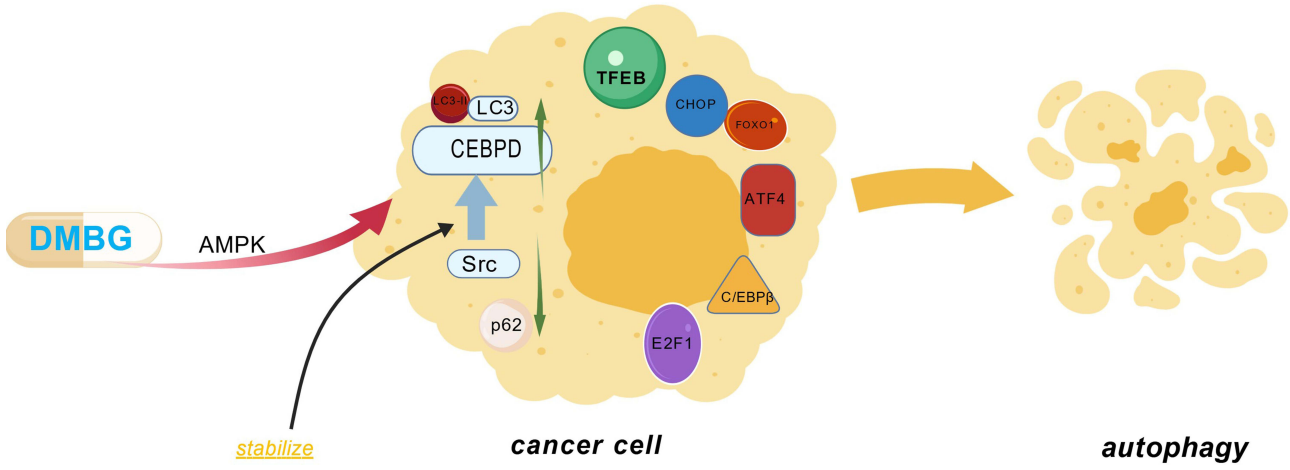

Figure 1 Mechanisms by which metformin induces autophagy through the AMPK pathway. On one hand: metformin can promote tumor cell autophagy by inducing CEBPD and maintain the stability of CEBPD by inhibiting Src; on the other hand: metformin can cause autophagy by reducing p70S6 and increasing LC3-II and LC3. Figure Created with BioGDP.com.

Abbreviations: DMBG, metformin; AMPK, AMP-activated protein kinase; CEBPD, transcription factor (CCAAT) enhancer-binding protein delta; Src, non-receptor tyrosine kinase; LC3, microtubule-associated protein light chain 3; LC3-II, a form of LC3; p62, selective autophagy receptor; TFEB, transcription factor EB; FoxO1, transcription factor Forkhead family member; E2F1, transcription factor E2F family member; ATF4, activating transcription factor 4; CEBPβ, transcription factor (CCAAT) enhancer-binding protein beta; CHOP, C/EBP homologous protein.

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ULK1, while the inhibition of mTORC1 relieves its suppression of ULK1, ultimately leading to the phosphorylation of downstream autophagy-related proteins and the initiation of autophagy.[104] As liver cancer progresses, accumulated stress and metabolic pressure lead to the upregulation of chaperone-mediated autophagy(CMA) as a compensatory mechanism. At this stage, macroautophagy activity decreases, activating NRF2 and promoting mTOR signaling pathways, which drive tumor cell proliferation and growth.[105]

Cell Cycle Arrest

Blocking the cell cycle can promote apoptosis of tumor cells. Studies have shown that one of the effects of metformin treatment for liver cancer lies in cell cycle arrest.[33] Subsequent more in-depth research has indicated that metformin can cause liver cancer cells to arrest at the G0/G1 phase of the cell cycle.[79] CAI et al believe that the transition from G1 to S phase of the cell cycle is regulated by cyclins and cyclin-dependent kinase inhibitors (CDKIs), including cyclin D1, cyclin E, p21CIP, and p27KIP. Cyclin D1 and cyclin E promote cell DNA synthesis and cell growth, and the over-expression of cyclin D1 and cyclin E promotes cancer progression.[106,107] Conversely, the downregulation of cyclin D1 and cyclin E expression limits the progression of the cell cycle to the G0/G1 phase and inhibits tumor cell proliferation.[56,108] Metformin can reduce the levels of cyclin D1 and cyclin E through the AMPK pathway, thereby blocking the cell cycle and inhibiting the growth of tumor cells.[32,56,79,109] Chen et al found that metformin inhibits the proliferation of liver cells through AMPK and its upstream kinase LKB1, thereby causing cell cycle arrest at the G0/G1 phase and upregulating P21clp1 and P27kip1 in a dose-dependent manner, while downregulating cyclin D1.[32] Another study has shown that metformin reduces BrdU (Thymidine analogues) incorporation in a AMPK-dependent manner, leading to cell cycle arrest at G0/G1, with the possible mechanism being the reduction of DNA production or induction of cell cycle arrest or apoptosis.[71] The Department of Medicine at Japan's Kagawa University confirmed that metformin induces cell cycle arrest both in vitro and in vivo. After treating Huh7 cells with 10 mM metformin for 24 to 72 hours, it was found that the expression of cell cycle regulators (cyclin D1, Cdk4, and cyclin E) decreased, and Huh7 cells accumulated in the G0/G1 phase with a reduction in the proportion of cells in the S phase and G2/M phase. Subsequently, 30 nude mice were subcutaneously inoculated with 5×10^6 Huh7 cells, and after the transplanted tumors reached a maximum diameter of over 6 mm, the mice were randomly divided into three groups and treated with metformin at doses of 1 mg per mouse, 2 mg per mouse, and phosphate-buffered saline (PBS), respectively. The results still showed a decrease in the expression of cell cycle regulators. This confirmed that metformin can arrest tumor cells in the G0/G1 phase.[110] The molecular mechanisms of metformin exerting cell cycle arrest and EMT are shown in Figure 2.

EMT

Epithelial-interstitial transformation (EMT) can cause significant changes in the morphology and phenotypes of epithelial cells, including cytoskeletal reorganization and changes in cell polarity, which increase the aggressiveness of cells.[111] It is an ongoing problem in the study of liver cancer. Therefore, EMT-Regulation has become one of the key targets for controlling the development of HCC.[112] Metformin activation of AMPK can not only increase cell differentiation, but also increase E-cadherin and reduce the expression of vimentin in HCC cells to inhibit EMT. These findings show that metformin partially inhibits the metastasis and invasion of HCC cells by reversing EMT.[113–116]

Similarly, in intrahepatic cholangiocarcinoma (including large duct type and small duct type), metformin can prevent EMT by activating the AMPK-FoxO3 pathway, increasing CK19 and E-Cadherin, and decreasing Vimentin, SNAIL1/2, and TWIST1 (EMT mesenchymal markers). In experiments, metformin at concentrations of 10–100 μM was applied to intrahepatic cholangiocarcinoma cells (derived from surgical specimens) for 48–96 hours, and its inhibitory effect was observed, showing a positive correlation with time and dose. When metformin-treated (metformin pretreatment for 57 days at 10 μM) and untreated intrahepatic cholangiocarcinoma cells were injected into mice, tumor growth was observed in the control group after 10 weeks, while no tumor growth was seen in the experimental group, thereby validating metformin's inhibition of EMT both in vitro and in vivo.[117] However, research regarding metformin's inhibition of EMT remains limited and requires further verification.

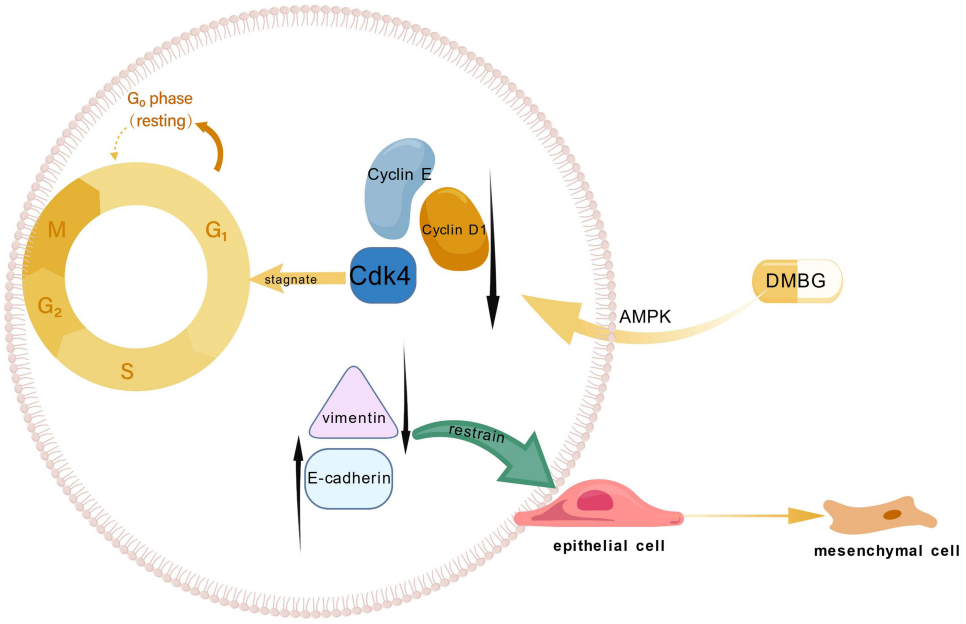

Figure 2 The mechanism by which metformin acts on the AMPK pathway leading to cell cycle arrest and EMT. Figure Created with BioGDP.com.
Abbreviations: DMBG, metformin; AMPK, AMP-activated protein kinase; Cyclin D1, cell cycle protein D1; Cyclin E, cell cycle protein E; Cdk4, cyclin-dependent kinase (a cell cycle regulator); E-cadherin, one of the markers of epithelial-mesenchymal transition; vimentin, intermediate filament protein (one of the markers of epithelial-mesenchymal transition); p21CIP and p27KIP, cell cycle regulators.

Glucose Metabolism

The antidiabetic effect of metformin depends on the activation of AMPK, which lowers blood sugar levels by reducing liver gluconeogenesis and increasing glucose uptake by skeletal muscles.[46,118] It is worth noting that metformin leads to a decrease in the expression of the glucose transporter Glut1, which can also lead to a decrease in glucose uptake to lower blood sugar, and this is also essential to regulate the fate of $CD4^+$T cells. Metformin can also cause reduced differentiation of T cells through the AMPK-mTOR pathway,[119] therefore, the potential connection between AMPK and Glut1 deserves our attention. As mentioned above, metformin can reverse EMT, thereby partially inhibiting the metastasis and invasion of HCC cells.[113–116] The study found that glucose starvation combined with metformin can increase metformin's ability to reverse EMT, antiproliferation, and migration.[120]

The relationship between AMPK and reactive oxygen species (ROS) is complicated. Although studies have shown that metformin can inhibit the accumulation of reactive ROS in an AMPK-dependent manner, studies have also reported that metformin can promote the accumulation of cytotoxic reactive oxygen species in a non AMPK-dependent manner.[121–123] This may be determined by specific signaling networks under different conditions, and it aligns with glucose's dual regulation of ROS—both glucose deficiency and high glucose concentration can induce intracellular ROS production through different signaling pathways that affect cellular metabolism and gene regulation. It has been shown that metformin reduces ROS production induced by high glucose, while simultaneously triggering and exacerbating ROS generation caused by low glucose.[124]

Studies on metformin's effect on glucose metabolism via AMPK are limited, and research has found that the lysosomal pathway can activate AMPK under low glucose conditions.[41,125] Since metformin can lower blood sugar and create a low-glucose environment, this suggests a direction for our future research.

Drug Combination

The treatment methods for liver cancer are diverse, with surgical resection being the preferred treatment for liver cancer.[126] In recent years, immunotherapy and targeted therapy have also played a crucial role in the treatment of advanced liver cancer. However, the existing treatments still face challenges such as drug resistance and the recurrence

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and metastasis of liver cancer.[127–129] Therefore, there is an urgent need for new drugs or combination therapies to overcome the existing limitations.

Sorafenib

Sorafenib is a first-line oral multitargeted kinase inhibitor for the treatment of advanced hepatocellular carcinoma, with evidence showing that it can block the Raf/MEK/ERK signaling pathway to inhibit tumor cell proliferation and also target tyrosine kinase receptors such as VEGFR-2 or PDGFR to produce anti-angiogenic effects.[130–133] However, long-term use of sorafenib can lead to sorafenib resistance, resulting in a decline in treatment efficacy and a lack of significant improvement in patient overall survival.[130,134] Therefore, there is an urgent need for new drugs to replace sorafenib or to be used in combination to counteract its limitations. Studies have shown that sorafenib can induce a shift from HIF-1α to HIF-2α, leading to an increase in HIF-2α, which is one of the reasons for sorafenib resistance.[135,136] HIF-2α can also lead to a decrease in TIP30, where TIP30, as a tumor suppressor factor,[137] is significantly associated with the invasion and metastasis of hepatocellular carcinoma. It not only regulates cell proliferation and apoptosis genes but also participates in cellular glucose tolerance, cancer metastasis, and invasion.[137–141] You et al found that combining metformin with sorafenib can inhibit the induction of HIF-2α by sorafenib, thereby reducing resistance to sorafenib and reducing cancer invasion and metastasis.[142] Guo et al also found that downregulating TIP30 can increase thioredoxin (TXN) expression, and increased TXN expression can accelerate tumor metastasis and increase tumor invasiveness.[143,144] Combining metformin with sorafenib can activate the AMPK pathway, leading to upregulation of TIP30 and downregulation of TXN.[145] Additionally, Ling et al believe that combining metformin with sorafenib can inhibit the activation of mTORC2 by metformin and increase the inhibition of mTORC1 by metformin, achieving synergistic effects. Compared to monotherapy, the combination not only blocks the cell cycle at the G0/G1 phase but also reduces key factors such as CyclinD1, CDK4, and Ki-67, and induces autophagy, significantly promoting the antiproliferative ability of hepatocellular carcinoma cells.[100] This process is achieved through the AMPK/mTORC1/2 pathway. In summary, the combination of metformin and sorafenib exerts synergistic effects through multiple pathways, effectively reducing HIF-2α levels, upregulating TIP30, and downregulating TXN, inducing cell cycle arrest and autophagy mechanisms, thus significantly inhibiting the progression and metastasis of hepatocellular carcinoma. Mechanism of metformin combined with sorafenib is shown in Figure 3.

5-Fu

5-Fu as an adenine synthase inhibitor has been approved as a chemotherapeutic drug for hepatocellular carcinoma and is widely used clinically.[146] HIF-1 is a basic transcription factor that regulates the transcription of various target genes in hypoxic responses.[147] HIF-1α is an oxygen-regulated subunit that mediates the basic function of HIF-1. Previous studies have shown that overexpression of HIF-1α may be involved in the pathogenesis of tumor chemoresistance,[148] and HIF-1α can increase the expression of multidrug resistance-related proteins P-gp and MRP1, thereby playing an important role in drug multidrug resistance.[149–151] Studies have found that metformin can reduce the expression of HIF-1α through the p-AMPK/mTOR pathway,[152,153] thereby reducing the expression of P-gp and MRP1, and consequently reducing hepatocellular carcinoma resistance to 5-Fu.[154]

Other Hypoglycemic Drugs

Metformin and thiazolidinediones are currently commonly used oral hypoglycemic drugs.[155–157] Rosiglitazone (Ros) is a representative of thiazolidinediones, which can improve insulin resistance and stimulate insulin secretion. Metformin is also a representative drug of biguanides. However, they have different side effects. Metformin can cause minor gastrointestinal adverse reactions and rare lactic acidosis, while rosiglitazone increases the risk of cardiovascular and fracture events.[158] Therefore, these two drugs are often used in combination in clinical practice to improve efficacy and reduce side effects.[156,159] Both drugs have been found to have anti-tumor effects. Metformin, as mentioned above. Rosiglitazone has been found to have anti-tumor capabilities in patients with lung cancer, prostate cancer, and colorectal cancer.[160] Therefore, some studies have found that a 1:1 mixture of the two drugs can inhibit hepatocellular carcinoma proliferation through the AMPK/p21 pathway with no obvious side effects.[161]

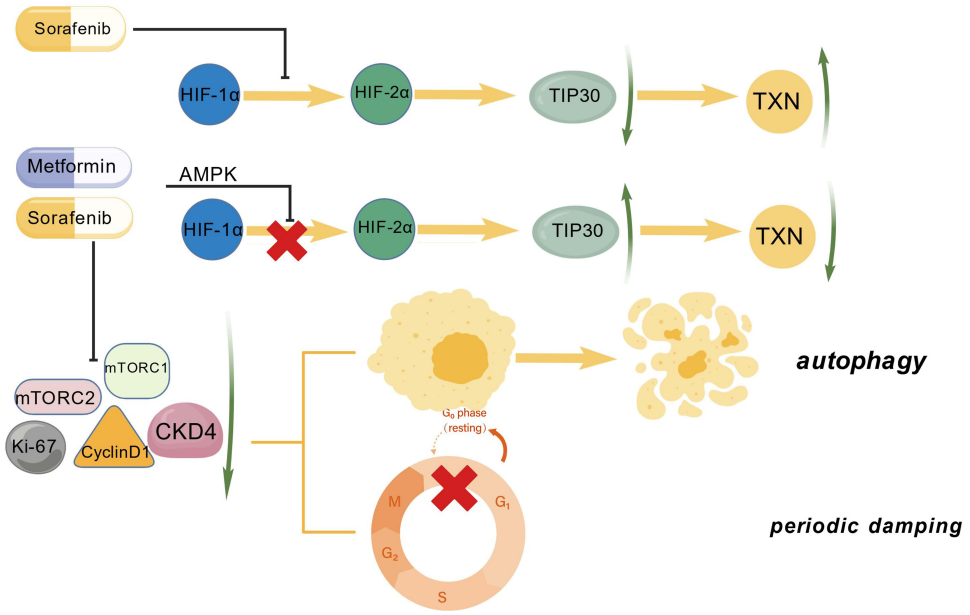

Figure 3 The mechanism of combined action of metformin and sorafenib on the AMPK pathway for hepatocellular carcinoma treatment. Figure Created with BioGDP.com.
Abbreviations: AMPK, AMP-activated protein kinase; HIF-1α, one of the hypoxia-inducible factors; HIF-2α, one of the hypoxia-inducible factors; TIP30, tumor suppressor factor; TXN, thioredoxin; Cdk4, cyclin-dependent kinase (a cell cycle regulator); Cyclin D1, cell cycle protein D1; Ki-67, cell proliferation marker; mTORC1, one of the rapamycin target protein complexes; mTORC2, another of the rapamycin target protein complexes.

The latest research has found that empagliflozin, as an antidiabetic drug, works by inhibiting the sodium-glucose cotransporter-2 (SGLT-2) in the proximal tubules of the kidney.[162] When used in combination with metformin, the two drugs complement each other to reduce the expression of liver injury indicators such as ALT, AST, and AFP, as well as tumor-related indicators such as ERK1/2, VEGF, and Ki67. This combination therapy also upregulates the ratio of p-AMPKα1/AMPKα1 (a subunit of AMPK) and Bax/Bcl-2, thereby inhibiting the progression of liver cancer. The mechanism of this combination treatment lies in the inactivation of the NF-κB (nuclear transcription factor) pathway via the AMPK pathway.[163] The mechanism of metformin combined with other drugs for liver cancer treatment is shown Figure 4.

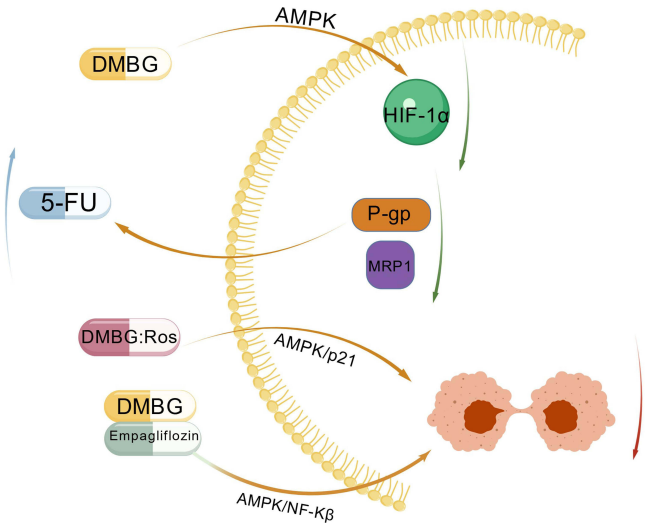

Figure 4 Mechanisms of Combined Treatment with Metformin and Other Drugs for Hepat-ocellular Carcinoma. Figure Created with BioGDP.com.
Abbreviations: DMBG, metformin; 5-Fu, fluorouracil; Ros, rosiglitazone; A-MPK, AMP-activated protein kinase; HIF-1α, one of the hypoxia-inducible factors; MRP1 and P-gp, multidrug resistance-associated protein; P21, cyclin-dependent kinase inhibitor; NF-κB, nu-clear factor-kappa B.

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Multiple studies have confirmed that metformin is a known antidiabetic drug with good antiproliferative activity, which can be used alone or in combination with other antiproliferative drugs to control tumors and is applicable to various cell types.[164–166] The mechanism of metformin combined drug therapy for liver cancer is shown in Table 1.

Combination with Cytokines

IL

Interleukin has a variety of important functions in the human body, including positive effects such as improving the body's immunity, but it also has side effects such as causing inflammation.[167] In the study by Zhao et al changes in interleukin in the plasma of 137 patients were monitored. The results showed that IL-6 and IL-8 were elevated in patients with liver cancer and were associated with liver cancer.[168] Among them, IL-8 as a chemokine in the human body has been shown to regulate the self-renewal and angiogenesis of tumor promoter cells.[169,170] In addition, IL-8 is positively correlated with the expression of PDL1 and PDL2. PD-L1 is a major immune checkpoint molecule that has a negative regulatory effect on T cell activity. High expression in a variety of cancers is associated with poor prognosis.[171,172] Therefore, IL-8 can be used as a checkpoint for immunosuppression.[168] The study by Chen et al found that metformin can reduce the expression of IL-8 through the p-AMPK/p-JNK pathway,[168] thereby achieving the purpose of controlling tumor growth. As a member of the endopeptidase protein family, MMP9 has been found to promote EMT, resulting in increased aggressiveness and metastasis of HCC cells.[173] In the latest research, metformin can reduce IL-8-mediated MMP9 elevation through the AMPK/JNK/MMP9 pathway, thereby reducing the aggressiveness of liver cancer.[168]

IL-22 is considered an inducible factor derived from T cells, mainly produced by Th1 and Th17 cells.[174] Its primary physiological functions include wound healing and innate antibacterial responses, while its receptor is mainly expressed by non-hematopoietic cells such as lung, gastrointestinal epithelial cells, and keratinocytes.[175] Pan et al found that IL-22 can induce the expression of anti-apoptotic and mitotic genes in hepatocytes.[176] Additionally, the presence of IL-22 allows damaged hepatocytes to survive, and these damaged hepatocytes are often precursors to HCC, thus participating to some extent in the occurrence and progression of cancer.[177] Finally, the expression of IL-22 in HCC tissue is higher in stages III–IV than in stages I–II patients, suggesting that IL-22 may be associated with enhanced proliferative capacity and increased malignancy of tumors.[178] Zhao et al further found that metformin inhibits the phosphorylation of STAT3/STAT4 through the p-AMPK/p-mTOR pathway, thereby blocking the derivation of Th1 and Th17 cells into IL-22, ultimately reducing the phosphorylation level of STAT3, Bcl-2 expression, and CyclinD1 expression.[119]

miRNA

miRNAs is a small non-coding RNA molecule that contains about 22–25 nucleotides. They are widely present in all kinds of organisms. Its main functional mechanism lies in regulating the transcriptional activity of the target gene,

Table 1 Mechanism of Metformin in Combination with Other Drugs in the Treatment of Liver Cancer

Drug Combination	Mechanism	Refs
Sorafenib	1. Reduce HIF-2α levels and reduce resistance to sorafenib; 2. Down-regulate TIP30 and down-regulate TXN to reduce the invasion of liver cancer; 3. Inhibit the activation of mTORC2, increase the inhibition of mTORC1, and reduce many key factors including CyclinD1, CKD4, Ki-67, induce autophagy, and arrest the cell cycle in the G0/G1 phase.	[100,142,145]
5-Fluorouracil	Reducing the expression of HIF-1α, thereby lowering the expression of drug-resistant-related proteins such as P-gp and MRP1.	[154]
Rosiglitazone	Metformin and its 1:1 mixture can inhibit hepatocellular carcinoma proliferation through the AMPK/p21 pathway.	[161]
Empagliflozin	Through the AMPK-NF-Kβ pathway, it reduces the expression of ALT, AST, AFP, p38MAPKa, ERK1/2, VEGF, Ki67 and many other indicators, upregulates the ratio of p-AMPKa1/AMPKa1 and Bax/Bcl-2, and inhibits the progress of liver cancer.	[163]

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thereby achieving gene silencing or activation, and playing a key regulatory role in various cell behaviors, functions and diseases.[179–181] Studies have found that high expression levels of miRNA-122 can enhance the sensitivity of liver cells to metformin. On the contrary, low expression can cause liver cells to be insensitive to metformin. The reason is that when miRNA is low, the AMPK pathway can be activated in advance, thereby inhibiting the activity of metformin.[182] Other studies have shown that the expression level of miRNA-23a can be significantly induced after metformin treatment. When the expression of miRNA-23a is suppressed, the apoptosis process of liver malignancies induced by metformin can be eliminated.[183] Through the AMPK/P53/miRNA-23a pathway, metformin can regulate the expression of miRNA-23a, thereby regulating the cell cycle regulation, apoptosis, EMT and other key processes involved in miRNA.[184,185]

P53

In addition to inducing the expression of miRNA-23a, P53 also plays an important role in the occurrence and metastasis of cancer. Studies have shown that when P53 is lacking, the inhibitory effect of metformin on liver cancer cells is weakened.[120] In fact, metformin regulates the expression of P53 through the AMPK pathway.[37] Specifically, AMPK activates the activity of P53 by phosphorylating the 15th serine. The mechanism of metformin combined with cytokines is shown in Figure 5 and Table 2.

Tumor-Initiating Cells and Aging

Tumor stem cells (CSCs) or tumor initiation cells (TICs) are a class of cell populations with significant tumor-causing characteristics. These cells are similar to the human body's ancestor stem cells and have the ability to differentiate and renew themselves. Therefore, they play a role in the entire cycle of tumor occurrence, development, metastasis, invasion and recurrence.[186,187] The study found that metformin can directly act on the starting cells of the tumor and exert antitumor effects by impairing the self-renewal ability of the starting tumor cells. At least part of the mechanism is related to the increase in the number of AMPK caused by metformin, which leads to a decrease in the level of mTOR and p70S6 kinases.[99] In addition, CD133 is one of the identification markers of CSCs.[188–190] Metformin reduces CD133

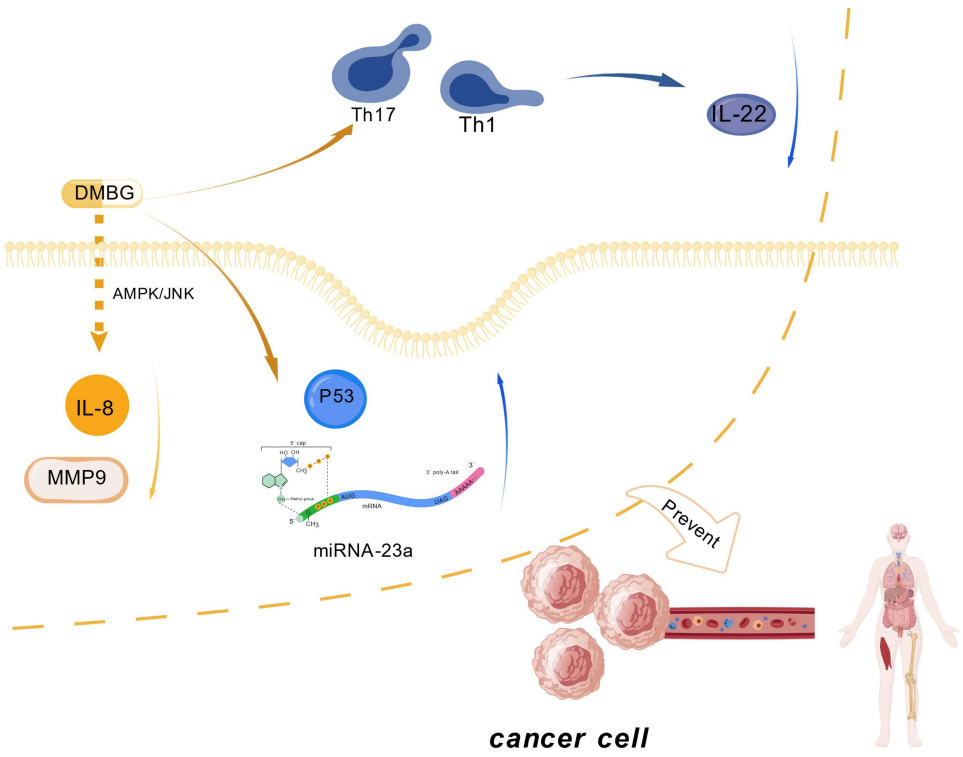

Figure 5 Mechanism of action of metformin in combination with other factors. Figure Created with BioGDP.com.
Abbreviations: DMBG, metformin; JNK, Amino-terminal kinase; Th1 and Th17, subtypes of T lymphocytes; IL22, interleukin 22; IL8, interleukin 8; MMP9, a member of the endopeptidase protein family; P53, p53 gene; miRNA-23a, microRNA 23a.

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Table 2 Mechanism of Action of Metformin Combined with Cytokines in the Treatment of Liver Cancer

Joint Cytokines	Mechanism	Refs
IL-8	Reducing the expression of IL-8 through the AMPK/JNK pathway and further reducing the expression of MMP9 not only inhibits the growth of liver cancer, but also reduces the aggressiveness of tumors.	[168]
IL-22	Inhibits STAT3/STAT4 phosphorylation through the p-AMPK/p-mTOR pathway, thereby blocking the differentiation and transformation of Th1 and Th17 cells to IL-22, reducing the occurrence of liver cancer.	[119]
miRNA-122	High expression levels of miRNA-122 can enhance the sensitivity of liver cells to metformin.	[182,183]
miRNA-23a	Through the AMPK/P53/miRNA-23a pathway, it participates in key processes such as cell cycle regulation, apoptosis, and EMT.	[183]
P53	Metformin activates the activity of P53 by phosphorylating the 15th serine through the AMPK pathway, thereby inhibiting the occurrence and development of tumors.	[37]

expression through two mechanisms: by regulating the CD133-P1 promoter region or by inactivating STAT3,[191,192] thereby targeting CSCs to achieve the treatment of liver cancer. The AMPK/CEBPβ pathway is a key mechanism by which metformin regulates the CD133 promoter.[193] CEBPβ consists of three isomers, namely LAP*LAP, and LIP,[194] where LAP can increase CD133 expression, while LIP can cause CD133 expression to decrease, so the ratio of LAP/LIP is the key to regulating CD133.[193] For the cutting-edge field of therapeutic aging, metformin can induce the aging of liver cancer cells through the AMPK/SIRT1 pathway to achieve the purpose of treatment. The specific mechanism is AMPK activation, which leads to a decrease in SIRT1 phosphorylation and inactivation of SIRT1 acetylase.[195] SIRT1 is a histone deacetylase, a member of the sirtuin family, which has important biological functions in regulating cell metabolism, aging, and stress response.[196] Its potential effects include regulating the acetylation of a variety of substrates, such as p53 and FoxO.[197–199] SIRT3 is also a member of the sirtuin family. As a key effector of the AMPK/mTOR pathway,[200] it can reverse regulate p-mTOR and downstream HIF-1α, resulting in cancer-suppressing effects.[201] At the same time, SIRT3 is also involved in the regulation of metabolism and aging,[202] so SIRT-3 is regarded as a key predictor of liver cancer.[203]

Combined with Radiofrequency Ablation

Zhang et al research found that metformin can significantly weaken the proliferation and invasion ability of liver cancer in patients with insufficient radiofrequency ablation.[204] Specifically, when radiofrequency ablation (RFA) is insufficient, the expression level of P-AKT in HCC cells increases and plays an important role in EMT.[205] Studies have shown that metformin can reduce the expression of P-AKT by up-regulating AMPK and PTEN, and further down-regulate PCNA and VEGF to overcome the increased invasiveness caused by insufficient radiofrequency ablation.[204]

Microenvironment and Inhibition of Angiogenesis

It is worth noting that the production of tumor blood vessels is essential for the growth of tumors, and this mechanism is also applicable in liver cancer.[206] In addition, the study of tumor microenvironment has become a hot topic in research. In liver cells, the microenvironment is mainly composed of hepatic stellate cells (HSC), fibroblasts, myofibroblasts, immune cells, and endothelial cells.[207] The study found that the interaction between HCC and HSC can promote tumor angiogenesis, while metformin targets hepatic stellate cells through the AMPK pathway to inhibit the activity of hepatic stellate cells, thereby effectively reducing the ability of tumor blood vessels to generate.[208]

Immunotherapy

Immunotherapy has become a hot topic in current research and has emerged as a novel approach for cancer treatment. Metformin can induce T cell differentiation through the AMPK-mTOR pathway to participate in immune regulation.[119] In patients with primary hepatocellular carcinoma and colorectal cancer liver metastasis, metformin treatment can

upregulate the expression of metabolism-related genes (Mpc1, Pck1, Adh4) in NASH (non-alcoholic steatohepatitis) mice, increase $CD8^+$ T cell motility, and restore the efficacy of anti-PD-1 therapy. In combination therapy with anti-PD-L1 and anti-VEGFR2 (simulating the clinical first-line regimen of atezolizumab plus bevacizumab), NASH also impairs its effectiveness, while metformin can reverse this inhibitory effect.[209,210] However, the lack of clinical data on the combination of metformin with immunotherapeutic drugs provides us with new insights for future research.

Non-AMPK Pathway

As shown above, metformin can trigger ROS accumulation in an AMPK-independent manner. Even when using an AMPK inhibitor to block AMPK phosphorylation, it cannot suppress the reduction of mTOR expression, indicating that metformin affects mTOR through pathways other than AMPK.[211] Previous reports have shown that metformin induces REDD1 through both AMPK-independent and p53-dependent mechanisms.[212] Numerous findings suggest that AMPK-dependent and AMPK-independent pathways may coexist.[79]

Future Directions and Challenges

In recent years, metformin has emerged as a potential liver cancer treatment drug, demonstrating multiple anti-liver cancer mechanisms by activating the AMPK pathway. Studies show that its mechanisms of action are complex and multi-dimensional, but many issues remain unexplained. HIF-1α and HIF-2α have both been confirmed to be associated with drug resistance. It is worth further research to explore their relationship, whether they act synergistically or antagonistically under certain conditions. Regarding the application of metformin in liver cancer treatment, clear standard guidelines are currently lacking. Studies have indicated that the specific effects of metformin in clinical settings are closely related to its usage concentration: high concentrations (5–30 mM) may induce cell apoptosis and block the cell cycle; while low concentrations (0.1–0.2 mM) tend to inhibit the proliferation of tumor stem cells.[192,213] And the dosage of metformin varies in different articles, with no unified standard. Additionally, although multiple clinical studies have confirmed the potential value of metformin as a liver cancer treatment drug, its safety issues still need further exploration. Current evidence is insufficient, and some studies even suggest that metformin may be ineffective or harmful for certain types of liver cancer, which to some extent increases its controversial application in the future clinical setting. Therefore, future research should focus on the following directions: first, draw on research from multi-omics and causal inference to validate the causal association between key downstream effect molecules of metformin (such as CEBPD, TIP30) and the risk or prognosis of liver cancer in large clinical cohorts, providing higher-level evidence-based medical evidence for the clinical application of metformin;[214] second, exploring more precise dosing regimens to optimize its therapeutic effects and reduce side effects. Only with a comprehensive understanding of its mechanisms can more accurate safety and efficacy evidence be provided for its clinical application.

Limitation

This review has attempted to collect as many literature reports as possible on the mechanisms related to metformin and AMPK, and explores its potential multitarget effects. However, although metformin has been preliminarily revealed in existing studies to regulate the metabolism of liver cancer cells by activating the AMPK pathway, there may still be limitations in the scope of literature collection. In addition, the mechanism of action of metformin is not limited to the AMPK pathway, and the synergistic effects of the signal conduction pathway are not described in detail in this review. It is worth noting that although current studies have shown that metformin has antitumor effects, its application in clinical practice still faces many challenges, including the lack of clear safety and conclusive evidence of effectiveness. The results of this part of the research suggest that its true clinical efficacy still needs to be further verified by more in-depth and detailed research.

Conclusion

This article provides a systematic review of metformin's effects on the AMPK pathway in the treatment of liver cancer, summarized as 1) inducing autophagy, 2) causing cell cycle arrest, 3) affecting glucose metabolism, 4) drug synergy, 5) inhibiting angiogenesis, 6) suppressing EMT, 7) combination with cytokines, and 8) immunotherapy combination. In

addition, metformin has been widely used in clinical practice for the treatment of diabetes. Study results suggest it may have potential therapeutic effects on liver cancer, but its true efficacy still needs further validation. This finding provides us with a new research direction: first, in the clinical aspect, exploring through large-scale clinical models. The efficacy and safety of metformin, and to identify the appropriate dose range and verify key downstream effector molecules; second, in terms of molecular mechanisms, to explore in detail the specific molecular mechanisms through which metformin acts via the AMPK pathway and its relationship with other pathways, such as the interaction between AMPK and non-AMPK pathways, whether they antagonize or synergize under specific conditions, and the coordination or antagonism between HIF-1α and HIF-2α in drug resistance; third, research on metformin in specific areas is limited, such as epithelial-mesenchymal transition, and more experiments are needed to support these studies.

Abbreviations

HCC, hepatocellular carcinoma; 5-Fu, 5-Fluorouracil; AMPK, AMP-activated protein kinase; DMBG, metformin; Ros, Rosiglitazone.

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Disclosure

The authors report no conflicts of interest in this work.

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