Cancer Cell International

REVIEW

Open Access

Non-coding RNAs as potential targets in metformin therapy for cancer

Yihan Zhang[1,2†], Yunhao Wu[2†], Zixu Liu[3†], Kangping Yang[2], Hui Lin[4] and Kai Xiong[1*]

Abstract

Metformin, a widely used oral hypoglycemic drug, has emerged as a potential therapeutic agent for cancer treatment. While initially known for its role in managing diabetes, accumulating evidence suggests that metformin exhibits anticancer properties through various mechanisms. Several cellular or animal experiments have attempted to elucidate the role of non-coding RNA molecules, including microRNAs and long non-coding RNAs, in mediating the anticancer effects of metformin. The present review summarized the current understanding of the mechanisms by which non-coding RNAs modulate the response to metformin in cancer cells. The regulatory roles of non-coding RNAs, particularly miRNAs, in key cellular processes such as cell proliferation, cell death, angiogenesis, metabolism and epigenetics, and how metformin affects these processes are discussed. This review also highlights the role of lncRNAs in cancer types such as lung adenocarcinoma, breast cancer, and renal cancer, and points out the need for further exploration of the mechanisms by which metformin regulates lncRNAs. In addition, the present review explores the potential advantages of metformin-based therapies over direct delivery of ncRNAs, and this review highlights the mechanisms of non-coding RNA regulation when metformin is combined with other therapies. Overall, the present review provides insights into the molecular mechanisms underlying the anticancer effects of metformin mediated by non-coding RNAs, offering novel opportunities for the development of personalized treatment strategies in cancer patients.

Keywords Metformin, Non-coding RNA, Long non-coding RNA, Circular RNA microRNA, Cancer

Introduction

In recent years, the number of cancer cases and deaths has remained high. It is estimated that there will be 1,958,310 new cancer cases and 609,820 cancer deaths in the U.S [1]. Compared with those in 2022, both figures have increased [2]. These findings indicate that cancer has emerged as a significant menace to human health, necessitating the immediate development of targeted and efficacious treatments.

Despite substantial progress in novel therapies, such as targeted therapy and immunotherapy for tumors over the past few decades, there continue to be numerous constraints in the precise diagnosis and treatment of tumors. Factors such as tumor heterogeneity and dynamics present formidable challenges for tumor treatment [3]. At the same time, the interactions and dynamics between the tumor and the tumor microenvironment make the treatment of tumors more complex and difficult [4]. In recent decades, numerous scientists have unearthed a multitude of novel applications for the age-old drug known as metformin, potentially heralding a new era in cancer treatment. Metformin, with the chemical formula $C_4H_{11}N_5$, is a naturally occurring

†Yihan Zhang, Yunhao Wu and Zixu Liu contributed equally.

*Correspondence:
Kai Xiong
xiongkai1@jxndefy.cn
[1] Department of Gastroenterology, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330008, Jiangxi, China
[2] The Second School of Clinical Medicine, Jiangxi Medical College, Nanchang, China
[3] The First School of Clinical Medicine, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi 330006, People's Republic of China
[4] Jiangxi Province Key Laboratory of Tumor Pathogenesis and Molecular Pathology, Department of Pathophysiology, School of Basic Medical Sciences, Nanchang, China

compound derived from the leguminous perennial herb goat's rue (*Galega officinalis* L.) [5]. Metformin inhibits hepatic gluconeogenesis and reduces insulin resistance and has long been widely used as an oral hypoglycemic agent and the first-line drug for the treatment of type 2 diabetes mellitus because of its safety, efficacy and tolerability [6]. As research continues, different new uses for metformin are being discovered, including its role in cancer treatment.

In 2005, Evans et al. [7] reported for the first time in a case–control study that metformin reduced the risk of cancer in diabetic patients and that this effect was positively correlated with the dose of metformin. Since then, there have been an increasing number of studies on the role of metformin in the treatment of various types of cancer.

Metformin has been shown to have a positive effect on the treatment of malignancies such as liver [8], osteosarcoma [9], colorectal [10], melanoma [11], pancreatic [12], bladder [13] and prostate [14] cancers. In the case of hepatocellular carcinoma (HCC), a retrospective cohort study revealed that metformin use reduced the risk of HCC by 21% compared with no treatment [15]; a meta-analysis of 11 studies by Ma et al. [16] revealed that metformin use was associated with a significant 41% reduction in mortality among 3452 patients with HCC. In addition, in another retrospective multicenter cohort study [17], 1566 patients with unresectable HCC treated with sorafenib at nine tertiary centers in South Korea were included. The patients were mostly male (83.8%), had chronic hepatitis B (77.3%) and were classified as Barcelona Clinical Liver Cancer stage C (BCLC-C) (78.3%) [17]. The results revealed improved long-term survival in patients treated with metformin [adjusted hazard ratio (aHR) = 3.464; $P < 0.001$], thus highlighting the important synergistic effect of metformin in combination with sorafenib [17].

With further research, it has been found that non-coding (nc)RNAs play important roles in metformin antitumor therapy. A number of ncRNAs involved in metformin antitumor therapy have been identified, but a number of mechanisms still need to be further elucidated as a basis for the individualized use of metformin and multimorbidity combination therapy [18, 19]. Therefore, the present review provides a detailed summary of the ncRNAs that play a role in the treatment of cancer with metformin and explores the ncRNAs that are regulated when metformin is combined with other therapeutic regimens (Fig. 1).

ncRNAs

ncRNAs are functional RNA molecules that are not translated into proteins and mainly include transfer RNAs, ribosomal RNAs, small RNAs and long non-coding (lnc)RNAs. Among them, microRNAs and lncRNAs are currently the most widely and intensively studied [20]. The present review also focused on the production process and mechanism of action of small interfering (si) RNA and circular (circ)RNA (Fig. 2).

micro(mi)RNA

miRNAs are a class of short endogenous ncRNAs that are extensively involved in the post-transcriptional regulatory activities of genes. miRNA genes are transcribed by RNA polymerase II to produce primary transcripts (pri-miRNAs) that are approximately several thousand bases in length. Subsequently, miRNA dimers are formed by the successive action of the protein complexes Drosha-DGCR8, Ran-GTP-Exportin-5 transporter protein, and Dicer-TRBP (Fig. 2). One strand is then rapidly degraded, and the other is translocated into the Argonaute 2 (Ago2) protein to form a miRNA-induced silencing complex (miRISC) [21–23]. RNA-induced silencing complexes (RISCs) bind to complementary target mRNAs and inhibit their translation, but studies have found that miRNAs can reversely amplify translation and bind to DNA under certain conditions, thereby controlling gene expression at the transcriptional level [24, 25].

lncRNA

In addition to miRNAs, the role of lncRNAs in metformin antitumor therapy has also gained attention. Many studies have shown that the mechanism of action of lncRNAs can be classified into two main categories: (i) Regulation of gene expression. First, guide lncRNAs activate or repress gene expression through repositioning of regulatory factors. Second, scaffolding lncRNAs contribute to the formation of ribonucleoprotein complexes. Third, decoy lncRNAs remove regulatory factors bound to the genome, thereby terminating their regulation. (ii) Regulation of protein activity and cell function. First, binding to specific proteins can regulate the activity of corresponding proteins, change the localization of proteins in the cell, or act as a structural component to form nucleic acid-protein complexes. Second, the complementary double stranded RNA formed by lncRNA binding to miRNA can generate endogenous siRNA under the action of Dicer enzyme. Third, it is a precursor molecule of other small molecule RNAs (such as miRNAs and Piwi-interacting RNAs) [26–30].

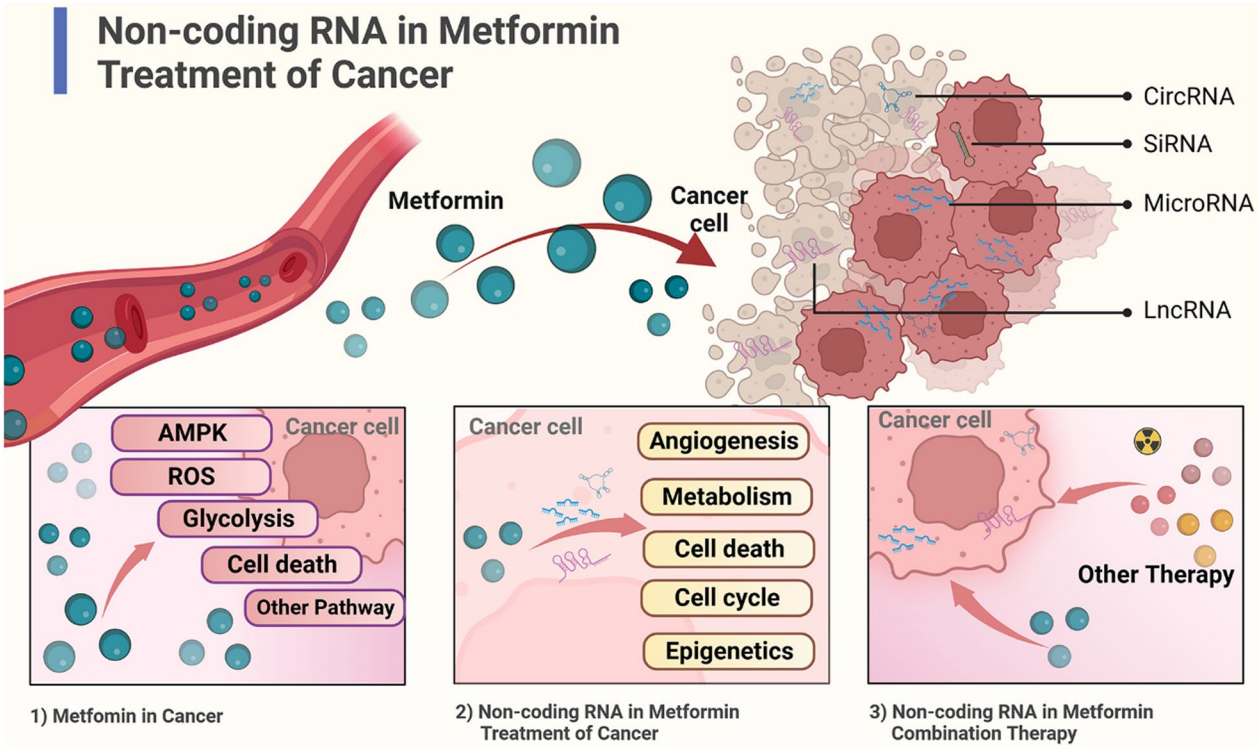

Fig. 1 Graphical abstract. Metformin can affect a wide range of cancer cell functions by modulating ncRNA. Metformin has been found to affect cancer progression by affecting AMPK, ROS, glycolysis, cell death, and other indirect pathways. Recently, ncRNAs have also been found to be involved in multiple pathways such as angiogenesis, cell metabolism, cell death, cell cycle and epigenetics in the treatment of cancer with metformin and can also be involved in the regulation of ncRNAs in combination with chemotherapy, radiotherapy and other therapeutic regimens, and can be used as predictive molecular markers of therapeutic efficacy. ncRNA: non-coding RNA; AMPK: adenosine monophosphate-activated protein kinase; ROS: reactive oxygen species; circRNA: circular RNA; siRNA: small interfering RNA; miRNA: microRNA; lncRNA: long non-coding RNA

circRNA

circRNAs are circular lncRNAs. The roles of circRNAs are very rich and include two main aspects: (i) Regulation of gene expression. First, circRNAs act as miRNA sponges to regulate gene expression by binding to their downstream target genes and releasing miRNAs [31, 32]. Second, they interact with RNA-binding proteins and regulate gene expression, such as the repression of PABPN1 translation by circPABPN1 [33]. Third, they regulate the process of variable shearing [34]. Fourth, they are associated with epigenetic inheritance and can regulate DNA methylation and histone modification [35]. (ii) It is capable of being translated into proteins, such as circ-ZNF609 [36], circ-SHPRH [37], circ-FBXW7 [38] and circMbl [39].

siRNA

siRNA is a common double-stranded ncRNA that is similar in length to miRNA and is usually 20–24 nucleotides in length [40]. The enzyme that catalyzes the generation of endogenous siRNA is Dicer enzyme, which cleaves dsRNA or short hairpin RNA to generate siRNA. SiRNAs bind to proteins such as Ago2 in the cytoplasm to form RISCs [41]. Single-stranded siRNAs in RISCs can bind complementary miRNAs and cleave them for gene silencing [42].

Metformin in cancer

Metformin achieves antitumor effects mainly through direct and indirect pathways (Fig. 3).

Direct pathways

AMP-activated protein kinase (AMPK) pathway

Metformin inhibits mitochondrial complexI and increases intracellular AMP levels, thereby activating AMPK [43]. Activation of AMPK by metformin inhibits the PI3K/AKT/mTOR signaling pathway, thereby inhibiting mRNA translation and lipid synthesis. Therefore, metformin can suppress tumor growth. It can also activate AMPK by activating hepatic kinase B1 [44]. In addition, presenilin enhancer 2 can also act as a target of metformin by binding to metformin and then forming a

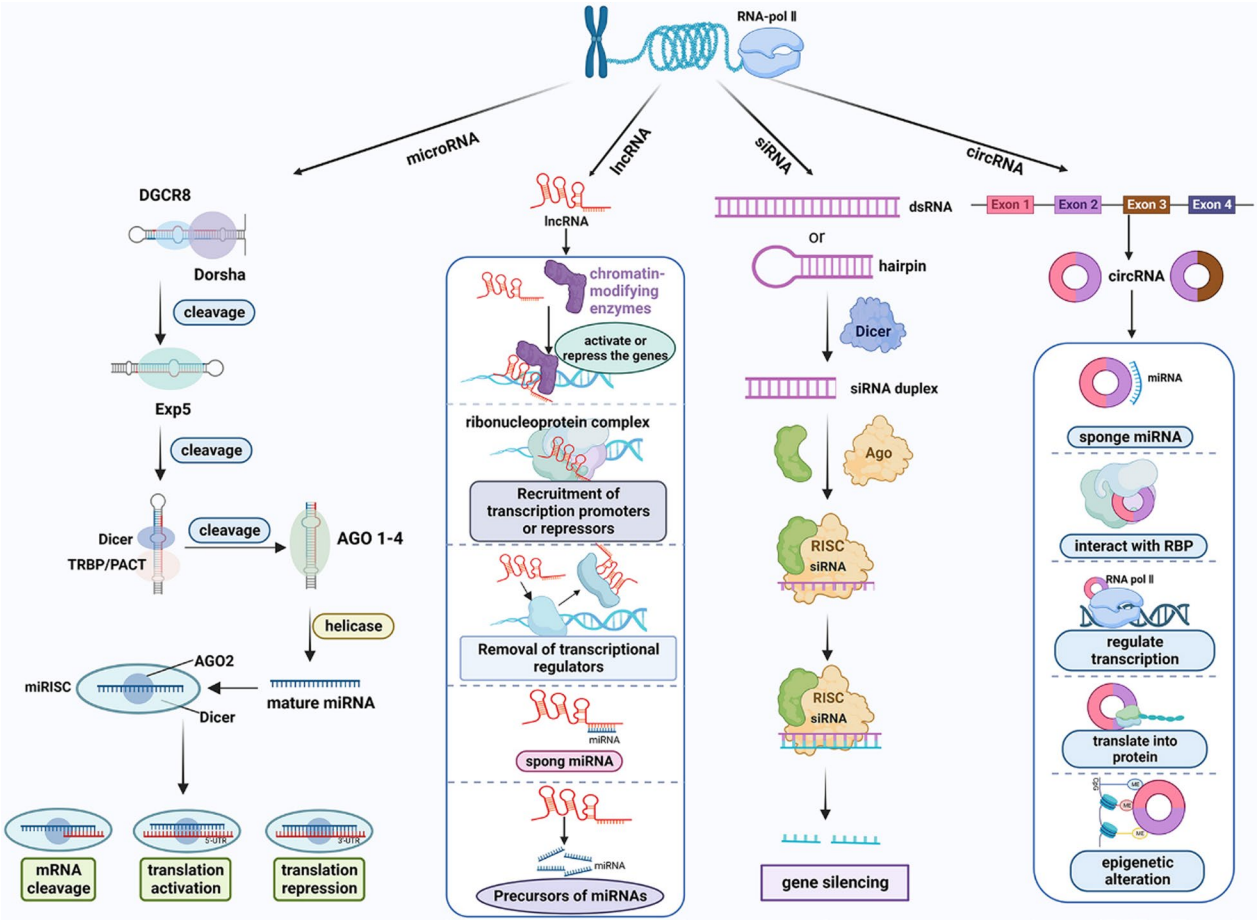

Fig. 2 Generation process and function of ncRNAs. The diagram illustrates the generation and main mechanism of action of miRNAs, lncRNAs, siRNAs and circRNAs. (1) miRNA: The miRNA gene is transcribed by RNA polymerase II to produce pri-miRNA. Pri-miRNA is processed into pre-miRNA with a stem-loop structure by Drosha-DGCR8. Pre-miRNA is transported into the cytoplasm with the assistance of the Ran-GTP-Exportin-5 transporter protein. Dicer-TRBP recognizes pre-miRNA and forms miRNA dimers by shearing and modifying stem-loop structures. Then, one strand is rapidly degraded and the other strand is translocated to AGO2 protein to form miRISC, which then exerts its function. (2) lncRNA: (A) Guide lncRNAs activate or repress gene expression through repositioning of regulatory factors. (B) Scaffold lncRNAs contribute to the formation of ribonucleoprotein complexes. (C) Decoy lncRNAs can remove regulatory factors that bind to the genome, thereby terminating their regulation. (D) They bind to miRNAs to form complementary double strands, which, in the presence of the enzyme Dicer, can produce endogenous siRNA. (E) siRNA and be used as precursor molecules for miRNAs and piRNAs. (3) siRNA: Dicer enzyme cleaves dsRNA or short hairpin RNA to generate siRNA. siRNA binds to proteins in the cytoplasm, such as Ago2, to form a RISC. Single-stranded siRNA in the RISC binds to and cleaves complementary miRNAs to achieve gene silencing. (4) circRNA: (A) Acts as a miRNA sponge and regulates gene expression by binding to its downstream target genes and releasing miRNAs; (B) interacts with RNA-binding proteins to regulate gene expression; (C) regulates variable shearing processes; (D) can be translated into proteins and (E) is associated with epigenetic inheritance and can regulate DNA methylation and histone modification. ncRNA, non-coding RNA; miRNA, microRNA; lncRNA, long non-coding RNA; siRNA, small interfering RNA; circRNA, circular RNA; pri-miRNA, primary miRNAs; pre-miRNA; precursor miRNA; dsRNA; double-stranded RNA; Ago2, Argonaute 2; RISC, RNA-induced silencing complex; RBP, RNA-binding protein

complex with ATP6AP1, a subunit of v-ATPase, which in turn inhibits v-ATPase and activates AMPK without affecting the intracellular AMP levels [45, 46]. The activation of AMPK by metformin inhibits the PI3K/AKT/mTOR signaling pathway, thereby inhibiting mRNA translation and lipid synthesis. Therefore, metformin can suppress tumor growth [47]. In addition, AMPK can induce the activation of the tumor suppressor protein p53, which inhibits tumor proliferation [48].

Reactive oxygen species (ROS)

ROS contain unpaired electrons, which are highly chemically reactive and can easily cause DNA damage, leading to cancer [49]. Metformin inhibits NAD(P)H oxidase activity, thereby reducing ROS production [50, 51]. Another review revealed that in normal cells with MnSOD, metformin increases the intracellular MnSOD expression level, thereby decreasing the ROS levels. By contrast, in AsPC-1 cancer cells without MnSOD, ROS

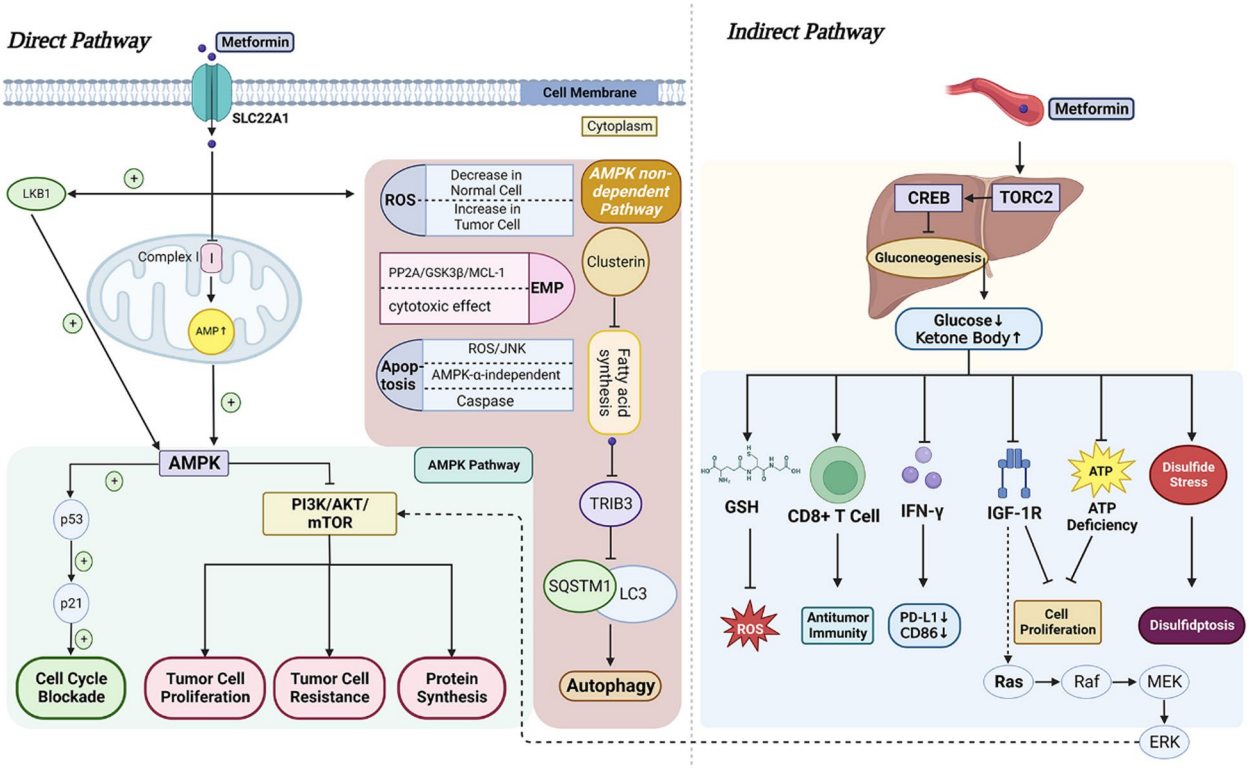

Fig. 3 The role of metformin in cancer. The direct pathway mainly includes two pathways. In the AMPK-dependent pathway, metformin can activate AMPK by activating LKB1, or by inhibiting mitochondrial complexl and increasing AMP levels. The activation of AMPK can inhibit the PI3K/AKT/mTOR pathway and activate p53, thus inhibiting tumor growth. In the AMPK-independent pathway, metformin reduces ROS level in normal cells and increases it in cancer cells, triggering apoptosis in tumor cells. Metformin can be combined with intermittent fasting to inhibit tumor growth via the PP2A/GSK3β/MCL-1 axis, or be combined with short-term starvation to induce a cytotoxic effect in vivo. Metformin also induces apoptosis in tumor cells through activation of the ROS/JNK pathway, or through downregulation of cell cycle protein D1 and upregulation of p53 expression via an AMPK-α-independent mechanism, or through activation of cysteine asparaginase. Metformin also binds to clusterin, which blocks fatty acid synthesis. Metformin also inhibits TRIB3 expression, enhancing the binding of SQSTM1 to LC3 and ubiquitinated proteins, resulting in anti-tumor effects. The indirect pathway consists mainly of combating oxygen free radicals by improving the glutathione redox status of normal cells. Indirectly combating tumors through ketone body-immune cell approaches such as promoting the expansion of CD8+T cells. Maintaining secondary lymphoid organ T cell activation and adaptation by inhibiting IFN-γ induction of PD-L1 and CD86. Inhibition of the hypoxia-inducible factor pathway by reducing the expression of IGF-1R, thereby inhibiting the tumor growth. In addition, without glucose or glutamine, ketone bodies alone cannot sustain tumor cell proliferation. Finally, glucose deprivation induces disulfidptosis in cancer cells. AMPK: adenosine monophosphate-activated protein kinase; LKB1: hepatic kinase B1; PP2A: protein phosphatase 2A; GSK3β: glycogen synthase kinase-3 β; MCL-1: myeloid cell leukemia-1; EMP: Embedn-Meyerhof-Parnas pathway; TRIB3: Tribbles homolog 3; SQSTM1: sequestosome 1; LC3: Microtubule-associated proteins 1 light chain 3; CD: cluster of differentiation; PD-L1: programmed death-ligand 1; IGF-1R: insulin-like growth factor 1

levels are elevated, which triggers tumor cell apoptosis [52].

Glycolysis and oxidative phosphorylation

It has been demonstrated that tumor cells can adapt to a more difficult metabolic environment by alternating between glycolysis and oxidative phosphorylation [53]. Metformin, an oxidative phosphorylation inhibitor, can be combined with intermittent fasting to inhibit tumor growth via the protein phosphatase 2A (PP2A)/glycogen synthase kinase-3 β (GSK3β)/myeloid cell leukemia-1 axis and does not cause weight loss or toxicity [54, 55]. In addition, treatments combining metformin and short-term starvation significantly affect glycolysis and oxidative phosphorylation, inducing significant cytotoxic effects and leading to a significant reduction in tumor growth in vivo [56].

Cell death

Apoptosis is a form of programmed cell death that is mediated by genes. There are a number of studies on metformin-mediated apoptosis in tumor cells. JNK can initiate apoptosis through an extrinsic pathway initiated by death receptors and an intrinsic pathway initiated

by mitochondrial events. Metformin can activate the NK/c-Jun signaling pathway by inducing ROS production in human osteosarcoma cells, thereby inducing their apoptosis [57]. The cell cycle protein d1 is essential for the G_1/S transition of the cell cycle [58]. Metformin can induce apoptosis in breast and cervical cancer cells by downregulating the cell cycle protein D1 and upregulating p53 expression through an AMPK-α-independent mechanism [59, 60]. In addition, it can selectively induce apoptosis in pancreatic cancer cells by activating cysteine asparaginase [61].

Other mechanisms

In addition, Apo J (clusterin) is also a target of metformin. When metformin binds to clusterin, SREBP-1c and its downstream target FASN can be inactivated, thus blocking fatty acid synthesis and inhibiting tumor growth. Previous studies have indicated that sequestosome 1 (SQSTM1) binds directly to microtubule-associated protein 1A/1B-light chain 3 (MAP1LC3/LC3) and promotes the autophagic degradation of ubiquitinated protein aggregates [5, 62]. Studies have found that the enhanced pseudokinase Tribbles homolog 3 (TRIB3) blocks the binding of SQSTM1 to LC3 and ubiquitinated proteins, leading to the accumulation of SQSTM1 and the inhibition of the clearance of ubiquitinated proteins, which in turn inhibits autophagic flux and the ubiquitin–proteasome system and promotes tumor growth and metastasis. Metformin inhibits SMAD3 phosphorylation and hinders the KAT5-SMAD3 interaction, thereby attenuating KAT5-mediated K3 acetylation of SMAD333 to inhibit SMAD3 transcriptional activity and TRIB3 expression, resulting in antitumor effects [63, 64]. Notably, the antiproliferative effect of metformin in prostate cancer is not mediated by AMPK but through the p53/REDD1 axis, which provides a new direction for the study of AMPK-independent pathways [65].

Indirect pathways

In addition to its direct action, metformin also has antitumor effects through indirect action. Metformin, a widely used oral hypoglycemic agent, causes a decrease in glucose levels accompanied by an increase in ketone body levels, which improves mitochondrial respiration and glutathione redox status in normal cells [66]. Glutathione can fight against oxygen free radicals, thus preventing cells and tissues from becoming cancerous due to damage [67]. Ketone bodies also promote the expansion of CD8 + T cells due to immune checkpoint blockade while suppressing the expression of activation/depletion markers such as CTLA-4 in the spleen [68, 69]. Moreover, 3-hydroxybutyrate inhibits the induction of programmed death-ligand 1 (PD-L1) and cluster of differentiation (CD)86 by IFN-γ, decreasing the negative feedback signaling resulting from T-cell receptor (TCR) involvement. Low expression of ligands for PD-1 and CTLA-4 may maintain secondary lymphoid organ T-cell activation and adaptation [68, 70]. Thus, metformin can fight tumors indirectly through a ketone body-immune cell approach. In addition, Seyfried and Shelton [71] noted that for cells to use ketone bodies effectively, they must have intact normal mitochondrial respiration. Thus, in the absence of glucose or glutamine, ketone bodies alone cannot sustain tumor cell growth and proliferation. The same results were observed in human glioma cells [72]. Alongside this, Seyfried and Shelton [71] proposed that ketone bodies also protect normal cells from damage induced by aggressive tumor growth through various neuroprotective mechanisms [71]. Insulin-like growth factor 1 (IGF-1) is a cell surface receptor associated with rapid tumor growth. The reduction in blood glucose levels induced by the hypoglycemic effect of metformin reduces the expression of IGF-1R and inhibits the hypoxia-inducible factor signaling pathway, thereby suppressing tumor cell growth [73]. In conclusion, by lowering glucose levels and elevating blood ketone body levels, metformin also has an indirect positive effect on antitumor therapy, as well as protecting and nourishing normal cells. Additionally, studies have also revealed that glucose deprivation can induce cancer cell death characterized by high SLC7A11 expression, i.e., disulfidptosis [74, 75]. This may inspire new research ideas for metformin anticancer therapy.

miRNA in metformin treatment of cancer

Metformin can inhibit cell growth, survival, clonogenicity, wound healing ability and sphere formation and enhance the chemosensitivity of tumor cells (Fig. 4) [76, 77]. On this basis, metformin can not only directly regulate miRNA-mediated inhibition of tumor activity [78, 79] but also indirectly affect downstream genes in metabolic or precancerous pathways [76, 80]. The underlying mechanisms may be as follows (Table 1) [81].

Tumor angiogenesis

Metformin-mediated miRNA can also affect tumor angiogenesis [82]. Angiogenesis is an important target for chemotherapy intervention in tumors with poor prognosis. Increasing evidence has shown that miRNAs are closely related to the normal function of the cardiovascular system and angiogenesis, represented by miR-21 [83], which affects the proliferation and migration of vascular cells and inhibits endothelial cells while also regulating angiogenesis-related genes [84, 85]. Metformin can affect the occurrence and development of tumors by regulating miRNA-mediated angiogenesis and cell proliferation. This may be because miRNAs can regulate the expression

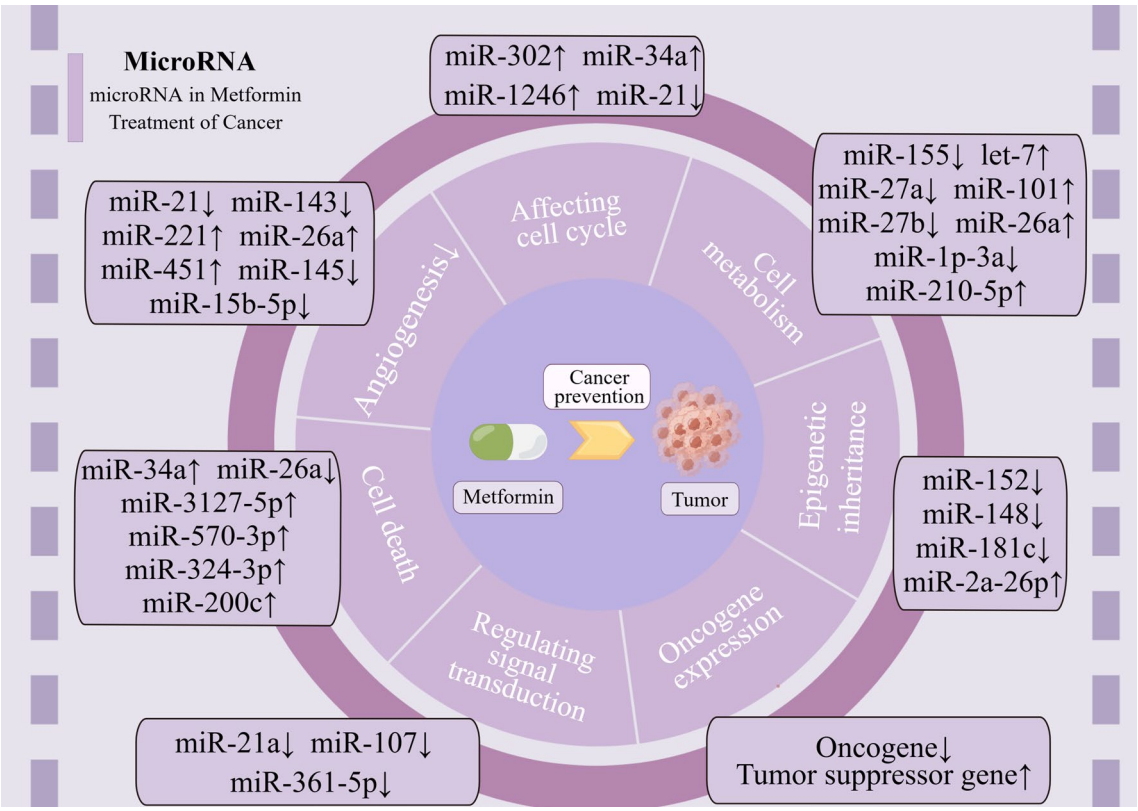

Fig. 4 miRNAs in metformin-treated cancer. Metformin can play an anti-tumor role by inhibiting tumor angiogenesis, regulating signal transduction, affecting cell metabolism, regulating oncogene expression, promoting tumor cell death, affecting epigenetics and cell cycle. More and more studies have found that miRNAs play a crucial role in this process. As shown in the figure, metformin achieved anti-tumor effect by downregulating miR-21, miR-143, miR-145, miR-15b-5p and upregulating miR-221, miR-26a, miR-451 to inhibit tumor angiogenesis. Metformin has anti-tumor effects by downregulating miR-21a, miR-107 and miR-361-5p to regulate downstream signal transduction. Metformin exerted anti-tumor effects by downregulating miR-155, miR-1p-3a, miR-27a, miR-27b and upregulating let-7, miR-210-5p, miR-101, miR-26a. Metformin can affect the occurrence and development of tumors by downregulating tumor promoting genes and upregulating tumor suppressor genes. Metformin can downregulate miR-152, miR-148, miR-181c and upregulate miR-2a-26p to affect epigenetics. Metformin has anti-tumor effects by upregulating miR-302, miR-1246, miR-34a and downregulating miR-21 to affect cell cycle. Metformin exerted anti-tumor effects by upregulating miR-34, miR-3127-5p, miR-200c, miR-570-3p, miR-324-3p and downregulating miR-26a to affect tumor cell death. miRNA: microRNA; PTEN: phosphatase and tensin homolog; HIF-1α: hypoxia-inducible factor 1 α; AMPK: activated protein kinase

of a variety of vascular-related genes, thereby regulating the proliferation, migration, differentiation and apoptosis of vascular cells [82]. For example, the upregulation of miR-26a inhibits angiogenesis in human HCC by affecting the hepatocyte growth factor-cMet pathway, while the upregulation of miR-221 increases p27 expression to play a role in the inhibitory effect of metformin on endothelial progenitor cell angiogenesis [86, 87]. In addition, metformin reverses the inhibitory effect of metformin on angiogenesis by downregulating miR-21 and negatively regulating the expression of phosphatase and tensin homolog and SMAD7, revealing a potential miR-21-dependent regulatory mechanism [88]. Notably, the development and angiogenesis of xenogeneic SiHa grafts in nude mice were significantly reduced after the administration of metformin. Metformin significantly attenuated the development and angiogenesis of xenografts in nude mice. This implies that metformin can reduce tumor blood supply by interfering with tumor blood vessel formation, thereby limiting tumor growth and spread [88]. Metformin has antitumor effects on cervical cancer cell migration, invasion and graft growth in nude mice by inhibiting the MALAT1/miR-142-3p interaction and reducing high mobility group AT-hook 2 expression, which reveals a new antitumor mechanism of metformin [88]. In the case of vascular endothelial cells, metformin inhibits the angiogenic capacity of endothelial progenitor cells by activating the AMPK-mediated autophagy pathway and increasing miR-221-mediated p27 expression [87].

Glucose also affects miRNAs that affect tumor angiogenesis. Hyperglycemia induces the upregulation of miR-34a, regulates the expression of sirtuin 1 and ultimately affects angiogenesis in vascular endothelial cells [89].

Table 1 Metformin regulates cancer through microRNAs

Author, year	miRNA	Expression	Cancer	Target	Pathway	Result	Model	(Refs.)
Jin D, 2020	miR-381	↑	NSCLC	YAP	YAP-snail	Proliferation↓, reverse EMT phenotype and migration and invasion↓	A549, H1299	[99]
Lee BB, 2023	miR-148/-152	↑	NSCLC	DNMTs	DNA methylation	Methylation↓, overall survival ↑	A549, H1650	[129]
Meng J, 2023	miR-9	↓	HCC	–	Twist1-YY1-p300/miR-9	The expression of oncogenes↓; malignant progression↓	PLC-PRF-5, Hep3B, HepG2	[130]
Cabello P, 2016	miR-26a	↑	BC	PTEN, EZH2	–	Proliferation, migration and invasion↓	MDA-MB-231 and MDA-MB-468 (both TNBC) and MCF-7 (intracavity)	[95]
Pulito C, 2017	miR-21-5p	↓	BC	CAB39L & Sestrin-1	–	Proliferation and metastasis↓	SUM159PT, MCF-7, BT-474, and BT-549	[78]
Zhang J,2017	miR-200c	↑	BC	AKT2 & Bcl-2	–	Growth, migration and invasion↓ and apoptosis↑	MCF-7, T-47-D, MDA-MB-231, BT549	[115]
Fujimori T, 2015	miR-302	↑	ICC	Rb, cyclin D1& Cdk4	–	Invasion↓	TFK-1 and HuCCT-1	[120]
Wang Y, 2018	miR-34a	↓	CRC	SNAIL1	p53-miR-34a-Sirtuin	Proliferation, migration and invasion↓	SW480 and HCT116	[121]
Feng YH, 2012	miR-21	↓	CRC	Spry2	MAPK/ERK	Proliferation and migration↓, apoptosis↑	HCT116	[123]
Dong J, 2020	miR-7	↑	NSCLC	AMPK	AKT/mTOR, MAPK/Erk, and NF-κB	growth, migration and invasion↓	A549	[79]
Zhang Z, 2020	miR-107	↓	NSCLC	Eomes	AMPK-miR-8-Eomes-PD-107	Cytotoxicity, PDCD1 transcription in CD8+T↑ and tumor growth↓	A459 and H460	[81]
Yan L, 2015	let-7	↑	OV	c-Myc, HMGA2 & Imp3	H7/let-19, Lin7/let-28	migration and invasion↓	A2780, Tara R127, ARK2, and HEK293	[104]
Yu Z, 2022	miR-3127-5p	↑	OV	LC3-I & LC3-II & Beclin 1	SNHG7/miR-3127-5p	Cell viability, migration, invasion and autophagy↓ and apoptosis↑	SKOV3, A2780	[108]
Ma M, 2020	miR-210-5p	↑	PCPG	PFKFB2	Glycolytic pathway	Proliferation and activity↓ and apoptosis↑	,PANC-1	[105]
Tanaka R, 2015	miR-221	↓	PCPG	P27	p2/CDK143/mTOR, PTEN, AKT	Growth↓ and apoptosis↑	MIAPaCa-2, PANC-1 and AsPC-1	[93]
Kalogirou C, 2016	miR-21	↓	RCC	PTEN	PTEN/Akt	Growth and reproduction↓ and apoptosis↑	CAKI-1 and CAKI-2	[83]
Deng Y, 2018; Melnik BC, 2020	miR-21	↓	SKCM	PTEN	PTEN/Akt	Cell proliferation, migration and angiogenesis↓	HaCaT	[91, 92]
Hong XL, 2023	miR-361-5p	↓	CRC	SHH	MYC/miR-361-5p/sonic hedgehog	Reverse dryness and eliminate chemical resistance triggered by Fusarium nuclear	HT-29, HCT 116	[77]

Table 1 (continued)

Author, year	miRNA	Expression	Cancer	Target	Pathway	Result	Model	(Refs.)
Suwei D, 2022	miR-5100	↓	Melanoma	STAT3	SPINK5/STAT3	Epithelial-mesenchymal transition↑ and metastasis↓	A2058, G361, B16-F10	[11]
Borzi C, 2021	miR-17	↑	NSCLC	LKB1	LKB1/AMPK	Modulating apoptosis	H1299, H358, and PC9	[114]
Kong Y, 2020	miR-2a-26p	↑	PCa	EZH2	AR and EZH2	Treat androgen refractory prostate cancer cells, growth and gene transcription↓	LNCaP, 22Rv1, PC3-Neo, and PC3-AR	[131]
Chen D, 2020	miR-141-3p	↑	PCa	TR4	QKI/circZEB1/miR-141-3p/ZEB1	Progression↓ and alters radiation sensitivity	C4-2, PC3, LNCaP, and Du145	[163]
Tan W, 2019	miR-708	↓	BC	CD47	CD47	Sphere formation, CD44/CD24 ratio, and tumor initiation↓ and chemosensitivity of BCSCs↑	MDA-MB-231 and MCF-7	[125]
Wang L, 2019	miR-497	↑	ESCA	PELP1	PELP1	Pyrodeath↑, cancer progression↓ and affect patient prognosis	–	[111]
Bao X, 2018	miR-570-3p	↑	OS	LCMR570& ATG3	–	Metastasis and autophagy↓, which facilitates the invasion of osteosarcoma cells	MG63, U2OS, 143B, KHOS, MNNG and SAOS2	[110]
Do MT, 2014	miR-34a	↑	wild-type p53 cancer	TRAIL	Sirt1/Pgc-1α/Nrf2	Sensitivity to oxidative stress and apoptosis↑	MCF-7, A549, SKOV3, HCT 116	[113]
Antognelli C, 2018	miR-101	↑	PCa	Glo1	MG-H1-AP and TGF-β1/Smad	It controls EMT, metastasis and invasion↓	DU145, PC3	[106]
Xie W, 2017	miR-34a	↑	RCC	Cyclin D1&p27KIP1	–	Growth ↓ and cell cycle arrest↑	ACHN, 769-P, and A498	[122]
Zhao W, 2016	miR-27a	↓	BC	AMPKα2	–	Growth ↓in a dose—and time-dependent manner	MCF-7	[102]
Wang F, 2016	miR-26a	↑	Oral cancer	Mcl-1	–	Apoptosis and antiproliferation↑	KB human oral cancer cell	[94]
Li W, 2012	miR-26a	↑	PAAD	HMGA1	–	Proliferation, migration and invasion ↓ and apoptosis↑	Sw1990 and Panc-1	[109]
Takahashi RU, 2015	miR-27b	↓	BC	ENPP1	–	Acquisition of CSC characteristics (e.g. drug resistance and tumor seeding ability)	MCF7, ZR75-1 and MDA-MB-231	[103]
Hou Y, 2021	miR-324-3p	↑	BC	GPX4	miR-324-3p/GPX4	Ferroptosis↑	MDA-MB-231 and MCF-7	[112]

miRNA: microRNA; NSCLC: non-small cell lung cancer; YAP: Yes-associated protein; EMT: epithelial-mesenchymal transition; DNMTs: DNA methyltransferases; HCC: hepatocellular carcinoma; BC: breast cancer; PTEN: phosphatase and tensin homolog; EZH2: Enhancer of zeste homolog 2; CAB39L: calcium-binding protein 39-like; AKT2: protein kinase B; Bcl-2: B-cell lymphoma-2; ICC: cholangiocarcinoma; AMPK: activated protein kinase; PDCD1: programmed cell death protein 1; OV: Ovarian serous cystadenocarcinoma; HMGA2: high mobility group AT-hook 2; CD: cluster of differentiation; LC3: Microtubule-associated proteins 1A/1B light chain 3B; PFKFB2: 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2; PCPG: Pheochromocytoma and Paraganglioma; RCC: Renal carcinoma; SKCM: skin cutaneous melanoma; SHH: sonic hedgehog; SPINK5: Serine Peptidase Inhibitor Kazal Type 5; LKB1: hepatic kinase B1; PCa: prostatic cancer; TR4: Testicular receptor 4; ESCA: Esophagus cancer; PELP1: Proline-, glutamic acid-, and leucine-rich protein 1; TRAIL: TNF-related apoptosis-inducing ligand; Glo1: glyoxalase 1; Mcl-1: myeloid cell leukemia-1; ENPP1: ectonucleotide pyrophosphatase/phosphodiesterase 1

Hypoglycemia may activate platelets to release miRNA, so it can be used as a potential biomarker for platelets in the hypoglycemic state [90]. In addition, metformin achieves an anti-tumor effect by downregulating miR-21 [91, 92], miR-143, miR-145 and miR-15b-5p and upregulating miR-221 [93], miR-26a [94, 95] and miR-451 [82] to inhibit tumors. However, much remains to be elucidated. Further studies are needed to explore the in-depth interactions and regulatory mechanisms between angiogenesis-related signaling pathways and miRNAs. In the future, miRNAs may be considered to predict or evaluate the efficacy or safety of metformin in regulating angiogenesis or to develop novel targeted drug delivery systems on the basis of these findings.

Tumor metabolism

Metformin has also been shown to inhibit tumors by affecting tumor metabolism. Metformin provides an important mechanism for the regulation of cancer metabolism and may represent a novel therapeutic target by regulating cancer cell metabolism, including reprogramming of cancer stem cells and drug resistance, involving the functional interactions of selective modulation of miRNAs [96]. Research has shown that metformin affects the proliferation and metabolism of colorectal cancer (CRC) cells by inhibiting oxidative phosphorylation and affecting metabolic pathways, especially glycolysis and respiration. This effect can be achieved by regulating the functional interactions of specific miRNAs, providing a new direction for potential therapeutic strategies [97]. In addition, a reduction in miR-155 can increase the sensitivity of MCF7-LTED cells to metformin treatment and reduce motility [96]. AMPK acts as a guardian of metabolism and mitochondrial homeostasis. It regulates miRNA expression in part by activating AMPK [98]. It specifically inhibits miR-1a-3p expression by activating the C/EBPβ/miR-1a-3p signaling pathway. At the same time, it also phosphorylates AUF1, thereby weakening the inhibitory effect of AUF1 on Dicer1 and eventually leading to changes in the expression of a series of miRNAs [97]. Notably, metformin also blocks the epithelial-mesenchymal transition (EMT) of non-small cell lung cancer and slows tumor growth and metastasis by inducing the upregulation of miR-381 and inhibiting Yes-associated protein and Snail [99]. In terms of tumor stemness, metformin reduces the development of cancer stem cell properties in breast cancer cells, including drug resistance and tumor vaccination ability, by inhibiting miR-27b-regulated exonucleotide pyrophosphatase/phosphodiester family member 1 [99]. Metformin treatment is able to reduce the metabolic differences between resistant and sensitive subgroups of breast cancer, revealing the potential of metformin for metabolic remodeling that may affect cancer chemoresistance [98].

In terms of gene expression, metformin can directly regulate the activity of S-adenosine homocysteine hydrolase (SAHH) to promote genome-wide changes in DNA methylation. In other words, the use of metformin in the treatment of tumors can not only downregulate the hypermethylation of oncogenes but also directly inhibit the proliferation of tumor cells [100]. Metformin can promote the expression of the tumor suppressor gene IGFBP7, thereby inhibiting the growth, proliferation, metastasis and invasion of cervical cancer [101]. In addition, metformin exerts anti-tumor effects by down-regulating miR-155, miR-1p-3a, miR-27a [94, 102], miR-27b [103] and upregulating let-7 [104], miR-210-5p [105], miR-101 [106] and miR-26a [94]. Specifically, metformin mainly directly regulates the insulin signaling pathway and indirectly interferes with cell proliferation and apoptosis [107].

Cell death

Metformin can inhibit the proliferation and migration of breast cancer cells, regulate the antioxidant system and mitochondrial pathway and may affect the expression levels of superoxide dismutase, Bax, Bcl-2, MMP-2, MMP-9, miR-21 and miR-155, which provides a new theoretical basis for the treatment and management of breast cancer [96]. Research has also shown that metformin promotes autophagy in ovarian cancer cells by altering SNHG7/miR-3127-5p. It can not only inhibit tumors but also improve the response of ovarian cancer to paclitaxel [108]. Metformin plays an important role in regulating miR-26a. As a cancer suppressor, miR-26a overexpression significantly inhibits cell proliferation, invasion, and migration and increases cell apoptosis, while downregulation of miR-26a has the opposite effect [109]. In addition, metformin targets LCMR1 and ATG12 by upregulating miR-570-3p [110]. It not only inhibits the metastasis of osteosarcoma but also reduces autophagy, which promotes tumor progression [110].

In terms of pyroptosis, studies have shown that proline-, glutamic acid- and leucine-rich protein-1 (PELP1), as a cancer-promoting factor, is closely related to the occurrence and development of late esophageal squamous-cell carcinoma (ESCC). Metformin or other apoptosis-inducing agents can affect the miR-497/PELP1 axis to inhibit the progression of ESCC [111].

Metformin induced ferroptosis in breast cancer cells by upregulating miR-324-3p and targeting GPX4, thereby showing a potential anticancer effect [112]. Notably, a study employing a xenograft model of mouse breast cancer demonstrated that metformin has the ability

to increase the expression of miR-324-3p [112]. This miRNA directly targets and suppresses the expression of GPX4, thereby leading to the initiation of ferroptosis [112]. This discovery not only highlights the potential of metformin as a potent inducer of ferroptosis but also suggests its ability to regulate other cell death pathways, including cuproptosis or disulfidptosis. In addition, metformin exerts anti-tumor effects by upregulating miR-34 [113], miR-17 [114], miR-497[111], miR-3127-5p [108], miR-200c [115], miR-570-3p [110], miR-324-3p and downregulating miR-26a [94] to affect tumor cell death.

Cell cycle

In terms of the cell cycle, to block the G0 to G1 cell cycle transition, metformin regulates the expression of miR-675-5p and significantly reduces the protein levels of cyclin D1 and cyclin-dependent kinase (Cdk) 4 and the phosphorylation level of the retinoblastoma protein [116]. Furthermore, metformin inhibits the proliferation of cholangiocarcinoma cell lines. Among them, metformin promotes the expression of the miR-302 cluster in tumors. The miR-302 cluster has been shown to inhibit the growth and proliferation of endometrial cancer by downregulating the expression of cyclin D1 and CDK1 [117]. miR-1246 is a target of the p53 family, and metformin may inhibit the metastasis and proliferation of human tumor cells by regulating miRNAs to inhibit cell cycle-related molecules [118]. For example, metformin has been found to promote the expression of miR-1246 in esophageal adenocarcinoma, ESCC and HCC cell lines, thereby exerting anticancer effects [119]. In addition, metformin has anti-tumor effects by upregulating miR-302 [120], miR-1246, miR-34a [121, 122] and downregulating miR-21 [123] to affect the cell cycle.

Epigenetics

In terms of epigenetics, metformin directly acts on epigenetic enzymes to change histone acetylation, histone and DNA methylation, and gene expression in tumor cells [124]. Specifically, metformin affects epigenomics and miRNA levels by activating AMPK and affecting the activity of various epigenetic modifying enzymes, such as histone acetyltransferases (HATs), histone deacetylases (HDACs) and DNA methyltransferases (DNMTs) [100]. It may play a role in the treatment of diabetes and the prevention of other diseases [124]. In addition, a study has also found that metformin inhibits the expression of DNMT in non-small cell lung cancer cells by upregulating the expression of miR-148/-152 family members, which may play a role in reducing the methylation level [19]. In terms of drug resistance, as one of the promoting regulators of mTOR and Akt transfection, miR-181c promotes Akt/AP-1 signaling and upregulates Raptor expression, which makes tumor cells more resistant to mTOR inhibitors and metformin [104].

Metformin can affect the occurrence and development of tumors by regulating miRNA-mediated angiogenesis, autophagy, the cell cycle, epigenetic inheritance, cell metabolism [125] and other cell activities [11]. The results of in vitro and in vivo experiments demonstrate that in addition to inducing tryptophan metabolic reprogramming [126], metformin can also directly enhance the activity of CD8+ T cells in a hypoxic environment, thereby increasing immunotherapeutic efficacy [127]. However, whether and what ncRNAs play a role in this process may be an area for further investigation. For example, existing research has found that a number of cells in the tumor microenvironment may exert regulatory effects on non-coding RNAs by releasing exosomes [128]. In addition, metformin can downregulate miR-152, miR-148 [129, 130] and miR-181c and upregulate miR-2a-26p [131] to affect epigenetics.

Finally, unlike the use and dosage of type 2 diabetes medications, the clinical use of tumor treatments, such as the determination of dose and the choice of treatment timing, has yet to be determined. In addition, how to combine metformin with other treatments to improve the effectiveness of treatment is also a challenge. Results of studies in xenograft models of breast cancer have shown that doses equivalent to 1500–2250 mg/day are required to inhibit tumorigenesis [132–134]. Nevertheless, most trials have based their studies on antidiabetic therapeutic doses of metformin, resulting in different treatment regimens and doses ranging from 1500 mg/day to 2250 mg/day. As a result, we do not really know whether these doses reach the concentrations required for antitumor effects. Also, the number of available clinical trials, which were conducted on different cancers at different times and in small numbers, makes it difficult to convince the public of the results. Furthermore, combining metformin with other treatments to enhance its efficacy is challenging. As an oral medication, metformin still presents certain challenges in terms of distribution and targeting within the body. For example, the chemosensitization/resensitization effects of metformin on various chemotherapeutic agents, which have different targeting effects on breast cancer cell carcinomas, are key drivers of intrinsic and acquired resistance [135–137].

lncRNA in metformin treatment of cancer

lncRNAs regulate vascular complications of diabetes and serve as biomarkers for cardiovascular disease and cancer [138], all of which are within the scope of metformin's

action (Fig. 5). Increasing evidence indirectly suggests that lncRNAs are closely related to the function of metformin [138–141]. Among them, the role of lncRNAs in the prevention of cancer by metformin has received increasing attention (Table 2). For example, lncRNAs have been implicated in the development of pancreatic cancer. Among them, urothelial cancer associated 1 (UCA1), LINC00976, TP73-AS1, XIST, LINC01559, HOXA-AS2, LINC00152, SNHG12, LUCAT1, LINC00462 and MACC1-AS1 have all been found to promote the occurrence and development of pancreatic cancer [119, 142]. By contrast, GAS5, LINC00261, LINC01111, DGCR5, MEG3 and LINC01963 inhibit the occurrence and development of pancreatic cancer [142]. Subsequently, a number of studies have explored the specific mechanism of metformin-mediated lncRNA [143–145]. H19 lncRNA promotes tumor cell migration and invasion by inhibiting let-7, a tumor suppressor miRNA [146]. Metformin downregulates H19 through DNA methylation and inhibits tumor cell migration and invasion, revealing a new mechanism of metastasis regulation [143, 144]. The expression of lncRNA taurine upregulated gene 1 (TUG1) is significantly increased following the use of metformin [144]. In bladder cancer, drug sensitivity tests and in vitro drug experiments have shown that the expression level of AC006160.1 is closely related to the

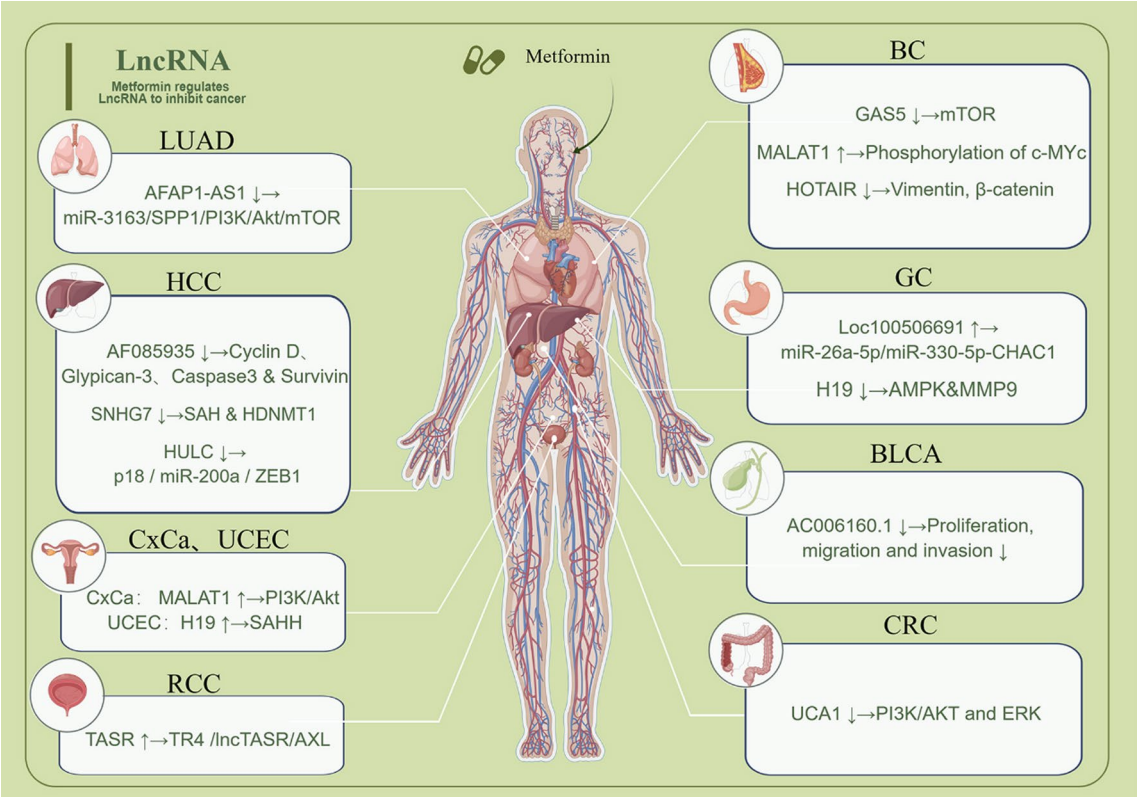

Fig. 5 lncRNA in metformin-treated cancer. More and more studies have shown that metformin can inhibit tumors by regulating lncRNA. Among them, metformin eventually affects the occurrence and development of LUAD, HCC, CxCa, UCEC, RCC, BC, GC, BLCA and CRC by mediating single or multiple lncRNAs. The specific mechanism is shown in the figure above and described in detail below. Metformin downregulates AFAP1-AS1 to regulate the miR-3163/SPP1/PI3K/Akt/mTOR axis to play a role in LUAD. In HCC, metformin downregulates AF085935 to downregulate cyclin D, glypican-3, caspase3 and survivinSNHG7 and downregulates HULC to regulate the p18/miR-200a/ZEB1 axis. In CxCa, metformin upregulates MALAT1 to regulate the PI3K/Akt axis. Metformin upregulates H19 to affect the expression of SAHH in UCEC. In RCC, metformin upregulates TASR to regulate the TR4/lncTASR/AXL axis. In BC, metformin upregulates MALAT1 to affect the phosphorylation of c-Myc, downregulates GAS5 to regulate the expression of mTOR and downregulates HOTAIR to affect vimentin and B-catenin. Metformin upregulates Loc100506691 to regulate the miR-26a-5p/miR-330-5p-CHAC1 axis and downregulates H19 to affect the expression of AMPK and MMP9 in GC. In BLCA, metformin downregulates AC006160.1 to inhibit tumor proliferation, migration and invasion. Metformin downregulates UCA1 to affect the amount of PI3K/AKT and ERK in CRC. lncRNA long non-coding RNA; LUAD: adenocarcinoma of lung; HCC: hepatocellular carcinoma; CxCa: cervical cancer; UCEC: uterine corpus endometrial carcinoma; RCC: renal carcinoma; BC: breast cancer; GC: Gastric cancer; BLCA: bladder cancer; CRC: colorectal cancer; HULC: highly upregulated in liver cancer; TASR TR4: testicular receptor 4; CHAC1: ChaC glutathione specific gamma-glutamylcyclotransferase 1; UCA1: urothelial cancer associated 1

Table 2 Metformin affects the growth and division of cancer cells by regulating the expression of LncRNAs (long-chain non-coding RNAs), thereby inhibiting tumor development

Author, year	lncRNA	Expression	Cancer	Target	Pathway	Effect on Cancer	Model	Refs.
Jiang Y, 2022	GAS5	↓	BC	mTOR, p-AMPK2 PTEN, p-mTOR and p-P6S7K	mTOR	Growth↓, apoptosis↑	MCF-7	[151]
Tseng HH, 2021	Loc100506691	↑	GC	CHAC1	miR-26a-5p/miR-330-5p-CHAC1	Growth, proliferation and movement↓	AGS, AZ-521, HR, NCI-N87, SNU-1 and TSHG	[152]
Huang Y, 2021	MALAT1	↑	BC	BECN1, VDAC1, LC3-II, CHOP and Bip	Phosphorylation of c-MYc	Proliferation↓, apoptosis↑ and cell cycle arrest↑	MCF7	[126]
Xia C, 2020; Xia C, 2018	MALAT1	↑	CxCa	IGFBP7	PI3K/Akt	The spongy effect and proliferation↓	Human cervical cancer SiHa	[88, 107]
Shi H, 2020	TASR	↑	RCC	TR4 and AXL	TR4/lncTASR/AXL	Progression↓	OSRC-2 and SW839	[155]
Wu P, 2019	SNHG7	↓	HCC	SAH and HDNMT1	–	Overcoming drug resistance and preventing recurrence of HPC	HPC cell line, FaDu	[149]
Li P, 2019	H19	↓	GC	AMPK and MMP9	–	Reduce metastasis and invasion	AGS, SGC7901	[153]
Zhong T, 2017	H19	↑	UCEC	SAHH&H19	H19/SAHH	DNA methylation↑ and proliferation↓	ARK2 and MCF-7	[100]
Qiu C, 2022	AFAP1-AS1	↓	LUAD	SPP1	miR-3163/SPP1/PI3K/Akt/mTOR	Proliferation, migration and invasion ↓	A549 and H3122	[150]
Golshan M, 2021	HOTAIR	↓	BC	Vimentin, β-catenin	–	Invasion, metastasis, migration↓, control EMT	MDA-MB-231	[154]
Liu J, 2022	AC006160.1	↓	BLCA	–	–	Proliferation, migration and invasion ↓	SV-HUC-1 (CL-0222), BIU-87 (CL-0035), HT-1376 (CL-0672), T24 (CL-0227), RT4 (CL-0431), RT-112 (CL-0682), 5637 (CL-0002), and UMUC3 (CL-0463)	[128]
Sabry D, 2019	AF085935	↓	HCC	Cyclin D 1, glypican-3, caspase3 and survivin	–	Proliferation↓, apoptosis↑	HepG2	[156]
Jiang Z, 2018	HULC	↓	HCC	miR-200a-3p	HULC/p18/miR-200a/ZEB1	Proliferation↓, apoptosis↑	LO2, HepG2 and HCCML3	[157]

lncRNA: long non-coding RNA; miRNA: microRNA; p-: phosphorylated; AMPK: activated protein kinase; PTEN: phosphatase and tensin homolog; BC: breast cancer; GC: gastric cancer; CHAC1: ChaC glutathione specific gamma-glutamylcyclotransferase 1; BECN1: beclin1; VDAC1: voltage-dependent anion-selective channel 1; LC3-II, microtubule-associated protein 1 light chain 3; CxCa: cervical cancer; IGFBP7: insulin-like growth factor binding protein 7; TR4: testicular solitary nuclear receptor 4; AXL: tyrosine protein kinase receptor; SAH: S-Adenosylhomocysteine; HDNMT1: human DNA methyltransferase1; RCC: renal carcinoma; HCC: hepatocellular carcinoma; HPC: hypopharyngeal cancer; UCEC: uterine corpus endometrial carcinoma; LUAD: adenocarcinoma of lung; HULC: highly upregulated in liver cancer; ZEB1: zinc finger E-box binding homeobox 1

body's sensitivity to metformin; the higher the expression level, the greater the sensitivity. Ac006160.1 is a tumor suppressor in the occurrence and development of bladder cancer [145]. For example, by promoting or inhibiting invasion and migration, DANCR, H19, HOTAIR, LINC00152, LINC00461, NEAT1 and LINC01857 are facilitating factors. GAS5, MT1JP, NEF, NKILA, LET, TFAP2A-AS1, and LncKLHDC7B are inhibited [147]. In addition, metformin inhibits the expression of specific protein (Sp) transcription factors in pancreatic cancer to exert anticancer effects, such as downregulating the expression of the lncRNA HULC. The same downregulation is also found in HCC cell lines [148, 149].

In lung adenocarcinoma, metformin suppresses tumor growth by downregulating AFAP1-AS1 and upregulating miR-3163 to regulate the secreted phosphoprotein

1/PI3K/Akt/mTOR axis [150]. In breast cancer, metformin can prevent over-activation of the mTOR signaling pathway by up-regulating lncRNA GAS5 to eventually suppress tumors [151]. Metformin inhibits the proliferation of gastric cancer (GC) cells by inhibiting the Loc100506691-miR-26a-5p/miR-330-5p-ChaC glutathione-specific gamma-glutamylcyclotransferase 1 axis [152]. In addition, metformin can downregulate H19, upregulate AMPK activation and downregulate MMP9 to inhibit the development of gastric cancer [153]. In breast cancer, metformin upregulates lncRNA MALAT1, HOTAIR, DICER1-AS1, LINC01121 and TUG1, thereby affecting the decrease of c-Myc phosphorylation to inhibit proliferation [126]. Another study showed that metformin can regulate the expression of the oncogene lncRNA HOTAIR and affect tumor growth [154]. Metformin can affect the sponge effect of lncRNA MALAT1/miR-142-3p to inhibit the proliferation of cervical cancer [88, 107]. In renal carcinoma, metformin can reduce sunitinib resistance by targeting the testicular receptor 4 (TR4) nuclear receptor and subsequently affecting the lncTASR/ tyrosine protein kinase receptor signaling axis. This is beneficial for inhibiting the occurrence and development of tumors [155]. In hypopharyngeal carcinoma, metformin downregulates SNHG7 expression by upregulating SAHH, thereby decreasing the resistance of hypopharyngeal cancer cells to paclitaxel and irradiation [149]. In EECs, metformin suppresses tumor growth by regulating the H19/SAHH axis and affecting genome-wide DNA methylation [100]. In bladder cancer, the overexpression of AC006160.1 significantly inhibits the proliferation and invasion of tumor cells and increases their sensitivity to metformin [128]. Metformin significantly reduces cyclin D1, lncRNA-AF085935 and glypican-3 to promote tumor apoptosis in HCC [156]. In addition, another study found that metformin regulated the HULC/p18/Zinc finger E-box binding homeobox 1 (ZEB1) signaling axis by inhibiting HBx-induced HULC overexpression, thereby suppressing HBV-induced HCC tumorigenesis [157].

Metformin regulates lncRNA expression and ultimately inhibits cancer through multiple mechanisms. The specific mechanism by which metformin mediates the suppression of tumorigenesis by lncRNAs is not fully understood. Although some studies have shown that metformin can inhibit the growth and invasion ability of tumor cells by activating the AMPK signaling pathway (the specific regulatory mechanism needs to be further studied) [100, 124]. A study has applied lncRNAs to establish a tumor prognosis model, and survival analysis results show that the model is well differentiated and that the best sensitive drugs can be selected based on this model [156]. This provides a new way to analyze the relationship between lncRNAs and cancer. Also, the model still needs to determine the most appropriate test standards and there is still a distance from the clinic.

Other ncRNAs in the treatment of cancer with metformin

In addition, some other ncRNAs, such as siRNAs and circRNAs, have also been reported in some studies. For example, Zhao et al. [141] developed a polycationic biguanide composed of metformin for in vivo siRNA delivery, which can systematically deliver RNAi therapies to cancerous tissues. Additionally, the combination of metformin and ornithine decarboxylase (ODC) siRNA has been reported to inhibit the proliferation and metastasis of human melanoma [158]. There are few studies on the role of circRNAs in metformin antitumor therapy. Some studies show that circRNAs can bind to miRNAs via sponge RNA-binding proteins, thereby affecting transcription, mRNA turnover and translation of coding genes and thus regulating gene expression [39, 159–162]. Some studies have indicated that metformin alters circRNA expression [143, 163]. For example, circZEB1 sponges miR-141-3p during radiation therapy in combination with metformin for prostate cancer treatment, ultimately enhancing radiosensitivity, as mentioned in detail in the combination therapy section below.

Mechanisms of ncRNAs in metformin combination therapy

ncRNAs also play important roles in metformin combination therapy (Table 3). *miRNA in metformin and rapamycin combination therapy.* Metformin can be combined with the mTOR inhibitor, rapamycin, to enhance the anticancer effect. Rapamycin reduces mTOR-regulated growth and survival signaling, including enhancing the expression of let-7b and miR-34a [144]. miR-34a expression is significantly reduced in a mouse model, as demonstrated by the tumor sphere test and luciferase assay associated with the mTOR pathway [144]. In addition, cell cycle analysis shows a significant decrease in miR-34a expression, inhibition of the mTOR pathway, and a decrease in the levels of its direct targets Notch, SNAI2 and Snail. Consequently, these factors lead to a reduction in the size of pancreatic cancer tumors and a decrease in the number of cancer cells [151]. MiR-221, which plays a role in pancreatic cancer treatment, is upregulated by metformin in p27 in PANC-1 and AsPC-1 cell models. This upregulation results in a decrease in p27 content, causing G_1 phase arrest and increasing the sensitivity of pancreatic cancer cells to TNF-related apoptosis-inducing ligand-induced apoptosis [143]. These findings contribute to the potential treatment of pancreatic cancer. These experiments provide the possibility of a

Table 3 Combination therapy of metformin via RNA for the treatment of cancer

Author, year	Combined treatment	ncRNA	Expression	Cancer	Model	Pathway	Result	Refs.
Cifarelli, 2015	Metformin and rapamycin	miR-34a	↓	PC	Panc02 pancreatic tumor cell transplant model	mTOR, Notch, Slug, Snail	Pancreatic tumor growth in obese, prediabetic mice↓; Glucose and insulin↓	[144]
Salgado-García, 2021	Metformin, doxorubicin and sodium oxamate	miR-106a	↓	CRC	CRC-derived HCT116 and SW480 colorectal cancer cells	Autophagy; HCT116/SW480/ULK1	ULK1 and LC3↑, Autophagy ↑	[147]
Jiang, Y.,2022	Metformin and OHT(4-hydroxytamoxifen)	lncRNA GAS5	↑	BC	tamoxifen-resistant MCF-7R cells	mTOR	mTOR↓; apoptosis↑	[151]
Zhan, Q,2022	cPLA2 siRNA and metformin	cPLA2- siRNA	–	GBM	PDX GBM	The mitochondrial energy metabolism	anticancer effects↑	[165]
Chen, D, 2020	RT with metformin	circZEB1 and miR-141-3p	↑	PC	mouse	TR4-mediated QKI/circZEB1/miR-141-3p/ZEB1	the progression of PCa↓	[163]
Coronel-Hernández, J.,2021	Metformin, Sodium oxamate and Doxorubicin	mir-26a	↓	CRC	CRC-derived HCT116 cells (ATCC CCL-247)	mTOR/AKT	Apoptosis and Autophagy↑	[148]
Tanaka, R.,2015	Metformin and TRAIL	miR-221	↓	PC	PANC-1 and AsPC-1 cells	TRAIL-DR5	TRAIL-induced apoptosis↑	[93]
Bhardwaj, A.,2018	Aspirin, metformin and fluvastatin	isomiR-140-3p	↓	TNBC	resistant to flusatatin preneoplastic cells	AMPK	Resistant level of cells to fluvastatin↓	[145]
Sabry, D.,2019	EGCG and metformin	lncRNA-AF085935	↓	HC	HepG2 cells	glypican-3	Proliferation↓ and apoptosis↑ of HepG2 cells	[156]
Wu, P.,2019	Metformin and paclitaxel or irradiation	lncRNA SNHG7	↓	HPC	FaDu cells	DNA methylation	HPC resistance↓; Recurrence↓	[149]
Lee, B.B.,2023	metformin and cisplatin	miR-148/-152 family members	↓	NSCLC	A549 lung cancer cell	DNMTs	DNMTs level↓, NSCLC Survival↓	[129]
Huang, Y.,2021	metformin and MALAT1 knockdown	lncRNA MALAT1, HOTAIR, DICER1-AS1, LINC01121 and TUG1	↓	BC	The human breast cancer cells MCF7	phosphorylation of c-Myc	cell proliferation↓; Apoptosis↑	[126]

ncRNA: non-coding RNA; siRNA: short interfering RNA; OHT: 4-hydroxytamoxifen; RT: radiation therapy; TRAIL: TNF-related apoptosis-inducing ligand; EGCG: epigallocatechin gallate; circ: circular RNA; PC: prostate cancer or pancreatic cancer; CRC: colorectal cancer; BC: breast cancer; GBM: glioblastoma; TNBC: triple-negative breast cancer; HC: hepatocellular carcinoma; HPC: hypopharyngeal cancer; NSCLC: non-small cell lung cancer; AMPK: activated protein kinase; DNMTs: methyltransferases

combination strategy for pancreatic cancer treatment [145].

miRNA in metformin and fluvastatin combination therapy

Metformin modifies resistance to fluvastatin in cancer cells by modulating the miRNA pathway [147]. In a model of fluvastatin-resistant preneoplastic cells, combination therapy with metformin and aspirin was shown to activate isomiR-140-3p. This activation resulted in a reduction in cellular resistance to fluvastatin [145]. Furthermore, the activation of AMPK by aspirin and metformin effectively inhibits the statin-induced abnormal upregulation of HMG-CoA reductase (HMGR), a key enzyme in the cholesterol biosynthesis pathway [147]. During the transition from normal to preneoplastic lesions, dysregulation of the 5' isomiRNA and its direct gene targets HMGR and HMGCS1 is observed in normal subjects. By activating AMPK, aspirin and metformin disrupt the cholesterol feedback pathway, leading to the inhibition of statin-induced abnormal upregulation of the HMGR. This sensitizes resistant cells to fluvastatin. In this pathway, the reduction of miR-140-3p-1 regulates

its target gene HMGR and its corresponding proteins. It is noteworthy that miR-140-3p-1 acts mainly during the transition from normal to pretumorigenic cells and has a lesser effect on the later development of the disease [154].

miRNA in combination therapy with metformin and chemotherapy drugs

Metformin enhances the efficacy of chemotherapeutic agents, such as cisplatin and doxorubicin [129, 130, 164]. In A549 cells, the combination of metformin and cisplatin synergistically upregulates the expression of miR-148/-152 family members and downregulates the posttranscriptional levels of DNMTs (DNMT1, DNMT3a and DNMT3b) [148]. miRNAs are susceptible to methylation-related silencing, which is regulated by the feedback loop of DNMT1, and miRNA expression can be affected by copy number alterations [129, 130]. Furthermore, in a CRC cell model (SW480), the combination of metformin, doxorubicin and sodium oxalate induces autophagy through the hyperlipidemic effects of ULK1 and LC3 [149]. In another CRC cell model (HCT116), the combination of metformin, doxorubicin and sodium oxalate significantly inhibited CRC cell proliferation through inhibition of the mTOR/AKT pathway, downregulation of hypoxia-inducible factor (HIF)-1α, reduction in the expression of miR-26a and the subsequent upregulation of ULK1, which led to apoptosis and autophagy [156]. These studies provide insights into the mechanisms underlying the cellular autophagy induced by combination therapy and highlight the roles of the miRNA-106a/ULK1 and HIF-1α/mTOR/AKT pathways in CRC [147]. Overall, these studies contribute to the understanding of metformin combination therapy as a potential approach to overcome drug resistance in CRC.

Other ncRNAs in metformin combination therapy

lncRNAs, circRNAs and siRNAs regulate the combination of metformin and other drugs. In the MCF7 model of human breast cancer cells, metformin upregulates lncRNAs, including HOTAIR, DICER1-AS1, LINC01121 and TUG1. Phosphorylation of c-Myc was further reduced in cells co-treated with metformin and MALAT1 knockout compared with those treated with metformin alone, further reducing breast cancer cell proliferation and increasing apoptosis. Specifically, MALAT1 knockdown increases the Bax/Bcl2 ratio and increases p21 expression while decreasing the expression of the cell cycle protein B1 [127]. Analysis of the combined effects of metformin with paclitaxel or irradiation by CCK-8 and membrane-associated protein-V/PI double staining revealed that downregulation of the lncRNA SNHG7 and enhancement of DNA methylation led to a reduction in hypopharyngeal cancer (HPC) resistance and relapse in the FaDu model [165]. Metformin upregulates DNMT1 expression through the activation of SAHH activity, leading to hypermethylation of the lncRNA SNHG7 promoter. As a result, reduced expression of the lncRNA SNHG7 sensitizes FaDu cells to paclitaxel and irradiation. This combination of metformin and paclitaxel represents a promising therapeutic strategy to overcome drug resistance and prevent the recurrence of HPC. It also sheds new light on the anticancer effects of EGCG and metformin, specifically their effects on glypican-3 and lncRNA-AF085935 in HCC. In the PDX glioblastoma (GBM) model, dual inhibition of phospholipid and mitochondrial metabolism through cytoplasmic phospholipase A2 (cPLA2)-siRNA knockdown and metformin treatment emerged as a potential therapeutic strategy [130]. This approach targets cPLA2 and demonstrates the potential of metformin in GBM treatment. Furthermore, combining radiation therapy with metformin induces TR4 nuclear receptor-mediated QKI/circZEB1/miR-141-3p/ZEB1 signaling, which promotes radiosensitivity to inhibit prostate cancer progression [163]. Consequently, prostate cancer cells exhibit increased radiosensitivity, thereby improving the efficacy of radiation therapy for prostate cancer.

Clinical trials related to ncRNA in metformin for cancer treatment

Current clinical trials are still only at the stage of measuring differences in ncRNA expression in metformin-treated cancers. The present review accessed all currently registered clinical studies related to non-coding RNA, metformin, and cancer through the ClinicalTrials.gov (https://classic.clinicaltrials.gov/) and found four studies that used metformin for cancer treatment in which microRNAs were measured and analyzed (Table 4).

One study [166] investigated cancer chemoprevention of potentially malignant lesions in the oral cavity by measuring the effect of systemic metformin hydrochloride on the change in millimeters of maximum diameter of potentially malignant lesions in the oral cavity (trial NCT03685409) and also measured the differential expression of miR-21 and miR-200 by three methods, namely, immunohistochemical analysis, miRNA analysis (PCR) as well as saliva miRNA analysis, which showed in the differential expression of miR-21 and miR-200 and then investigated the effect of metformin hydrochloride on the prevention of oral cancer through miR-21 and miR-200. A total of 62 men and women between the ages of 20–70 were enrolled in the Phase III clinical trial of metformin hydrochloride 500 mg orally in the test group and a placebo oral tablet in the control group.

Another oral cancer-based study [167] is investigating the preventive effect of metformin hydrochloride on oral

Table 4 Clinical trials related to non-coding RNA in metformin for cancer treatment

NCT Number	Conditions	Interventions	Outcome measures	Sex	Age, years	Phases	Enrollment	Study designs	Start date	Completion date
NCT03685409	Oral cancer	Drug: Metformin Hydrochloride 500 mg Drug: Placebo oral tablet	Clinical Outcomes Immunohistochemical analysis microRNA analysis Salivary microRNA	All	20–70	Phase 3	62	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Single (outcomes assessor) Primary Purpose: Prevention	1 October 2018	30 September2020
NCT03684707	Oral cancer	Drug: Metformin HCl 500 mg 24 h Sa Tab Other: Starch tablet	Evaluate lesion size in millimeters Measurement of salivary microRNA Measuring immunohistochemical marker	All	20–60	Phase 4	30	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Single (Outcomes Assessor) Primary Purpose: Prevention	15 September 2018	15 September 2019
NCT05468554	Thyroid cancer	Drug: metformin Radiation: Radioactive iodine (I-131)	Changes in AMH, Inhibin B and FSH levels due to the action of metformin Assessment of the effect of metformin on the parameters of oxidative stress The evaluate of difference in serum concentration of selected parameters of apoptosis The evaluate of difference in expression in selected microRNA	Female	18–45	Phase 3	160	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Triple (Participant, Care Provider, Investigator) Primary Purpose: Treatment	1 November 2022	30 April2026
NCT06044025	Castration sensitive prostate cancer	Drug: Metformin Drug: Turmeric	Assess feasibility of recruitment Evaluate time to PSA relapse with nutritional intervention on iADT Quality of life assessed by the Functional Assessment of Cancer Therapy-Prostate (FACT-P), version 4 Quality of Life assessed by the Aging Male Symptoms Questionnaire	Male	18–100	Phase 1	30	Allocation: N/A Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Supportive Care	February 2024	October 2026

cancer (trial NCT03684707), in which the trial combines measurements of salivary microRNAs (measuring salivary markers 31 and 210 in saliva and tissue biopsies) and measurements of immunohistochemical markers (measuring cytosolic protein A2 markers in tissues) over the course of a year, linking oral cancer to miRNAs. A total of 30 men and women between the ages of 20–60 were selected for the trial, with 500 mg of metformin hydrochloride and 500 mg of glucophage once daily in the test group and oral starch tablets in the control group for the phase IV clinical trial.

The third trial [168] explored the evaluation of metformin effect on the fertility of women treated with 131I for thyroid cancer (trial NCT05468554), with the primary outcome measure being changes in AMH, inhibin B and FSH levels and the primary endpoints were to assess the effect of metformin on serum AMH, inhibin B and FSH concentrations and differences in sinus follicle counts in a study group consisting of women with papillary thyroid cancer treated with 131I and to assess the effect of metformin on the recovery of the ovarian reproductive system in patients with thyroid cancer. This trial will assess the effect of metformin on oxidative stress parameters and differences in serum concentrations of selected parameters of apoptosis and assess differences in the expression of selected miRNAs.

A feasibility study to assess the maintenance role of intermittent androgen deprivation therapy in patients with biochemically progressive castration-sensitive prostate cancer [169] will assess the inhibitory effect of metformin and curcumin as a nutritional regimen for the maintenance treatment of prostate cancer, primarily through the assessment of time to prostate cancer recurrence by iADT nutritional intervention. The effect of metformin on miRNA, a tumor influencing factor, was explored by measuring the expression levels of biomarkers (including EMT and miRNAs) in blood samples with the expression levels of cancer dormant biomarkers (miR-200, TGF-β, BMP, immune and inflammatory factors) in blood samples, and observing the effects of metformin on the changes of the content of miRNAs. A total of 30 male prostate cancer patients with a history of prior treatment, aged 18–60 years, were selected for the trial, which was conducted as a single-group OPEN LABEL intervention, where subjects would start metformin and curcumin within 14 days. The trial takes the objective evaluation criteria of the serological tests described above as a supplement, somewhat compensating for the adverse effects of the Hawthorne effect associated with unmasked trials [170].

At present, the clinical research on ncRNA in metformin treatment of cancer has not been fully performed and all of the above trials have not yet published the results. In the future, a wider range of experiments is needed, and lncRNAs and circRNAs also need to be included in the scope of the study.

A metformin-based therapeutic approach compared to the direct delivery of ncRNAs

In comparison to the direct administration of lncRNA for the treatment of cancer, the modulation of lncRNA by metformin presents several advantages for this purpose.

Superior safety profile. Metformin is a widely utilized pharmaceutical agent with a well-established safety record. By contrast, the long-term safety of direct delivery of lncRNA, a relatively novel therapeutic approach, has yet to be fully substantiated. The potential risks to the patient's body, when metformin is used to modulate ncRNA for cancer treatment, are relatively minimal. The side effects of metformin are typically mild and predictable, predominantly gastrointestinal discomfort. This can be mitigated by adjusting the dosage or administering appropriate treatment. In contrast, direct delivery of ncRNA may elicit unforeseen immune responses, cytotoxicity, and other adverse effects. For example, a clinical study using MRX3, a synthetic miR-34a mimic, for the treatment of tumors (ClinicalTrials.gov identifier NCT01829971), including a variety of solid tumors and hematologic malignancies, was terminated prematurely due to severe immune-related adverse effects that resulted in the death of four patients [171, 172].

The convenience and low risk of administration are additional benefits of metformin. It is typically administered orally, which is highly compliant with patient preferences. It does not necessitate intricate injections or other sophisticated administration techniques, and its dosage range is precise. The direct delivery of lncRNA typically requires a more intricate administration method, including injection, the use of nanoparticle carriers, and other techniques. While the utilization of vectors permits in vivo cellular targeting and effective uptake of ncRNA therapeutics, it also has the potential for incompatibility and immunogenicity of disparate vector materials and advanced materials with ncRNA. Viral transduction and expression vector construction carry the risk of genomic integration.

The drug has multiple mechanisms of action. Metformin has been demonstrated to inhibit cancerous growth in several ways. First, it can regulate the expression of ncRNA, which in turn affects the metabolism, proliferation and survival of cancer cells. Second, it activates the AMPK pathway [43]and the ROS pathway [50, 51], both of which play a role in the direct inhibition of cancer cells. Third, it activates the glycolysis-oxidative phosphorylation pathway through an indirect mechanism [53].

To avoid off-target effects, it is essential to consider that a single ncRNA has the capacity to direct the entire cellular pathway by interacting with a vast number of target genes. However, the multitude of cellular effects of ncRNAs renders off-target effects virtually inevitable. The critical role of miRNAs in regulating the pleiotropic effects within the network represents a significant challenge for miRNA-based therapeutic approaches [173].

The potential for off-target effects can be mitigated by selecting an appropriate dose of metformin, which will achieve the desired benefits through the activation of endogenous ncRNAs.

Conclusion

Metformin plays a pivotal role in cancer treatment through the regulation of ncRNAs. In promoting the routine clinical application of therapeutic ncRNAs, it is essential to address several key issues, including the assurance of proper targeting, the reduction of immunogenic responses, and the determination of the optimal dosage required to achieve the desired effect while minimizing side effects. Metformin mediates endogenous ncRNA sequences through different mechanisms to ensure physiological adaptations and tissue-specific expression levels. These findings provide a strong theoretical basis and clinical rationale for the clinical use of ncRNAs in ncRNA therapy. In the future, these ncRNAs may play a variety of potential roles in cancer therapy through approaches such as prognostic prediction and RNA vaccine-drug combinations. Metformin is currently being studied in a wide range of clinical trials, which will lay the groundwork for its future use as a therapeutic or adjuvant treatment and for cancer prevention. Nonetheless, current investigations have certain limitations. First, although some studies have demonstrated the capacity of metformin to regulate ncRNAs for cancer treatment, the specific regulatory mechanisms involved remain largely unknown. Second, despite some studies exploring the regulatory influence of ncRNAs in metformin-based combination therapy, these investigations remain relatively scarce. Third, owing to the large number of ncRNAs and the relative complexity of experimental design, more studies are needed to validate these regulatory mechanisms and to further elucidate the interaction of ncRNAs in metformin combination therapy with other drugs. Moreover, while ncRNAs hold significant potential in cancer therapy, most studies remain confined to laboratory settings and lack powerful clinical validation. Hence, increased emphasis on clinical studies is imperative to evaluate the practical applicability of ncRNAs in cancer treatment. As an oral drug, metformin still has some challenges in terms of its distribution and targeting in vivo. How to increase the concentration of metformin in tumor tissues and how to accurately deliver it into tumor cells are problems that need to be solved. Studies suggest that long-term use of metformin may cause tumor cells to become resistant to it. Therefore, how to overcome the resistance to metformin and how to improve its antitumor effect are further research directions. In the future, by further studying the regulatory effect of metformin on miRNA, it is possible to improve the understanding of its mechanism in tumor prevention and treatment and provide a theoretical basis for the development of new treatment strategies.

Abbreviations

miRNA	MicroRNA
lncRNA	Long non-coding RNA
DNMTs	DNA methyltransferases
c-Myc	Proto-oncogene Myc
SAHH	S-adenosylhomocysteine hydrolase
LC3	Microtubule-associated proteins 1A/1B light chain 3B
P27	Cyclin-dependent kinase inhibitor 1B
sonic hedgehog	Sonic hedgehog protein
TR4	Testicular receptor 4
PELP1	Proline-, glutamic acid-, and leucine-rich protein 1
HULC	Highly upregulated in liver cancer
HCC	Hepatocellular carcinoma
BC	Breast cancer
CRC	Colorectal cancer
GC	Gastric cancer
AMPK	Activated protein kinase
p-	Phosphorylated
UCA1	Urothelial cancer associated 1

Acknowledgements
The figures were created with BioRender software (BioRender.com) and Figdraw 1.0 software (https://www.figdraw.com/#/).

Author contributions
All authors have discussed the proposed scope and content of the present study before drafting. YHZ, YHW and ZXL wrote and revised the manuscript. KX and KPY reviewed and edited the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Funding
The present study was supported by Science and Technology Program of Jiangxi Health Commission (Grant No. 202210566 to KX).

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Patient consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 17 May 2024 Accepted: 24 September 2024
Published online: 01 October 2024

References

1. Siegel RL, et al. Cancer statistics, 2023. CA A Cancer J Clin. 2023;73(1):17–48.
2. Siegel RL, et al. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7–33.
3. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. Nat Rev Clin Oncol. 2018;15(2):81–94.
4. Arneth B. Tumor microenvironment. Medicina. 2019;56(1):15.
5. Triggle CR, et al. Metformin: is it a drug for all reasons and diseases? Metabolism. 2022;133: 155223.
6. Gonzalez-Lopez C, Wojeck BS. Role of metformin in the management of type 2 diabetes: recent advances. Pol Arch Intern Med. 2023. https://doi.org/10.20452/pamw.16511.
7. Evans JM, et al. Metformin and reduced risk of cancer in diabetic patients. BMJ. 2005;330(7503):1304–5.
8. Papadakos SP, et al. The emerging role of metformin in the treatment of hepatocellular carcinoma: is there any value in repurposing metformin for HCC immunotherapy? Cancers (Basel). 2023;15(12):3161.
9. Zhao B, et al. Metformin suppresses self-renewal ability and tumorigenicity of osteosarcoma stem cells via reactive oxygen species-mediated apoptosis and autophagy. Oxid Med Cell Longev. 2019;2019:9290728.
10. Xiao Q, et al. Metformin suppresses the growth of colorectal cancer by targeting INHBA to inhibit TGF-β/PI3K/AKT signaling transduction. Cell Death Dis. 2022;13(3):202.
11. Suwei D, et al. Metformin inhibits melanoma cell metastasis by suppressing the miR-5100/SPINK5/STAT3 axis. Cell Mol Biol Lett. 2022;27(1):48.
12. Szymczak-Pajor I, et al. Metformin induces apoptosis in human pancreatic cancer (PC) cells accompanied by changes in the levels of histone acetyltransferases (particularly, p300/CBP-Associated Factor (PCAF) Protein Levels). Pharmaceuticals. 2023;16(1):115.
13. Liu CQ, et al. Metformin use on incidence and oncologic outcomes of bladder cancer patients with T2DM: an updated meta-analysis. Front Pharmacol. 2022;13: 865988.
14. Jo JK, et al. Risk analysis of metformin use in prostate cancer: a national population-based study. Aging Male. 2023;26(1):2156497.
15. Kramer JR, et al. Effect of diabetes medications and glycemic control on risk of hepatocellular cancer in patients with nonalcoholic fatty liver disease. Hepatology. 2022;75(6):1420–8.
16. Ma SJ, et al. Metformin use improves survival of diabetic liver cancer patients: systematic review and meta-analysis. Oncotarget. 2016;7(40):66202–11.
17. Cho YY, et al. Clinical characteristics of long-term survivors after sorafenib treatment for unresectable hepatocellular carcinoma: a Korean national multicenter retrospective cohort study. J Hepatocell Carcinoma. 2021;8:613–23.
18. Alimoradi N, Firouzabadi N, Fatehi R. How metformin affects various malignancies by means of microRNAs: a brief review. Cancer Cell Int. 2021;21(1):207.
19. Conceição I, et al. Metformin treatment modulates long non-coding RNA isoforms expression in human cells. Noncoding RNA. 2022;8(5):68.
20. Ratti M, et al. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) as new tools for cancer therapy: first steps from bench to bedside. Target Oncol. 2020;15(3):261–78.
21. Peng Y, Croce CM. The role of MicroRNAs in human cancer. Signal Transduct Target Ther. 2016;1:15004.
22. Shah MY, et al. microRNA therapeutics in cancer—an emerging concept. EBioMedicine. 2016;12:34–42.
23. Dexheimer PJ, Cochella L. MicroRNAs: from mechanism to organism. Front Cell Dev Biol. 2020;8:409.
24. Handa H, et al. The role and function of microRNA in the pathogenesis of multiple myeloma. Cancers. 2019;11(11):1738.
25. Jame-Chenarboo F, et al. High-throughput analysis reveals miRNA upregulating α-2,6-sialic acid through direct miRNA-mRNA interactions. ACS Cent Sci. 2022;8(11):1527–36.
26. Gao N, et al. Long non-coding RNAs: the regulatory mechanisms, research strategies, and future directions in cancers. Front Oncol. 2020. https://doi.org/10.3389/fonc.2020.598817.
27. Ishteyaq Majeed S et al. Long non-coding RNAs: biogenesis, mechanism of action and role in different biological and pathological processes, in recent advances in noncoding RNAs, T. Lütfi, Editor. 2022, IntechOpen: Rijeka. p. Ch. 2.
28. Mattick JS, et al. Long non-coding RNAs: definitions, functions, challenges and recommendations. Nat Rev Mol Cell Biol. 2023;24(6):430–47.
29. Statello L, et al. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol. 2021;22(2):96–118.
30. Yang K, et al. LncRNAs in Kawasaki disease and Henoch-Schönlein purpura: mechanisms and clinical applications. Mol Cell Biochem. 2024;479(8):1969–84.
31. Hansen TB, et al. Natural RNA circles function as efficient microRNA sponges. Nature. 2013;495(7441):384–8.
32. Li Z, et al. CircRNA regulation of T cells in cancer: unraveling potential targets. Int J Mol Sci. 2024;25(12):6383.
33. Abdelmohsen K, et al. Identification of HuR target circular RNAs uncovers suppression of PABPN1 translation by CircPABPN1. RNA Biol. 2017;14(3):361–9.
34. Ashwal-Fluss R, et al. circRNA biogenesis competes with pre-mRNA splicing. Mol Cell. 2014;56(1):55–66.
35. Lv J-F, et al. Epigenetic alternations and cancer chemotherapy response. Cancer Chemother Pharmacol. 2015;77(4):673–84.
36. Legnini I, et al. Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. Mol Cell. 2017;66(1):22-37.e9.
37. Zhang M, et al. A novel protein encoded by the circular form of the SHPRH gene suppresses glioma tumorigenesis. Oncogene. 2018;37(13):1805–14.
38. Yang Y, et al. Novel role of FBXW7 circular RNA in repressing glioma tumorigenesis. J Natl Cancer Inst. 2017;110(3):304–15.
39. Pamudurti NR, et al. Translation of CircRNAs. Mol Cell. 2017;66(1):9-21.e7.
40. Laganà A, et al. Computational design of artificial RNA molecules for gene regulation. In: Methods in molecular biology. New York: Springer; 2014. p. 393–412.
41. Elbashir SM, et al. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001;411(6836):494–8.
42. Friedrich M, Aigner A. Therapeutic siRNA: state-of-the-Art and future perspectives. BioDrugs. 2022;36(5):549–71.
43. Pecinová A, et al. Mitochondrial targets of metformin-are they physiologically relevant? BioFactors. 2019;45(5):703–11.
44. Hasanvand A. The role of AMPK-dependent pathways in cellular and molecular mechanisms of metformin: a new perspective for treatment and prevention of diseases. Inflammopharmacology. 2022;30(3):775–88.
45. Ma T, et al. Low-dose metformin targets the lysosomal AMPK pathway through PEN2. Nature. 2022;603(7899):159–65.
46. Foretz M, Guigas B, Viollet B. Metformin: update on mechanisms of action and repurposing potential. Nat Rev Endocrinol. 2023;19(8):460–76.
47. Lord SR, Harris AL. Is it still worth pursuing the repurposing of metformin as a cancer therapeutic? Br J Cancer. 2023;128(6):958–66.
48. Yi Y, et al. Role of p53 family proteins in metformin anti-cancer activities. J Cancer. 2019;10(11):2434–42.
49. Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front Environ Sci. 2014. https://doi.org/10.3389/fenvs.2014.00053.
50. Mogavero A, et al. Metformin transiently inhibits colorectal cancer cell proliferation as a result of either AMPK activation or increased ROS production. Sci Rep. 2017. https://doi.org/10.1038/s41598-017-16149-z.
51. Bost F, Ben-Sahra I, Tanti J-F. Prevention of mutagenesis: new potential mechanisms of metformin action in neoplastic cells. Cancer Prev Res. 2012;5(4):503–6.
52. Warkad MS, et al. Metformin-induced ROS upregulation as amplified by apigenin causes profound anticancer activity while sparing normal cells. Sci Rep. 2021. https://doi.org/10.1038/s41598-021-93270-0.
53. Jose C, Bellance N, Rossignol R. Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? Biochimica et Biophysica Acta BBA Bioenergetics. 2011;1807(6):552–61.
54. Elgendy M, et al. Combination of hypoglycemia and metformin impairs tumor metabolic plasticity and growth by modulating the PP2A-GSK3β-MCL-1 axis. Cancer Cell. 2019;35(5):798-815.e5.
55. Zhao H, Swanson KD, Zheng B. Therapeutic repurposing of biguanides in cancer. Trends Cancer. 2021;7(8):714–30.

56. Marini C, et al. Divergent targets of glycolysis and oxidative phosphorylation result in additive effects of metformin and starvation in colon and breast cancer. Sci Rep. 2016. https://doi.org/10.1038/srep19569.
57. Wang G, et al. Metformin prevents methylglyoxal-induced apoptosis by suppressing oxidative stress in vitro and in vivo. Cell Death Dis. 2022;13(1):29.
58. Baldin V, et al. Cyclin D1 is a nuclear protein required for cell cycle progression in G1. Genes Dev. 1993;7(5):812–21.
59. YenmİŞ G, et al. Metformin promotes apoptosis in primary breast cancer cells by downregulation of cyclin D1 and upregulation of P53 through an AMPK-alpha independent mechanism. Turkish J Med Sci. 2021;51(2):826–34.
60. Yudhani RD, et al. Metformin modulates cyclin D1 and P53 expression to inhibit cell proliferation and to induce apoptosis in cervical cancer cell lines. Asian Pac J Cancer Prev. 2019;20(6):1667–73.
61. Wang L-W, et al. Metformin induces apoptosis of pancreatic cancer cells. World J Gastroenterol. 2008;14(47):7192.
62. Pankiv S, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem. 2007;282(33):24131–45.
63. Li K, et al. Metformin reduces TRIB3 expression and restores autophagy flux: an alternative antitumor action. Autophagy. 2018;14(7):1278–9.
64. Hua F, et al. The TRIB3-SQSTM1 interaction mediates metabolic stress-promoted tumorigenesis and progression via suppressing autophagic and proteasomal degradation. Autophagy. 2015;11(10):1929–31.
65. Ben Sahra I, et al. Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. Cancer Res. 2011;71(13):4366–72.
66. Chen WH, et al. Glucose/glutathione co-triggered tumor hypoxia relief and chemodynamic therapy to enhance photothermal therapy in bladder cancer. ACS Appl Bio Mater. 2021;4(10):7485–96.
67. Pizzorno J. Glutathione! Integr Med. 2014;13(1):8–12.
68. Ferrere G, et al. Ketogenic diet and ketone bodies enhance the anticancer effects of PD-1 blockade. JCI Insight. 2021. https://doi.org/10.1172/jci.insight.145207.
69. Ding JT, et al. Landscapes and mechanisms of CD8(+) T cell exhaustion in gastrointestinal cancer. Front Immunol. 2023;14:1149622.
70. Chen GG, et al. Impact of metformin on immunological markers: Implication in its anti-tumor mechanism. Pharmacol Ther. 2020;213: 107585.
71. Seyfried TN, Shelton LM. Cancer as a metabolic disease. Nutr Metab. 2010;7(1):7.
72. Vallejo FA, et al. The contribution of ketone bodies to glycolytic inhibition for the treatment of adult and pediatric glioblastoma. J Neurooncol. 2020;147(2):317–26.
73. Kasprzak A. Insulin-like growth factor 1 (IGF-1) signaling in glucose metabolism in colorectal cancer. Int J Mol Sci. 2021;22(12):6434.
74. Liu X, et al. Actin cytoskeleton vulnerability to disulfide stress mediates disulfidptosis. Nat Cell Biol. 2023;25(3):404–14.
75. Liu T, et al. Exploring the role of the disulfidptosis-related gene SLC7A11 in adrenocortical carcinoma: implications for prognosis, immune infiltration, and therapeutic strategies. Cancer Cell Int. 2023;23(1):259.
76. Malinowski B, Musiała N, Wiciński M. Metformin's modulatory effects on miRNAs function in cancer stem cells—a systematic review. Cells. 2020;9(6):1401.
77. Hong XL, et al. Metformin abrogates *Fusobacterium nucleatum*-induced chemoresistance in colorectal cancer by inhibiting miR-361-5p/sonic hedgehog signaling-regulated stemness. Br J Cancer. 2023;128(2):363–74.
78. Pulito C, et al. Metformin-induced ablation of microRNA 21–5p releases Sestrin-1 and CAB39L antitumoral activities. Cell Discov. 2017;3:17022.
79. Dong J, et al. Metformin mediated microRNA-7 upregulation inhibits growth, migration, and invasion of non-small cell lung cancer A549 cells. Anticancer Drugs. 2020;31(4):345–52.
80. Zhou JY, Xu B, Li L. A new role for an old drug: metformin targets micro<scp>RNA</scp>s in treating diabetes and cancer. Drug Dev Res. 2015;76(6):263–9.
81. Zhang Z, et al. Metformin enhances the antitumor activity of CD8(+) T lymphocytes via the AMPK-miR-107-Eomes-PD-1 pathway. J Immunol. 2020;204(9):2575–88.
82. Wang G, et al. Mechanisms of action of metformin and its regulatory effect on microRNAs related to angiogenesis. Pharmacol Res. 2021;164: 105390.
83. Kalogirou C, et al. Metformin-derived growth inhibition in renal cell carcinoma depends on miR-21-mediated PTEN expression. Urol Int. 2016;96(1):106–15.
84. Liao Z, et al. Cardiac telocytes inhibit cardiac microvascular endothelial cell apoptosis through exosomal miRNA-21-5p-targeted cdip1 silencing to improve angiogenesis following myocardial infarction. Theranostics. 2021;11(1):268–91.
85. Varghese E, et al. Anti-angiogenic effects of phytochemicals on miRNA regulating breast cancer progression. Biomolecules. 2020;10(2):191.
86. Yang X, et al. MicroRNA-26a suppresses angiogenesis in human hepatocellular carcinoma by targeting hepatocyte growth factor-cMet pathway. Hepatology. 2014;59(5):1874–85.
87. Ni H-Z, et al. Metformin inhibits angiogenesis of endothelial progenitor cells via miR-221-mediated p27 expression and autophagy. Future Med Chem. 2019;11(17):2263–72.
88. Xia C, et al. Metformin, a first-line drug for type 2 diabetes mellitus, disrupts the MALAT1/miR-142-3p sponge to decrease invasion and migration in cervical cancer cells. Eur J Pharmacol. 2018;830:59–67.
89. Arunachalam G, et al. Molecular Interplay between microRNA-34a and sirtuin1 in hyperglycemia-mediated impaired angiogenesis in endothelial cells: effects of metformin. J Pharmacol Exp Ther. 2015;356(2):314–23.
90. Eyileten C, et al. Alteration of circulating platelet-related and diabetes-related microRNAs in individuals with type 2 diabetes mellitus: a stepwise hypoglycaemic clamp study. Cardiovasc Diabetol. 2022. https://doi.org/10.1186/s12933-022-01517-5.
91. Deng Y, Ma W. Metformin inhibits HaCaT cell viability via the miR-21/PTEN/Akt signaling pathway. Mol Med Rep. 2018;17(3):4062–6.
92. Melnik BC, et al. MicroRNA-21-enriched exosomes as epigenetic regulators in melanomagenesis and melanoma progression: the impact of western lifestyle factors. Cancers. 2020;12(8):2111.
93. Tanaka R, et al. Metformin causes G1-phase arrest via down-regulation of MiR-221 and enhances TRAIL sensitivity through DR5 Up-regulation in pancreatic cancer cells. PLoS ONE. 2015;10(5): e0125779.
94. Wang F, et al. Metformin induces apoptosis by microRNA-26a-mediated downregulation of myeloid cell leukaemia-1 in human oral cancer cells. Mol Med Rep. 2016;13(6):4671–6.
95. Cabello P, et al. The antitumor effect of metformin is mediated by miR-26a in breast cancer. Int J Mol Sci. 2016;17(8):1298.
96. Bacci M, et al. miR-155 drives metabolic reprogramming of ER+ breast cancer cells following long-term estrogen deprivation and predicts clinical response to aromatase inhibitors. Can Res. 2016;76(6):1615–26.
97. Zhang Y, et al. Metformin protects against H2O2-Induced cardiomyocyte injury by inhibiting the miR-1a-3p/GRP94 pathway. Mol Ther Nucleic Acids. 2018;13:189–97.
98. Zhou G, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001;108(8):1167–74.
99. Jin D, et al. Metformin-repressed miR-381-YAP-snail axis activity disrupts NSCLC growth and metastasis. J Exp Clin Cancer Res. 2020;39(1):6.
100. Zhong T, et al. Metformin alters DNA methylation genome-wide via the H19/SAHH axis. Oncogene. 2016;36(17):2345–54.
101. Laskov I, et al. Anti-diabetic doses of metformin decrease proliferation markers in tumors of patients with endometrial cancer. Gynecol Oncol. 2014;134(3):607–14.
102. Zhao W, et al. miR-27a-mediated antiproliferative effects of metformin on the breast cancer cell line MCF-7. Oncol Rep. 2016;36(6):3691–9.
103. Takahashi RU, et al. Loss of microRNA-27b contributes to breast cancer stem cell generation by activating ENPP1. Nat Commun. 2015;6:7318.
104. Yan L, et al. Regulation of tumor cell migration and invasion by the H19/let-7 axis is antagonized by metformin-induced DNA methylation. Oncogene. 2015;34(23):3076–84.
105. Ma M, et al. Low glucose enhanced metformin's inhibitory effect on pancreatic cancer cells by suppressing glycolysis and inducing energy stress via up-regulation of miR-210-5p. Cell Cycle. 2020;19(17):2168–81.
106. Antognelli C, et al. Glyoxalase 1 sustains the metastatic phenotype of prostate cancer cells via EMT control. J Cell Mol Med. 2018;22(5):2865–83.

107. Xia C, et al. iTRAQ-based quantitative proteomic analysis of the inhibition of cervical cancer cell invasion and migration by metformin. Biomed Pharmacother. 2020;123: 109762.
108. Yu Z, et al. Metformin affects paclitaxel sensitivity of ovarian cancer cells through autophagy mediated by long noncoding RNASNHG7/miR-3127-5p axis. Cancer Biother Radiopharm. 2022;37(9):792–801.
109. Li W, et al. Metformin alters the expression profiles of microRNAs in human pancreatic cancer cells. Diabetes Res Clin Pract. 2012;96(2):187–95.
110. Bao X, et al. Inhibition of LCMR1 and ATG12 by demethylation-activated miR-570-3p is involved in the anti-metastasis effects of metformin on human osteosarcoma. Cell Death Dis. 2018;9(6):611.
111. Wang L, et al. Metformin induces human esophageal carcinoma cell pyroptosis by targeting the miR-497/PELP1 axis. Cancer Lett. 2019;450:22–31.
112. Hou Y, et al. Metformin induces ferroptosis by targeting miR-324-3p/GPX4 axis in breast cancer. Acta Biochim Biophys Sin. 2021;53(3):333–41.
113. Do MT, et al. Metformin induces microRNA-34a to downregulate the Sirt1/Pgc-1α/Nrf2 pathway, leading to increased susceptibility of wild-type p53 cancer cells to oxidative stress and therapeutic agents. Free Radic Biol Med. 2014;74:21–34.
114. Borzi C, et al. LKB1 down-modulation by miR-17 identifies patients with NSCLC having worse prognosis eligible for energy-stress-based treatments. J Thorac Oncol. 2021;16(8):1298–311.
115. Zhang J, et al. Metformin inhibits tumorigenesis and tumor growth of breast cancer cells by upregulating miR-200c but downregulating AKT2 expression. J Cancer. 2017;8(10):1849–64.
116. Zhuang Y, Miskimins WK. Cell cycle arrest in Metformin treated breast cancer cells involves activation of AMPK, downregulation of cyclin D1, and requires p27Kip1 or p21Cip1. J Mol Signal. 2008;3:18.
117. Yan GJ, et al. MicroRNA miR-302 inhibits the tumorigenicity of endometrial cancer cells by suppression of Cyclin D1 and CDK1. Cancer Lett. 2014;345(1):39–47.
118. Cao M, et al. Research advances on circulating long noncoding RNAs as biomarkers of cardiovascular diseases. Int J Cardiol. 2022;353:109–17.
119. Ghafouri-Fard S, et al. LncRNAs: novel biomarkers for pancreatic cancer. Biomolecules. 2021;11(11):1665.
120. Fujimori T, et al. Antitumor effect of metformin on cholangiocarcinoma: In vitro and in vivo studies. Oncol Rep. 2015;34(6):2987–96.
121. Wang Y, Wu Z, Hu L. The regulatory effects of metformin on the [SNAIL/miR-34]:[ZEB/miR-200] system in the epithelial-mesenchymal transition(EMT) for colorectal cancer(CRC). Eur J Pharmacol. 2018;834:45–53.
122. Xie W, et al. Metformin induces growth inhibition and cell cycle arrest by upregulating microRNA34a in renal cancer cells. Med Sci Monit. 2017;23:29–37.
123. Feng YH, et al. MicroRNA-21-mediated regulation of Sprouty2 protein expression enhances the cytotoxic effect of 5-fluorouracil and metformin in colon cancer cells. Int J Mol Med. 2012;29(5):920–6.
124. Yang Q, et al. LINC02159 promotes non-small cell lung cancer progression via ALYREF/YAP1 signaling. Mol Cancer. 2023. https://doi.org/10.1186/s12943-023-01814-x.
125. Tan W, et al. Metformin mediates induction of miR-708 to inhibit self-renewal and chemoresistance of breast cancer stem cells through targeting CD47. J Cell Mol Med. 2019;23(9):5994–6004.
126. Huang Y, et al. lncRNA MALAT1 participates in metformin inhibiting the proliferation of breast cancer cell. J Cell Mol Med. 2021;25(15):7135–45.
127. Fan S, et al. Downregulation of the long non-coding RNA TUG1 is associated with cell proliferation, migration, and invasion in breast cancer. Biomed Pharmacother. 2017;95:1636–43.
128. Liu J, et al. Ferroptosis-related long noncoding RNAs have excellent predictive ability for multiomic characteristics of bladder cancer. Oxid Med Cell Longev. 2022;2022:9316847.
129. Lee BB, et al. Metformin regulates expression of DNA methyltransferases through the miR-148/-152 family in non-small lung cancer cells. Clin Epigenet. 2023;15(1):48.
130. Meng J, et al. Twist1-YY1-p300 complex promotes the malignant progression of HCC through activation of miR-9 by forming phase-separated condensates at super-enhancers and relieved by metformin. Pharmacol Res. 2023;188: 106661.
131. Kong Y, et al. Inhibition of EZH2 enhances the antitumor efficacy of metformin in prostate cancer. Mol Cancer Ther. 2020;19(12):2490–501.
132. Anisimov VN, et al. Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice. Exp Gerontol. 2005;40(8–9):685–93.
133. Anisimov VN, et al. Metformin decelerates aging and development of mammary tumors in HER-2/neu transgenic mice. Bull Exp Biol Med. 2005;139(6):721–3.
134. Kim J, et al. Phase II randomized trial of neoadjuvant metformin plus letrozole versus placebo plus letrozole for estrogen receptor positive postmenopausal breast cancer (METEOR). BMC Cancer. 2014;14:170.
135. Bao B, et al. Metformin may function as anti-cancer agent via targeting cancer stem cells: the potential biological significance of tumor-associated miRNAs in breast and pancreatic cancers. Ann Transl Med. 2014;2(6):59.
136. Cioce M, et al. Metformin-induced metabolic reprogramming of chemoresistant ALDHbright breast cancer cells. Oncotarget. 2014;5(12):4129–43.
137. Shi P, et al. Metformin suppresses triple-negative breast cancer stem cells by targeting KLF5 for degradation. Cell Discov. 2017;3:17010.
138. Gholami M, et al. Metformin and long non-coding RNAs in breast cancer. J Transl Med. 2023;21(1):155.
139. Abd-Rabou AA, et al. Metformin-loaded lecithin nanoparticles induce colorectal cancer cytotoxicity via epigenetic modulation of noncoding RNAs. Mol Biol Rep. 2021;48(10):6805–20.
140. Liao W, et al. Identification of an m6A-related long noncoding RNA risk model for predicting prognosis and directing treatments in patients with colon adenocarcinoma. Front Cell Dev Biol. 2022. https://doi.org/10.3389/fcell.2022.910749.
141. Zhao Y, et al. PolyMetformin combines carrier and anticancer activities for in vivo siRNA delivery. Nat Commun. 2016. https://doi.org/10.1038/ncomms11822.
142. Gandhy SU, et al. Specificity protein (Sp) transcription factors and metformin regulate expression of the long non-coding RNA HULC. Oncotarget. 2015;6(28):26359–72.
143. Guo J, et al. Metformin-induced changes of the coding transcriptome and non-coding RNAs in the livers of non-alcoholic fatty liver disease mice. Cell Physiol Biochem. 2018;45(4):1487–505.
144. Cifarelli V, et al. Metformin and rapamycin reduce pancreatic cancer growth in obese prediabetic mice by distinct microRNA-regulated mechanisms. Diabetes. 2015;64(5):1632–42.
145. Bhardwaj A, et al. The isomiR-140–3p-regulated mevalonic acid pathway as a potential target for prevention of triple negative breast cancer. Breast Cancer Res. 2018. https://doi.org/10.1186/s13058-018-1074-z.
146. Kallen AN, et al. The imprinted H19 lncRNA antagonizes let-7 microRNAs. Mol Cell. 2013;52(1):101–12.
147. Salgado-García R, et al. Negative regulation of ULK1 by microRNA-106a in autophagy induced by a triple drug combination in colorectal cancer cells in vitro. Genes. 2021;12(2):245.
148. Coronel-Hernández J, et al. Combination of metformin, sodium oxamate and doxorubicin induces apoptosis and autophagy in colorectal cancer cells via downregulation HIF-1α. Front Oncol. 2021;11: 594200.
149. Wu P, et al. Metformin suppresses hypopharyngeal cancer growth by epigenetically silencing long non-coding RNA SNHG7 in FaDu cells. Front Pharmacol. 2019. https://doi.org/10.3389/fphar.2019.00143.
150. Qiu C, et al. Metformin suppresses lung adenocarcinoma by down-regulating long non-coding RNA (lncRNA) AFAP1-AS1 and secreted phosphoprotein 1 (SPP1) while upregulating miR-3163. Bioengineered. 2022;13(5):11987–2002.
151. Jiang Y, et al. Metformin reverses tamoxifen resistance through the lncRNA GAS5-medicated mTOR pathway in breast cancer. Ann Transl Med. 2022;10(6):366.
152. Tseng HH, et al. Metformin inhibits gastric cancer cell proliferation by regulation of a novel Loc100506691-CHAC1 axis. Mol Ther Oncolytics. 2021;22:180–94.
153. Li P, et al. Long noncoding RNA H19 participates in metformin-mediated inhibition of gastric cancer cell invasion. J Cell Physiol. 2019;234(4):4515–27.
154. Golshan M, et al. Metformin modulates oncogenic expression of HOTAIR gene via promoter methylation and reverses

epithelial-mesenchymal transition in MDA-MB-231 cells. J Cell Biochem. 2021;122(3–4):385–93.
155. Shi H, et al. Targeting the TR4 nuclear receptor-mediated lncTASR/AXL signaling with tretinoin increases the sunitinib sensitivity to better suppress the RCC progression. Oncogene. 2020;39(3):530–45.
156. Sabry D, et al. Anti-proliferative and anti-apoptotic potential effects of epigallocatechin-3-gallate and/or metformin on hepatocellular carcinoma cells: in vitro study. Mol Biol Rep. 2019;46(2):2039–47.
157. Jiang Z, Liu H. Metformin inhibits tumorigenesis in HBV-induced hepatocellular carcinoma by suppressing HULC overexpression caused by HBX. J Cell Biochem. 2018;119(6):4482–95.
158. Hsueh EC, Zhang Y, Peng G. Abstract 4066: Combination metformin and ODC siRNA inhibit human melanoma proliferation and migration. Cancer Res. 2011;71(8_Supplement):4066–4066.
159. Ma Q, et al. Emerging roles of circular RNAs in tumorigenesis, progression, and treatment of gastric cancer. J Transl Med. 2024;22(1):207.
160. Xu C, et al. N(6)-methyladenosine-modified circ_104797 sustains cisplatin resistance in bladder cancer through acting as RNA sponges. Cell Mol Biol Lett. 2024;29(1):28.
161. Yuan ZA-O, et al. Exosomal circ_0037104 derived from Hu-MSCs inhibits cholangiocarcinoma progression by sponging miR-620 and targeting AFAP1. J Biochem Mol Toxicol. 2024;38(2): e23656.
162. Liao XAO, et al. Tumor-derived exosomal circular RNA pinin induces FGF13 expression to promote colorectal cancer progression through miR-1225–5p. Gut Liver. 2024. https://doi.org/10.5009/gnl230304.
163. Chen D, et al. Targeting the radiation-induced TR4 nuclear receptor-mediated QKI/circZEB1/miR-141–3p/ZEB1 signaling increases prostate cancer radiosensitivity. Cancer Lett. 2020;28(495):100–11.
164. Li Z, et al. Research progress on the premature ovarian failure caused by cisplatin therapy. Front Oncol. 2023;13:1276310.
165. Zhan Q, et al. Blood exosomes-based targeted delivery of cPLA2 siRNA and metformin to modulate glioblastoma energy metabolism for tailoring personalized therapy. Neuro Oncol. 2022;24(11):1871–83.
166. 188. NCT03685409 . Cancer Chemoprevention by Metformin Hydrochloride in Oral Potentially Malignant Lesions,Clinicaltrials.gov. (2018-09-26)
167. NCT03684707. Cancer Chemoprevention by Metformin Hydrochloride Compared to Placebo in Oral Potentially Malignant Lesions, Clinicaltrials.gov.(2018-09-26)
168. NCT05468554. Evaluation of Metformin Effect on the Fertility of Women Treated With 131I for Thyroid cancer(METHYR), Clinicaltrials.gov. (2022-10-19)
169. NCT06044025. A Feasibility Study Evaluating a Role for Maintenance Therapy in Patients With Biochemically Progressive Castration Sensitive Prostate Cancer on Intermittent Androgen Deprivation Therapy, Clinicaltrials.gov.(2024-01-15)
170. Demetriou C, et al. Hawthorne effect on surgical studies. ANZ J Surg. 2019;89(12):1567–76.
171. Hong DS, et al. Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. Br J Cancer. 2020;122(11):1630–7.
172. Beg MS, et al. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. Invest New Drugs. 2017;35(2):180–8.
173. Zhang S, Zhu F. Comment on: "MicroRNA Mimics or Inhibitors as Antiviral Therapeutic Approaches Against COVID-19." Drugs. 2021;81(14):1691–2.

Publisher's Note