

Review

The Dual Role of Metformin: Repurposing an Antidiabetic Drug for Cancer Therapy

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Abstract

Maintaining glucose homeostasis is vital for normal physiological function, and any disturbance in this balance is associated with the development of degenerative and chronic diseases, like Type 2 Diabetes (T2D) and certain types of cancer, where altered glucose metabolism plays a central role. Epidemiological evidence indicates a positive association between diabetes and an increased risk of developing certain types of cancer. Such a correlation may be driven by shared risk factors, namely obesity, inflammation, and insulin resistance. The observed association between diabetes and an increased risk of certain cancers, along with the rising incidence of both diseases, has in recent years raised interest in treatments that may target both conditions. Among them, the biguanide metformin, the first-line drug prescribed for T2D, has attracted significant attention as a repurposed drug due to its potential role in cancer treatment. Metformin is a glucose-lowering drug that reduces hepatic glucose production and improves insulin sensitivity, promoting glucose uptake by the skeletal muscle, contributing to better glycemic control in individuals with T2D and prediabetic syndromes. However, beyond its metabolic effects, metformin also influences key signaling pathways involved in cell growth and survival, such as the AMP-activated protein kinase (AMPK)/mTOR axis, raising interest in its potential application as an anticancer agent. Furthermore, metformin inhibits mitochondrial complex I, disrupting cellular energy production, which is essential for cancer proliferation. This review aims to explore and clarify the multifunctional role of metformin in both T2D and cancer, focusing on the metabolic alterations observed in these diseases. It highlights how glucose metabolism dysregulation contributes to disease progression in both contexts and explores the molecular targets of metformin in each condition and its potential for dual therapeutic benefit. Finally, selected clinical trials concerning metformin use in cancer therapy, alone or in combination, will be presented, highlighting its potential to enhance treatment response, reduce resistance, and improve overall patient outcomes.

Keywords: glucose metabolism; diabetes; cancer; drug repurposing; metformin



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1. Introduction

Type 2 diabetes mellitus (T2D) is a chronic global health problem, characterized by decreased insulin response and persistently elevated blood glucose levels, which is associated with several long-term comorbidities such as retinopathy, nephropathy, neuropathy, cardiovascular disease, and non-alcoholic fatty liver disease (NAFLD) [1]. The prevalence of the disease has dramatically increased in recent years worldwide, mainly due to unhealthy lifestyles (increased consumption of processed and sugary foods coupled with reduced physical activity), growing obesity prevalence, and aging of the population. Nevertheless, T2D is a multifactorial disease, and several factors can be involved in its development, with environmental factors interacting with genetic predisposition. Many of the patients who develop T2D are obese, and the increase in sedentary lifestyle and obesity appears as risk factors for this type of disease, associated with heredity, being a strong link between diabetes mellitus and obesity consistently observed across different racial and ethnic groups [2]. The pathogenesis of T2D involves resistance to the action of insulin and may also sometimes involve failures in insulin secretion. Fat accumulation in adipose tissue contributes to insulin resistance, whereas the accumulation in the pancreas is related to pancreatic beta cells' dysfunction [3]. Such insulin resistance is closely associated with low-grade chronic inflammation, which contributes to the pathogenesis of the disease [4]. White adipose tissue, especially the visceral, produces several inflammatory molecules, like cytokines and chemokines, triggering inflammatory processes [3]. Such low-grade chronic inflammation, assessed by raised levels of C-reactive protein (CRP), can also contribute to other complications of diabetes, including the increased risk for other chronic degenerative diseases, like cancer, cardiovascular (CVD), and neurodegenerative diseases [5–7]. In fact, diabetes is associated with an increased risk of developing certain types of cancer and an increased cancer mortality, namely for colon, pancreatic, liver, kidney, bladder, endometrium, and breast cancers, although the risk varies between women and men [8–10]. T2D and cancer share some risk factors, including overweight and obesity, unhealthy diet, lack of physical activity, and aging. Moreover, several patients are diagnosed with both those cancers and diabetes more often than would happen just by chance, although no correlation was found with other cancers, or is inconclusive for lung cancer or non-Hodgkin lymphoma, and in some cases, like prostate cancer, the risk is actually lower [9]. Thus, T2D might impact the development of some types of cancer through various mechanisms, including low-grade chronic inflammation. Chronic inflammation is directly associated with cancer, creating conditions that support tumor growth and development through the release of reactive oxygen species (ROS), growth factors, and cytokines that promote cell growth and proliferation, altering the immune response and leading to changes in the tumor microenvironment [11,12]. Likewise, in the 2011 paper “Hallmarks of Cancer: The Next Generation”, revising the original identification of cancer hallmarks, authored by Douglas Hanahan and Robert A. Weinberg, tumor-promoting inflammation has been identified as a key enabling characteristic fostering multiple hallmark functions that lead to tumor proliferation and progression [13]. This inflammatory state can be found in either T2D or cancer, worsening outcomes in both diseases. Furthermore, other associations can be found in both diseases, like dysregulated glucose metabolism and signaling pathways, such as insulin/IGF or phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mechanistic target of rapamycin (mTOR) [14]. Some well-known antidiabetic drugs belonging to the biguanide class, like metformin (the most prescribed) or phenformin, have these pathways as targets [15–17]. Although originally developed for diabetes treatment, these drugs have demonstrated interesting antitumor properties, and several reports exploit these properties by repurposing them for cancer therapy. Since these drugs are well known in terms of their effects and side effects, their use in oncology could be safer and more predictable.

Accordingly, evidence from various studies suggests that the use of these compounds, namely metformin, was consistently linked to a lower risk of cancer development in diabetic patients, particularly pancreatic, colorectal, and liver cancers [18,19]. Furthermore, their use has also been associated with increased survival rates in cancer patients, decreased resistance, and a lower probability of cancer recurrence [19]. In this review, we address these multiple roles of metformin therapeutics, both in T2D and cancer, exploiting its effect on glucose metabolism and homeostasis, its cellular targets and mechanisms of action, as an antidiabetic or anticancer drug. Finally, we will review the clinical mechanisms of metformin decreasing cancer resistance, as well as clinical trials evaluating metformin in cancer therapy, combined with standard therapies.

2. Disturbance of Glucose Homeostasis in Diabetes and Cancer

As previously mentioned, a common feature of both diabetes and cancer is the disturbance of glucose homeostasis. However, the causes and consequences of this disturbance differ between the two diseases and have distinct implications. Glucose is a fundamental and universal fuel in the human body, particularly vital for tissues and cells like the brain, retina, erythrocytes, and renal medulla [20–22], being regulated through a complex network involving hormones, transporters, and metabolic and signaling pathways. The first step of glucose utilization consists of its uptake into the cell, a process mediated by glucose transporters (GLUTs). Different GLUT isoforms are expressed differently in specific tissues, ensuring that glucose delivery is adapted to the metabolic needs of each tissue. After being transported into the cell, glucose can be directed into distinct metabolic pathways according to the cell's energy status and functional requirements. It may be directed towards glycolysis to produce ATP, stored as glycogen, or enter the pentose phosphate pathway (PPP) to generate NADPH and ribose-5-phosphate, providing cells with energy and biosynthetic precursors. Together with glucagon, insulin is essential for glucose homeostasis. They play opposing roles in maintaining glucose homeostasis by promoting the storage or mobilization of glucose, respectively. Additionally, hormones such as cortisol, adrenaline, and growth hormone are also players in this regulation, particularly during stress or fasting, to help maintain metabolic balance [23,24]. Intracellular signaling pathways, including the PI3K/Akt and AMP-activated protein kinase (AMPK) pathways, also participate in glucose homeostasis [25]. In healthy individuals, such regulation ensures a balance between glucose supply and demand across different tissues, according to their needs and physiological status. However, in some diseases, like diabetes and cancer, this accurate regulation becomes disrupted (Figure 1). Although both disorders involve altered glucose metabolism, the inherent mechanisms and adaptive responses show marked differences, reflecting the distinct pathophysiological features of each disease.

GLUTs belong to the solute transporter (SLC2A) family of proteins and are present in many tissues/cells of the body, e.g., brain, erythrocytes, adipocytes, and liver, where they mediate glucose uptake [26]. The fourteen different isoforms of GLUTs are subdivided into three distinct protein classes, according to their sequence homology. Each GLUT isoform has a unique tissue distribution, a substrate specificity and a specific physiological function [27]. All GLUT proteins were originally assumed to catalyze the transport of hexoses into and out of cells. This is clearly the case for the class 1 GLUT proteins (GLUTs 1–4 and 14). However, class 2 (GLUTs 5, 7, 9 and 11) and class 3 (GLUTs 6, 8, 10, 12 and 13) GLUT proteins do not necessarily have a primary role in catalyzing glucose transport [28]. GLUT-1 is expressed in tissues with a high glycolytic rate, such as erythrocytes, being responsible for glucose uptake in high-need cells [26,28]. However, this transporter also plays a central role in tumorigenesis as it delivers glucose in hypoxic environments. In fact, GLUT-1, a target

gene of HIF-1, is highly expressed in hypoxic cancer cells, allowing the maintenance of a high metabolic rate in these cells [26].

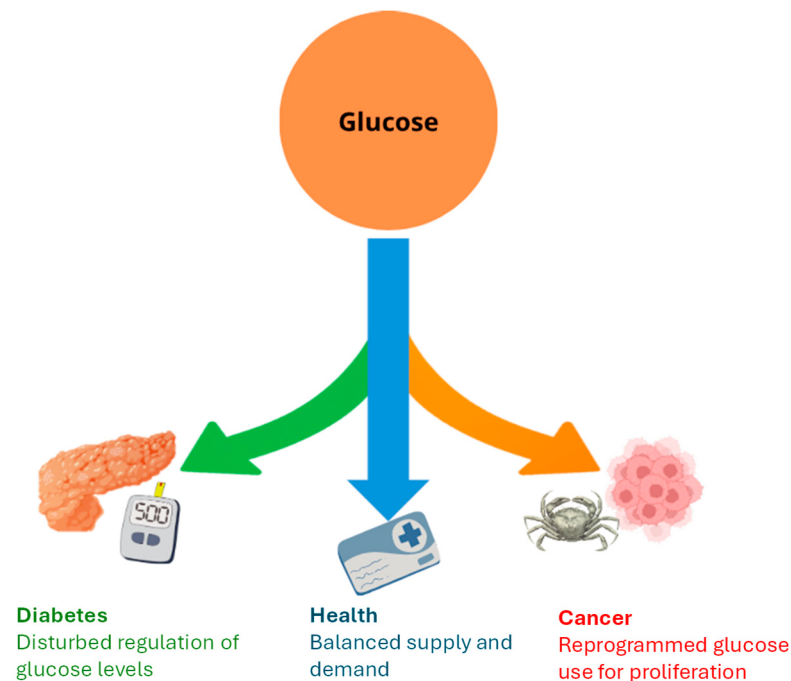


Figure 1. Glucose utilization in healthy individuals, in type 2 diabetes, and cancer cells. This diagram illustrates the differences in glucose metabolism under three different physiological and pathological conditions. In healthy individuals, glucose homeostasis is tightly regulated, ensuring balanced glucose supply, demand, and energy production. In T2D, insulin resistance impairs glucose uptake in peripheral tissues, leading to disturbed regulation and hyperglycemia. In cancer cells, a reprogrammed use of glucose occurs with a preference for aerobic glycolysis (the Warburg effect), supporting rapid proliferation and biosynthetic demands.

2.1. Diabetes

Diabetes mellitus is a metabolic disease characterized by a deficiency in insulin production, increased destruction of the hormone, or its inefficiency [29–32]. Its main consequence is the loss of cells’ ability to capture glucose from the blood, making hyperglycemia the most prominent symptom of the disease [29–32]. As previously mentioned, T2D results from a relative resistance to endogenous insulin—a condition promoted by environmental factors, namely obesity –, and from a decreased secretion of this hormone [29–32]. It has been associated with several defects in the early and intermediate steps of the insulin signaling pathway [32–37] and accounts for about 90% of diabetics [30,31,38].

Insulin is produced in the β cells of the pancreas in response to rising blood glucose levels [29]. Among insulin’s functions are storing fuel and facilitating glucose uptake by cells, particularly in adipose tissue and muscle [29,30]. Insulin binds to receptors on target cells, triggering a cellular response that causes vesicles containing glucose transporters to fuse with the plasma membrane. This increases the number of glucose transporters on the membrane, promoting glucose uptake from the blood [29,39].

2.1.1. Insulin Signaling Pathway, PI3K/Akt Signaling, and Glucose Metabolism

The insulin signaling pathway starts when insulin binds to its specific receptor on the cell surface. This binding is both necessary and sufficient to produce the biological effects of insulin, serving as the main trigger for the next steps in signaling. The insulin receptor is a heterotetrameric membrane glycoprotein, made up of two α - and two β -subunits linked by disulfide bonds [30–32,34,39–41]. The extracellular α -subunits contain

the insulin-binding sites. When insulin binds, these subunits are drawn closer together, causing a conformational change in the receptor. This conformational change activates the tyrosine kinase activity of the β -subunits, promoting ATP binding and receptor autophosphorylation on key tyrosine residues [30,32–34,37,39–41]. This step initiates downstream signaling [32–34,39–41]. It is also important to note that signal transduction through the insulin receptor is not limited to its activation at the cell surface since the ligand-receptor complexes move from the microvilli of the cell surface toward clathrin-coated pits and are then internalized into endosomes [40,42]. This process allows the tyrosine kinases to access substrates that might not otherwise be reachable. After the endosomal lumen is acidified by proton pumps, the complex dissociates, which, in hepatocytes, is followed by degradation of insulin by endosomal acidic insulinase [32,42]. This transient process contributes to the acute nature of insulin's metabolic effects [32,40,42].

Once insulin binds to the α chains of its receptor, the tyrosine kinase activity of the β chains is activated, and each $\alpha\beta$ monomer transfers a phosphoryl group from ATP to the hydroxyl group of three critical tyrosine residues located near the carboxyl terminus of the β chain of its partner in the dimer [34]. Autophosphorylation enables phosphorylation of downstream target proteins, including the insulin receptor substrate (IRS) family [32,34,43]. The IRS family is composed of four closely related members (IRS-1, IRS-2, IRS-3, and IRS-4)—associated with, to a greater or lesser extent, β -cell growth and function and insulin sensing—and a more distantly related homolog, Gab-1—more likely involved in hepatic growth factor signaling [32,33,40]. When phosphorylated, insulin receptor substrate-1 (IRS-1) becomes the nucleation point for a complex of proteins that trigger two primary pathways—the mitogen-activated protein kinase (MAPK) pathway and the PI3K/Akt pathway [33,40].

To activate the MAPK pathway, IRS-1 binds growth factor receptor binding protein 2 (Grb2). Grb2 binds to Son-of-sevenless (Sos) and then to Ras, causing GDP-GTP exchange and Ras activation [29,33,34,39,40,43]. Activated Ras recruits c-Raf, which phosphorylates and activates MAPK/Erk kinase (MEK). MEK then phosphorylates extracellular signal-regulated kinase (Erk). Once activated, Erk moves into the nucleus, where it undergoes further phosphorylation and activates transcription factors such as ELK1, which modulates the transcription of about 100 insulin-regulated genes. This sequence ultimately stimulates cell division, protein synthesis, and cell growth [29,32–34,39,40,43].

In turn, to activate the PI3K/Akt pathway, PI3K binds IRS-1 through its SH2 domain [32,39,40,43,44]. Upon activation, its catalytic subunit phosphorylates proteins at serine residues or converts the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP₂) to phosphatidylinositol 3,4,5-trisphosphate (PIP₃). In turn, PIP₃ functions as an intracellular messenger, being responsible for growth stimulation, changes in intracellular trafficking, and activation of phosphoinositide-dependent kinases [29,30,32,33]. In effect, PIP₃ binds to the pleckstrin homology domain of Akt and induces its translocation to the plasma membrane [29,30]; there, it also functions as an allosteric regulator of phosphoinositide-dependent protein kinase 1 (PDK1), which phosphorylates Akt and protein kinase A (PKA) [30].

Akt is a serine/threonine kinase that acts on more than 100 target proteins, one of them being glycogen synthase kinase 3 (GSK3) [29,30,40,43,45]. When phosphorylated by Akt, GSK3 loses its catalytic activity and is no longer able to phosphorylate and inactivate glycogen synthase (GS). By preventing GS inactivation, insulin signaling stimulates glycogen synthesis in the liver and muscle [29,30,43,45–47]. In addition, Akt promotes the translocation of glucose transporters (of which GLUT4 appears to be the predominant isoform in muscle and adipose tissues *in vivo*) from internal vesicles to the plasma membrane, stimulating glucose uptake from the blood by a facilitative

process [29,30,33,40,43,48,49]. Yet, it is not crucial to this function, since its inhibition does not completely abolish insulin's effects on glucose transport [50]. By phosphorylating and inhibiting tuberous sclerosis complex 2 (TSC2), Akt is equally responsible for mechanistic target of rapamycin complex 1 (mTORC1)-mediated activation of protein biosynthesis and cell proliferation [30,32,35,36,40,45,46].

Besides these activities, an increasing number of studies have assigned Akt a role in promoting cell survival and regulating cell growth and differentiation [45,46,51]. Since it can phosphorylate proteins that regulate lipid, protein, and glycogen synthesis and cell survival, Akt provides a direct link between insulin receptor signaling and insulin's biological effects [32,40,45,51].

2.1.2. Molecular Mechanisms of T2D Pathogenesis

The pathogenesis of T2D is complex and multifactorial, primarily involving insulin resistance—a reduced cellular response to insulin—and, in many cases, impaired insulin secretion and disrupted insulin signaling. Although insulin secretion is not completely absent, pancreatic β -cells often fail to compensate adequately for the increased demand, especially as the disease progresses [29–32]. These alterations are closely associated with obesity and chronic low-grade inflammation, as discussed below. Such factors collectively disrupt key metabolic pathways that regulate glucose homeostasis.

Insulin resistance may arise from abnormalities in insulin receptor function or defects in post-receptor signaling pathways, resulting in a marked reduction in glucose uptake by peripheral tissues, particularly muscle and adipose tissue, which become less responsive to insulin [29–32]. Moreover, there is impaired suppression of hepatic glucose production and increased lipolysis in adipose tissue. These disturbances contribute to persistent hyperglycemia, which in turn exacerbates β -cell stress and gradually impairs insulin secretion [29–32].

Regarding impaired insulin signaling, defects can occur at several points along the pathway. For example, serine phosphorylation of IRS-1, often triggered by pro-inflammatory cytokines such as tumor necrosis factor (TNF- α) and activation of stress-related kinases like c-Jun N-terminal kinase (JNK) and IKK β , impairs insulin signaling, contributing to insulin resistance [52,53]. Reduced expression of GLUT4 in muscle and adipose tissue also limits glucose uptake, while the upregulation of suppressors of cytokine signaling proteins promotes IRS degradation, further weakening insulin action [52,54].

Among the key risk factors, obesity constitutes a major contributor to T2D and plays a pivotal role in the development of insulin resistance through multiple interconnected mechanisms. In obesity, an enlargement of adipose tissue is observed, along with increased levels of free fatty acids and adipokines, namely resistin, that interfere with insulin signaling [52]. Furthermore, dysfunctional adipose tissue exhibits increased infiltration of immune cells, which secrete pro-inflammatory cytokines that exacerbate insulin resistance [52,54]. Elevated circulating levels of cytokines such as TNF- α , IL-6, and IL-1 β are commonly observed in individuals with metabolic syndrome and T2D, which impair insulin signaling via activation of inflammatory pathways [52–55]. These inflammatory molecules also promote insulin resistance in the liver and contribute to T2D-related complications like NAFLD and non-alcoholic steatohepatitis (NASH) [52]. In fact, in addition to disrupted glucose metabolism, lipid metabolism is also impaired, with increased lipolysis in adipose tissue and ectopic lipid accumulation in organs such as the liver and muscle, increasing insulin resistance [52,56]. Additionally, mitochondrial dysfunction is frequently observed in insulin-resistant tissues, impairing fatty acid oxidation and increasing ROS production [57]. This oxidative stress leads to the accumulation of lipotoxic intermediates and activation of stress and inflammatory pathways, further disrupting insulin signaling

and reducing β -cell viability [57,58]. As the disease progresses, pancreatic β -cell function worsens due to glucotoxicity, lipotoxicity, and cytokine-induced stress, ultimately resulting in insufficient insulin secretion relative to metabolic demand [52,56].

2.1.3. T2D Treatment

Lifestyle modifications, particularly diet and physical activity, are key components in T2D management. Weight loss often improves insulin sensitivity, as excess adipose tissue contributes to insulin resistance. One such mechanism involves adipokines, including resistin [59]. Exercise is especially beneficial, as increased muscle activity enhances glucose uptake independently of insulin, thereby helping to reduce blood glucose levels and alleviate hyperglycemia. In this context, both aerobic and anaerobic exercises have been shown to significantly improve glycemic control, leading to reductions in % hemoglobin A1c (HbA1c) levels, enhanced lipid profiles, and decreased blood pressure [60–62].

Concerning nutritional therapy, it should be focused on a calorie-controlled diet low in simple sugars and rich in dietary fiber to slow carbohydrate absorption and reduce postprandial glucose peaks. Diets rich in whole grains, legumes, fruits, vegetables, lean proteins, and healthy fats have been shown to improve glycemic control, significantly lower HbA1c, and promote weight loss [63,64].

Nevertheless, in addition to lifestyle changes, many patients require oral pharmacological treatment. These oral hypoglycemic agents include insulin secretagogues, such as sulfonylureas, and insulin sensitizers, primarily biguanides like metformin, which remains the first-line treatment for T2D [65–67]. Sulfonylureas act by stimulating insulin secretion through closure of ATP-sensitive potassium channels in pancreatic β -cells and are typically used when metformin is contraindicated [67]. However, they are associated with a higher risk of hypoglycemia, weight gain, and progressive loss of efficacy over time [66,67]. In contrast, metformin reduces hepatic glucose production, improves insulin sensitivity, is weight-neutral or may induce minor weight loss, carries a low risk of hypoglycemia, and is generally well-tolerated [67,68].

2.2. Cancer

In healthy, non-transformed cells, glucose serves as a primary source of energy. Under normoxic conditions, glucose metabolism proceeds through a tightly regulated sequence of biochemical pathways, including glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation (OXPHOS) [69]. While normal cells predominantly rely on mitochondrial OXPHOS under aerobic conditions, many cancer cells exhibit a fundamentally different metabolic behavior. In what is known as the Warburg effect, first described by Otto Warburg in the 1920s, tumor cells preferentially convert glucose into lactate via aerobic glycolysis, even when oxygen is available to support mitochondrial respiration [26,70,71]. This phenomenon is now understood as a strategic adaptation rather than a consequence of mitochondrial dysfunction. Despite its lower energetic efficiency, the Warburg effect offers several advantages to rapidly proliferating tumor cells. First, glycolytic intermediates are redirected into anabolic pathways such as the PPP and serine biosynthesis, supplying nucleotides, amino acids, and lipids [70]. Second, lactate production contributes to the acidification of the tumor microenvironment (TME), which facilitates tissue invasion, suppresses immune responses, and promotes angiogenesis [70,72]. Third, glycolysis can proceed at a much faster rate than OXPHOS, allowing for rapid energy generation to meet acute demands [72]. Collectively, the Warburg effect represents a hallmark of cancer metabolism and is closely associated with tumor progression, therapy resistance, and immune evasion.

To meet the elevated metabolic demands imposed by aerobic glycolysis, cancer cells increase their capacity to import glucose. This is primarily achieved through the upregulation

of GLUTs [26]. Among these, GLUT1 and GLUT3 are the most frequently overexpressed isoforms across various tumor types [73]. GLUT1, a high-affinity glucose transporter, is commonly upregulated in lung, breast, prostate, and brain cancers [74–76]. GLUT3, which has even higher glucose affinity than GLUT1, is particularly elevated in highly proliferative and aggressive tumors, including gastric cancers [77]. The overexpression of these transporters not only correlates with tumor grade and poor prognosis but also reflects the metabolic shift toward increased glycolytic flux. The expression of GLUTs in tumor cells is tightly regulated by oncogenic signaling pathways. For example, the MYC oncogene directly enhances the transcription of GLUT1 and several glycolytic enzymes. Similarly, activation of the PI3K/Akt/mTOR signaling axis promotes GLUT surface localization and stability, while also stimulating glycolytic enzyme activity. A central mediator in this regulation is hypoxia-inducible factor 1- α (HIF-1 α), a transcription factor stabilized under hypoxic conditions, common in solid tumors. HIF-1 α induces the expression of GLUT1 and glycolytic enzymes, facilitating adaptation to oxygen-poor microenvironments [26,78]. Together, these mechanisms ensure that tumor cells maintain continuous and elevated glucose uptake, supporting both energy production and the generation of biosynthetic precursors required for rapid proliferation. The dependency on GLUT-mediated glucose transport has emerged as a potential vulnerability in cancer, with therapeutic strategies under investigation to inhibit GLUT function or expression.

In addition to the upregulation of glucose transporters, cancer cells exhibit profound alterations in the expression and activity of key glycolytic enzymes, further enhancing the glycolytic flux that supports rapid growth and survival under adverse conditions. These enzymatic changes are not essential for maintaining high metabolic throughput but also enable the redirection of intermediates into biosynthetic pathways, contributing to the metabolic flexibility characteristic of cancer cells [70]. One of the most frequently upregulated enzymes in cancer is hexokinase II (HK2), which catalyzes the first committed step of glycolysis [79]. Similarly, phosphofructokinase-1 and the tumor-specific pyruvate kinase M2 isoform (PKM2) play key roles in regulating glycolytic flux and facilitating the diversion of intermediates into biosynthetic pathways. Together, these enzymes are tightly regulated by oncogenic signaling and contribute to tumor progression, proliferation, and survival under metabolic stress [80,81]. Among them, lactate dehydrogenase A (LDHA), which converts pyruvate to lactate, is typically overexpressed in tumor cells and strongly regulated by HIF-1 α . Shu et al. demonstrated that elevated LDHA activity supports the Warburg effect by regenerating NAD⁺ required for sustained glycolysis and by producing lactate, which contributes to microenvironmental acidification. This acidification promotes tumor invasion and immune evasion, reinforcing the malignant phenotype [82].

While the Warburg effect is a hallmark of cancer metabolism, mitochondrial function remains essential. Pyruvate and acetyl-CoA link glycolysis to the TCA cycle, fueling biosynthesis and maintaining redox homeostasis. Although the Warburg effect is the most recognized metabolic alteration in cancer, glycolysis is not the sole source of ATP in most tumors. In fact, solid tumors are composed of metabolically heterogeneous cell populations that may rely on aerobic glycolysis, anaerobic glycolysis, or OXPHOS, often coexisting within the same microenvironment [83,84]. This dual metabolic strategy, combining rapid but inefficient ATP production via glycolysis with mitochondrial oxidative metabolism for biosynthetic precursor generation, confers a metabolic advantage, particularly under fluctuating environmental conditions. Notably, OXPHOS plays a critical role in certain tumor subpopulations, such as cancer stem cells, and in tumors that develop resistance to glycolytic inhibition [85,86]. Several tumor types, including melanoma, ovarian cancer, and acute myeloid leukemia, have been shown to rely heavily on OXPHOS. In these contexts,

OXPHOS supports not only ATP production but also redox balance and survival under metabolic stress, making it a promising therapeutic target [87].

The TME is profoundly shaped by these metabolic adaptations. Hypoxia, a common feature of solid tumors, stabilizes HIF-1 α , which upregulates GLUT1, HK2, LDHA, and enzymes of the PPP, reinforcing the glycolytic phenotype and promoting survival under low oxygen tension [26,78,88]. Lactate accumulation and export by monocarboxylate transporters (MCTs) acidifies the extracellular environment, facilitating invasion, immune evasion, and angiogenesis [88–90]. Moreover, the high glucose consumption by tumor cells creates a competitive metabolic landscape. Immune cells, particularly activated T lymphocytes, require glucose for their effector functions. In glucose-depleted environments, immune cells become metabolically exhausted, which contributes to immune suppression and tumor immune escape [90,91].

These metabolic interactions within the TME underscore the dual role of glucose metabolism in supporting tumor cell fitness and modulating immune responses. Beyond the Warburg effect, mitochondrial metabolism also plays a central role in tumor progression and immune regulation. As such, targeting oxidative phosphorylation (OXPHOS) has emerged as a promising therapeutic approach. In this context, the antidiabetic drug metformin has gained attention for its ability to inhibit mitochondrial complex I, modulate tumor metabolism, and restore immune function.

3. Metformin Targets and Mechanism of Action

3.1. Metformin Targets and Mechanism of Action as an Antidiabetic Drug

Metformin is widely recognized as the first-line therapy for T2D, exerting its glucose-lowering effects through several mechanisms. A central mechanism of its antidiabetic action is the inhibition of hepatic gluconeogenesis, which involves both redox-dependent and mitochondrial pathways. Disruption of the cytosolic redox balance plays a central role in metformin's mechanism of action. At clinically relevant concentrations (50–100 μ M), metformin inhibits mitochondrial glycerol-3-phosphate dehydrogenase (GPD2), thereby altering the cytosolic NADH/NAD⁺ ratio and selectively suppressing hepatic gluconeogenesis from substrates such as glycerol and lactate [68,92,93]. In fact, the inhibition of GPD2 and the resulting redox imbalance are considered the predominant pathways at therapeutic concentrations [68]. Concerning the mitochondrial pathway, it involves the inhibition of mitochondrial complex I, resulting in decreased cellular ATP levels and thereby limiting the energy supply required for gluconeogenesis [94]. Another important component of metformin's antidiabetic action is the activation of AMPK in hepatocytes and other metabolically active tissues, which has significant effects on metabolic pathways. AMPK activation is associated with metformin's partial inhibition of mitochondrial complex I, leading to subtle changes in the cellular energy charge, specifically an increased AMP/ATP ratio, which promotes AMPK activation (Figure 2) [68,95,96]. The metabolic consequences of AMPK activation include: (1) reduced hepatic gluconeogenesis via downregulation of key gluconeogenic enzymes, leading to decreased hepatic glucose output; (2) enhanced fatty acid oxidation and inhibition of lipogenesis; (3) increased glucose uptake in skeletal muscle mediated by GLUT4 translocation to the plasma membrane; and (4) inhibition of mTOR signaling, leading to decreased protein synthesis and reduced cell growth [96,97]. However, it is important to note that complex I inhibition leading to AMPK activation has been observed at supra-pharmacological concentrations of metformin [68].

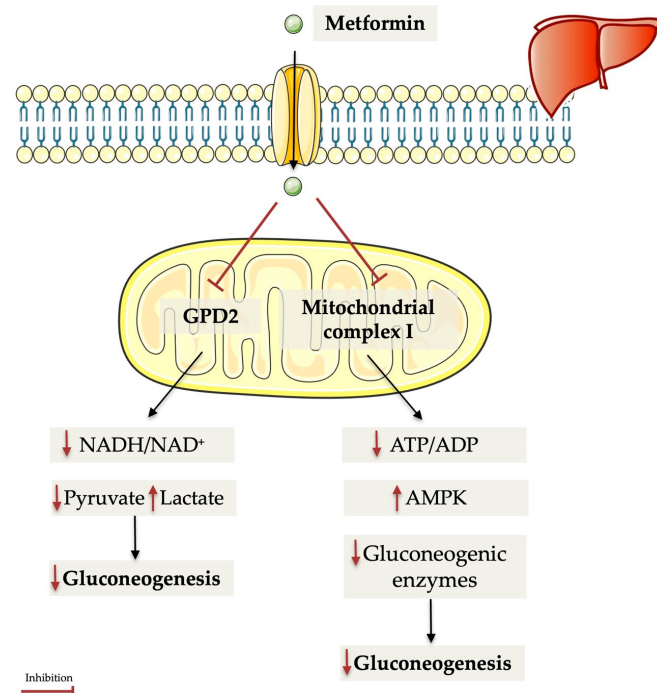


Figure 2. Inhibition of hepatic gluconeogenesis by metformin. The effects involve both redox-dependent and mitochondrial pathways. Mitochondrial glycerol-3-phosphate dehydrogenase (GPD2) is inhibited, leading to a decreased cytosolic NADH/NAD⁺ ratio and reduced availability of substrates such as lactate. Mitochondrial complex I is also inhibited, resulting in lower cellular ATP levels. Subsequent activation of AMPK leads to downregulation of key gluconeogenic enzymes and decreased hepatic glucose output. ↑: increased; ↓: decreased.

3.1.1. Metformin and Gut Microbiome

Since patients with metabolic diseases, such as T2D, often exhibit dysbiosis, the gut microbiome has acquired heightened importance in the context of metabolic disease treatment [98]. When administered orally, metformin levels in the gut are 100- to 300-fold higher than in serum, making the gut a major reservoir for the drug in humans. Furthermore, there is accumulating evidence that it also acts through gut-related pathways [99–101]. Its hypoglycemic effect is more pronounced when administered orally, and the lower bowel appears to be a major site of action [102]. Metformin's impact on gut microbiome comes as no surprise when considering the historic use of biguanides as antimicrobials, with folate metabolism impairment possibly being one of the mechanisms underlying such effects [103]. The bacterial reshaping observed after treatment may result from taxon-specific resistance/sensitivity to the bacteriostatic or bactericidal activity of the drug [104].

Although it is difficult to disentangle the effects of pharmacological treatment from those deriving from metabolic disease itself [98,105], different studies have successfully associated metformin use with alterations in the relative abundance of several bacterial strains, most of which are Proteobacteria and Firmicutes [106–108]. At the genus level, treatment consistently results in an increase in *Escherichia* and a decrease in *Intestinibacter* [103–105,109,110]. Microbial changes in individuals initially not taking metformin but later switched to receive the drug correlate with those observed upon longer-term treatment. Additionally, these individuals also experience an increase in *Bifidobacterium* [106]. Various studies also report treatment-induced abundance of *Akkermansia muciniphila*. This bacterial strain had already been associated with improved metabolic features in mice and humans [101,103,105,106,111]. Metformin is also associated with an increase in mucin-producing cells, and *A. muciniphila* occurs predominantly in the

mucus layer of the colon, where it enhances mucus secretion, promotes intestinal integrity, and decreases epithelial permeability and inflammation; in fact, it modulates the immune system and delays the onset of type 1 diabetes mellitus (Figure 3) [98,99,107,111,112]. Whole-genome shotgun analysis revealed that *Bifidobacterium adolescentis* was increased by metformin treatment, and a negative correlation was found between its abundance and HbA1c, suggesting a contribution to the drug's antidiabetic effect [106]. Interestingly, transferring microbiota from metformin-treated individuals to germ-free mice improves glucose tolerance, indicating causality [103,106].

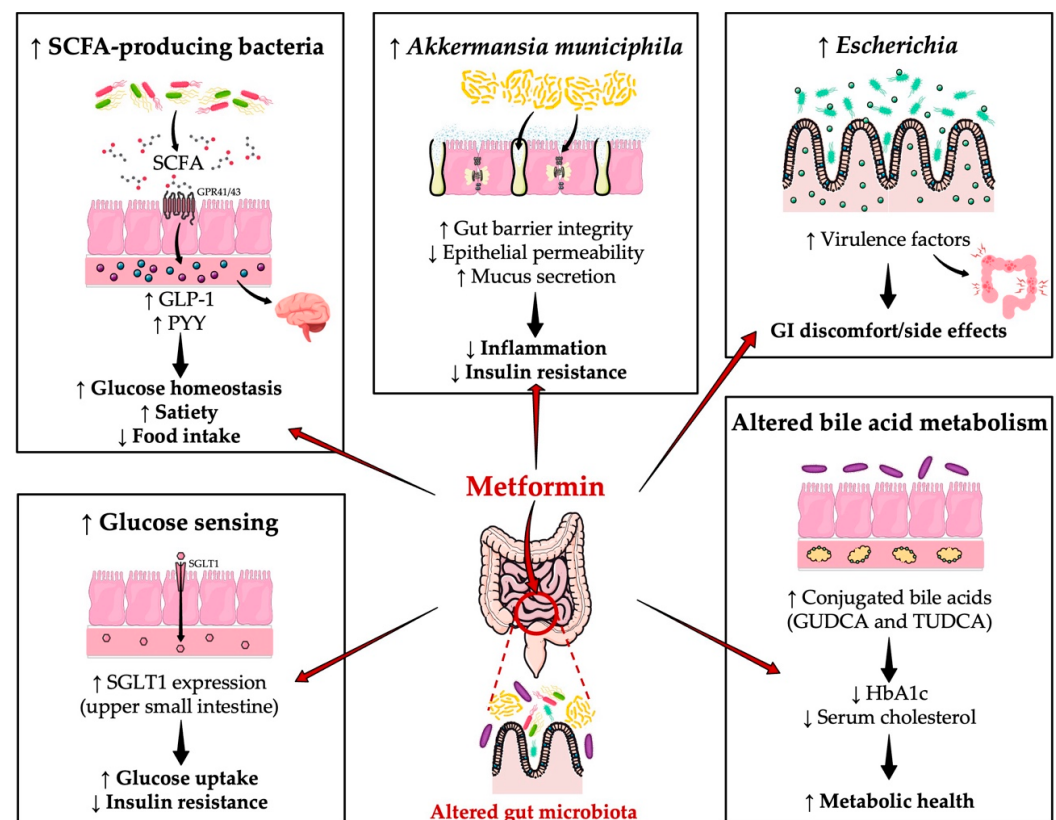


Figure 3. Metformin interferes with metabolism, glucose homeostasis and weight control by affecting the gut microbiota, namely by increasing the relative abundance of *Akkermansia*, *Bifidobacterium*, *Escherichia*, and SCFA-producing bacteria, and decreasing the proportion of *Intestinibacter*. For more details on each mechanism, please refer to the main text. GI: gastrointestinal; GLP-1: glucagon-like peptide 1; GPR41/43: G-protein-coupled receptors 41 and 43; GUDCA: glyoursodeoxycholic acid; HbA1c: % hemoglobin A1c; PYY: peptide YY; SCFA: short-chain fatty acids; SGLT1: sodium-glucose cotransporter 1; TUDCA: tauroursodeoxycholic acid; ↑: increased; ↓: decreased.

In vivo and in vitro metagenomics and metatranscriptomics evidenced consistent shifts in lipopolysaccharide (LPS) biosynthesis, incretin, and short-chain fatty acid (SCFA) metabolism; the latter was further confirmed by an increase in acetate, propionate, and butyrate [98,102,104–107,109,110,113]. In vivo data further support that metformin enhances the growth of SCFA-producing bacterial taxa in general, including *Allobaculum*, *Bacteroides*, *Bifidobacterium*, *Blautia*, *Butyricoccus*, *Butyrivibrio*, *Lactobacillus*, *Akkermansia*, *Phascolarctobacterium*, *Butyricimonas*, *Coproccoccus*, and *Ruminococcus* [98,99,103,111]. In rodents, augmented SCFA production was shown to increase intestinal gluconeogenesis and reduce hepatic gluconeogenesis, appetite, and body weight, thereby promoting beneficial metabolic effects [101,103,104]. A recent integrative perspective suggests the gut-kidney-brain axis is implicated in the concerted regulation of the anorexigenic signals behind metformin action [102]. The interaction between metformin and the gut microbiota modu-

lates epithelial cell activity, which produces metabolites, gut hormones, anorexigenic agents, SCFAs, and secondary bile acids that circulate in the bloodstream, targeting multiple organs and regulating glucose metabolism [102,107]. SCFAs, through G-protein-coupled receptors GPR41/43, lead to the intestinal secretion of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), regulating glucose homeostasis and food intake (Figure 3) [107]. In addition, butyrate has been shown to inhibit histone deacetylases, thereby having anticancer properties [109].

In parallel, bile acid reabsorption in the distal ileum decreases, whereas the plasma levels of conjugated bile acids, such as glycochenodeoxycholic acid (GUDCA) and taurochenodeoxycholic acid (TUDCA), increase with metformin treatment. Higher bile acid metabolism is negatively associated with HbA1c and serum cholesterol levels, suggesting a potential link between bile acid modulation and improved metabolic health (Figure 3) [98,106,107,109,111].

On the other hand, some microbiome alterations, namely increased abundance of *Escherichia* and associated virulence factors, also underlie metformin's side effects, such as bloating, diarrhea, nausea, and intestinal discomfort (Figure 3) [102,104,105].

Interindividual differences in gut microbiota, resulting from heredity, ethnicity, and lifestyle, among others, may account, at least in part, for the interindividual differences in metformin therapeutic success, tolerance or intolerance, and even for the development of side effects or toxicity. This opens potential for personalized medicine based on microbiome profiling [99,102]. It should also be noted that shifts in the microbiome are closely followed by metabolome changes, with metformin causing enrichment in microbial pathways involved in the TCA cycle, butanoate, arginine, and proline metabolism; this has been suggested to have implications in various aspects of the host's physiology and health, including their cognition, mood and behavior [108,112].

3.1.2. Metformin and Obesity

As previously mentioned, obesity, hyperinsulinemia, and insulin resistance are typical features of T2D. Since obesity and T2D share similarities in terms of insulin-receptor binding, tyrosine kinase signaling, and glucose transport, metformin is efficacious in both conditions [114,115]. Accordingly, insulin resistance is the underlying cause of obesity in T2D diabetic patients, but also in non-diabetic conditions, such as polycystic ovary syndrome. Metformin restores sodium-glucose cotransporter 1 (SGLT1) expression in the upper small intestine, improving glucose sensing [111]. By increasing insulin sensitivity, it also affects body weight, being useful in obesity treatment and prevention [114–116]. In line with this, metformin treatment has been shown to lead to lower caloric intake, as well as moderate weight loss in insulin-sensitive and insulin-resistant obese patients, with the percentage of weight loss being more pronounced in the latter [100,114,115,117].

Improved insulin sensitivity reduces delayed postprandial peaks of the hormone, which frequently cause hypoglycemia and consequent carbohydrate and compensatory food cravings. Furthermore, as stated earlier, metformin improves insulin-stimulated glucose distribution to skeletal muscle, inhibits hepatic gluconeogenesis and glucose output, and reduces glucose intestinal absorption [100,114,116,118]. Even when skeletal muscle and liver cells are resistant to insulin, adipose tissue remains relatively sensitive to the hormone, which justifies the accumulation of abdominal fat. In this context, as metformin restores peripheral insulin sensitivity, it prevents pancreatic hypersecretion of the hormone, attenuating insulin signaling in adipose tissue and accumulation of abdominal fat [114]. Collectively, these effects contribute to lower glucose availability and consequent fat storage in adipose tissue, explaining why metformin diminishes adipose tissue, but not lean body mass [114,117,118].

The fact that metformin treatment also significantly reduces body weight in non-diabetic patients suggests that additional mechanisms, besides decreasing insulin resistance, are involved in metformin-mediated weight loss. In this sense, metformin has been shown to suppress appetite in a dose-dependent manner, possibly because it acts by decreasing leptin levels, thereby improving leptin resistance [114,115,117]. The rise in GLP-1 and PYY levels under treatment with the biguanide, as well as fewer hyperinsulinemia events, are possible factors accountable for diminished hunger and increased satiety [100,114,115]. Lactate accumulation, due to the inhibition of the electron transport chain and increased anaerobic glycolysis, leads to mild metabolic acidosis, which is also appetite-suppressing [100]. On the other hand, metformin-induced weight loss seems to be independent of changes in energy expenditure [100]. There is also evidence that metformin modulates the activity of the central nervous system, namely the parahippocampal gyrus, ventromedial prefrontal cortex, fusiform gyrus, and hypothalamus. These areas are involved in semantic memory, reward formation, and orexigenic pathways; such interactions may then underlie altered food-reward relationships and decreased hunger [100,116].

As explored in the previous section, metformin's interaction with the gut microbiome may also promote appetite reduction and weight loss. Increased abundance of SCFA-producing species decreases hepatic gluconeogenesis, limits fatty acid release from adipocytes, and suppresses appetite via the incretin system [100,101].

Currently, the use of metformin with the sole purpose of weight reduction remains off-label; inconsistencies in weight loss, side effects, and the emergence of newer, more effective drugs have prevented its approval as a first-line treatment for obesity [117]. Still, it represents an option for patients at high risk of metabolic complications and who do not tolerate or comply with other interventions, either pharmacological or non-pharmacological [100]. It is also being investigated as a potential tool for counteracting sarcopenic obesity, a condition characterized by increased fat mass and decreased lean mass that is typical of aging. Interestingly, ongoing studies focus on this biguanide as a strategy to mitigate obesity's sequelae, such as NAFLD, obstructive sleep apnea, metabolic bone disease, and osteoarthritis [100,116].

3.2. Metformin Targets and Mechanism of Action as an Anticancer Drug

Although the Warburg effect or aerobic glycolysis is widely recognized as a cancer hallmark, it represents only one aspect of the metabolic reprogramming observed in malignant cells. In fact, and in opposition to Warburg's first postulate that cancer cells have defective mitochondria, it is now well established that cancer cells can also rely on mitochondrial metabolism to support their growth and survival [119–122]. It has already been reported that OXPHOS contributes more to total ATP production than aerobic glycolysis in many tumors [123]. Tumors are very heterogeneous and both pathways can contribute to ATP production, providing cells with metabolic flexibility to adapt and proliferate in response to environmental conditions [121]. Although many cancers primarily rely on aerobic glycolysis for proliferation, the contribution of this pathway to total ATP production can vary widely, ranging from as little as 1% to as much as 64% [123]. Recent studies have demonstrated that OXPHOS can be upregulated in different types of cancer, like leukemias, lymphomas, pancreatic ductal adenocarcinoma, and this can happen even in the context of elevated glycolytic activity, highlighting the ability of cancer cells to simultaneously activate diverse metabolic pathways for energy production [87]. Several agents that inhibit the glycolytic pathway—such as 3-bromopyruvate, dichloroacetate (DCA), and 2-deoxyglucose (2-DG)—have shown promising results as anticancer agents [124]. Nevertheless, others like phenformin, which targets mitochondrial complex I and thereby inhibits oxidative phosphorylation, have also been effective in reducing cell viability, ATP

production, and resistance to conventional anticancer drugs [125]. Consequently, in predominantly glycolytic tumors, targeting the Warburg effect may offer greater therapeutic benefit, whereas in cancers with a more oxidative metabolic profile, OXPHOS inhibitors such as metformin or phenformin may provide a more effective treatment strategy. In an in vitro study using glioma—a highly aggressive cancer known for its marked metabolic plasticity—as a model, it was observed that the glycolytic U251 cell line was more sensitive to the antiglycolytic agents DCA and 2-DG. However, treatment with phenformin did not significantly reduce intracellular ATP levels in these cells. In contrast, the more oxidative glioma cell line SW1088 showed reduced ATP levels and cell proliferation in response to all tested compounds, indicating a higher degree of metabolic plasticity [125]. Nevertheless, a stronger impact of OXPHOS inhibition on ATP production was observed, in alignment with the predominantly oxidative metabolism of this cell line. In both cell lines, all the tested agents increased the sensitivity to temozolomide, the gold standard treatment for glioma, suggesting their potential to enhance therapeutic efficacy when used in combination strategies [125]. However, despite the previous use of phenformin in lowering blood glucose levels, it has been withdrawn from clinical use in most countries due to its association with severe, and sometimes fatal, lactic acidosis [126]. Although metformin has been reported in some studies to be less potent in inhibiting cancer cell growth compared to phenformin [127], its well-established safety profile and extensive clinical experience have made it a standard treatment in medical practice. Therefore, several studies investigating metformin's potential as an anticancer agent are currently underway, and encouraging findings indicate that diabetic patients treated with metformin exhibit improved survival rates in lung, colorectal, and prostate cancers, along with a decreased risk of developing pancreatic, breast, colorectal, and liver cancers [128]. There are some well-established metformin targets, but its role in inhibiting the mitochondrial respiratory chain complex I, therefore impairing OXPHOS, causing bioenergetic stress, and altering metabolism in different cancer types, has been consistently reported (Table 1). Metformin is transported into the cell via an organic cation transporter (OCT) and accumulates primarily in the mitochondria, where it exerts its action by inhibiting complex I of the electron transport chain (Figure 2). Due to this inhibition, NADH accumulates and mitochondrial ATP production decreases, leading to an increase in the AMP/ATP ratio. This shift activates AMPK, a key energy sensor that helps restore energy balance by turning on catabolic energy-producing pathways and turning off anabolic energy-consuming ones [129]. Through these mechanisms, metformin influences cellular metabolism and may contribute to its therapeutic effects, not only in its primary role improving insulin sensitivity but also showing potential anticancer activity.

Table 1. Effects of metformin on different cancer types in in vitro and in vivo models, focusing on its action through mitochondrial complex I inhibition and metabolic effects.

Cancer Type	In Vitro Cell Lines/In Vivo Model and Dose Range	Effects on Cancer Characteristics	Mechanism	Metabolic Effects	References
Ovarian	In vitro studies with SKOV3 and A2780 cell lines	Reduces cell viability and proliferation; induces apoptosis and ferroptosis	Inhibition of mitochondrial complex I; energy depletion; ferroptosis under stress	Mitochondrial dysfunction and decreased ATP production; altered glucose metabolism	[130]
Head and Neck Squamous Cell Carcinoma (HNSCC)	In vitro studies with FaDU, Detroit 562 and HNSCC cell lines	Reduces growth and colony formation; cell cycle arrest	Complex I inhibition; loss of NAD ⁺ /NADH homeostasis	Reduced ATP levels; increased metabolic stress markers (increased AMP/ATP ratio)	[131,132]
Lung	In vitro studies with BEAS-2B, H170 and NSCLC H522, H2030, H1299, H2009, H838, A549 and H1975 cell lines In vivo studies with A/J mice (200 mg metformin /kg by intraperitoneal injection)	Decreases proliferation and colony formation; induces apoptosis through oxidative stress	Complex I inhibition causing energy depletion; increased mitochondrial ROS	Lower ATP production; altered lactate levels	[133]

Table 1. Cont.

Cancer Type	In Vitro Cell Lines/In Vivo Model and Dose Range	Effects on Cancer Characteristics	Mechanism	Metabolic Effects	References
Liver	In vitro studies with LO2, HepG2, Huh-7, LX-2 cell lines and with primary human hepatocytes	Reduces proliferation; induces autophagy and mitochondrial dysfunction; inhibition of cell cycle progression	Mitochondrial dysfunction; inhibition of mitochondrial complex I; disruption of mitochondrial voltage-dependent anion channel 1 (VDAC1)	Decreased ATP production; increased lactate production; influence on metabolite levels of lactate, alanine, glycerol-3-phosphate, glycerol, glycine, and glutamate, derived from glucose	[134–136]
Breast	In vitro studies with MCF-7, BT-474, SKBR-3 and TNBC Hs 578T and MDA-MB-231 cell lines	Suppresses proliferation; activates apoptosis; reduces cell growth and colony formation; potentiates the effect of conventional anticancer drugs; cell cycle arrest	Direct inhibition of complex I leading to metabolic stress; decreased mitochondrial respiration and increased aerobic glycolysis	Increased glucose consumption and lactate production; short-term exposure to metformin reduces cellular glucose uptake, but long-term exposure leads to the opposite	[137–140]
Pancreatic	In vitro studies with AsPC-1, BxPC-3, PANC-1 and MIAPaCa-2 cell lines and with primary pancreatic CSC In vivo studies with mice xenografted with pancreatic carcinomas (150 mg metformin/kg by intraperitoneal injection)	Limits tumor progression and proliferation; specifically eliminates pancreatic cancer stem cells	Complex I inhibition, reducing oxidative phosphorylation	Decreased ATP production; impaired mitochondrial metabolism; hyperglycemia protects against the metformin-induced growth inhibition	[141,142]
Leukemia	In vitro studies with REL NALM-6, HL60, MOLM14 and U937 cell lines	Induces apoptosis and energy stress; induces superoxide generation and oxidative stress; blockage of cell cycle and inhibition of cell proliferation and colony formation of leukemic cells	Inhibition of mitochondrial electron transport	Reduced oxygen consumption and mitochondrial ATP synthesis; increased glycolysis and lactate production, pentose phosphate pathway and fatty acid metabolism	[143,144]
Prostate	In vitro studies with LNCaP, P69, PC-3 and DU145 cell lines	Decreases proliferation and migration; induces energetic crisis; synergic effect with 2DG	Complex I inhibition induces energetic crisis	Decreased ATP levels; metabolic crisis indicators	[145]
Colorectal	In vitro studies with HCT116 p53 ^{−/−} cell line In vivo studies with mice xenografted with this cell line (drinking water containing 1–5 mg/mL of metformin)	Sensitizes cells to chemotherapy; reduces proliferation, migration, and invasion	Complex I inhibition causes energy depletion and enhances chemosensitivity	Reduced ATP; altered glucose metabolism	[146]

HNSCC, Head and neck squamous cell carcinoma NSCLC, non-small cell lung cancer; VDAC1, voltage-dependent anion channel 1 CSC; Cancer Stem Cells.

As said before, the Warburg effect is not the only metabolic strategy in cancer, and this model does not capture the full metabolic complexity, heterogeneity and plasticity of tumors. Moreover, emerging evidence supports the concept of the reverse Warburg effect, in which cancer-associated fibroblasts or other stromal cells perform aerobic glycolysis, exporting lactate that can be used by nearby cancer cells to fuel their oxidative metabolism [147]. Consequently, mitochondrial metabolism is not only preserved but often enhanced in several types of cancer, providing a metabolic plasticity that supports tumor growth, survival, and therapy resistance beyond the traditional Warburg paradigm. In this context, both antiglycolytic agents and electron transport chain inhibitors, such as metformin, may play a valuable role as anticancer therapies. Although the exact mechanism of action of metformin is not yet fully understood, one of the primary targets is believed to be the already mentioned mitochondrial complex I of the electron transport chain, leading to a reduction in ATP production [148,149]. Inhibition of complex I also increases the leakage of electrons and the production of ROS, contributing to oxidative stress, which further disrupts mitochondrial function [150]. Similar effects have been observed with phenformin, which, despite its discontinued clinical use due to the risk of lactic acidosis at high blood glucose levels, is a more potent inhibitor of mitochondrial complex I compared to metformin [87]. As a result of impaired mitochondrial respiration,

cells treated with metformin (or phenformin) increase their dependence on glycolysis for ATP generation. This metabolic reprogramming, unlike the Warburg effect, results in a moderate increase in lactate production, especially under conditions of high glucose availability or hypoxia [151–153]. Metformin can be particularly effective in a context of low glucose availability due to high glucose uptake by cancer cells. In these conditions, metformin enhances the metabolic stress, inhibiting mitochondrial ATP production. At the same time, the limited extracellular glucose restricts ATP generation through glycolysis. The combined impairment of both major energy-producing pathways leads to a critical energy crisis within the cell, ultimately triggering cell death [154].

Besides the respiratory chain (mostly complex I, though some studies suggest it may also inhibit complexes II and IV [155]), it was reported that metformin also inhibits HIF-1 activity, [156]. HIF-1 plays a central role in activating the transcription of GLUTs, glycolytic enzymes, MCTs, or vascular endothelial growth factor (VEGF), promoting angiogenesis, metabolic reprogramming, and cell survival under hypoxic conditions—processes that support tumor growth and progression [157,158]. By interfering with HIF-1 activity, metformin may also disrupt these adaptive responses, contributing to its potential anticancer effects. Furthermore, metformin has also been shown to inhibit glutaminase (GLS), an enzyme often overexpressed in cancer cells, essential to feed the TCA with α -ketoglutarate, supporting energy production and biosynthesis. Furthermore, blocking GLS activity, metformin may reduce ammonia production and limit ammonia-induced autophagy—a survival mechanism used by tumor cells under stress [159]. These effects on glutamine metabolism could contribute to metformin's antitumor action by disrupting a key energy and biosynthesis source for cancer cells. Besides, the bioenergetic stress induced by metformin can make tumor cells more vulnerable, thereby increasing the efficacy of conventional anticancer treatments.

3.2.1. Metformin-Induced Alterations in ROS Metabolism in Cancer Cells

Metformin has shown the ability to interfere with key cellular pathways, potentially inducing cell death, particularly in tumor cells. In addition to decreasing hepatic gluconeogenesis, metformin also modulates ROS levels, contributing to oxidative stress and impacting tumor cell viability [160]. Furthermore, metformin promotes autophagy via AMPK/mTOR signaling pathways, which suppresses the proliferation, migration, and invasion of cancer cells [161]. These diverse effects enhance metformin's potential for cancer prevention and treatment, in addition to its advantages in managing complications related to diabetes and processes associated with aging [162].

ROS are acknowledged for their essential function in maintaining homeostasis, as they regulate oxidative stress, intracellular signaling, and cell survival. In the context of cancer, ROS exhibit a dual function, facilitating both pro-tumorigenic and anti-tumorigenic signaling [163]. Cancer cells demonstrate increased levels of ROS because of metabolic changes and heightened proliferation rates. To preserve redox homeostasis and avoid apoptosis, cancer cells enhance their antioxidant mechanisms [164,165]. Tumor cells are recognized for exhibiting increased basal levels of ROS, which arise from metabolic changes, rapid proliferation rates, and enhanced survival abilities [166,167]. Nevertheless, metformin has demonstrated the potential to mitigate this characteristic, which contributes to the tumor cells' heightened resistance to apoptosis and improved proliferation capacity [167,168]. By interfering with the mitochondrial complex I, metformin leads to a decrease in ATP levels, which subsequently raises the AMP/ATP ratio, disrupts electron transport, and ultimately increases the production of ROS, such as superoxide anion and hydrogen peroxide, resulting in harmful oxidative stress [169,170]. The accumulation of ROS has various cellular consequences, particularly causing damage to DNA, lipids, and essential pro-

teins, and can also initiate cell cycle arrest, senescence, and apoptosis. It activates stress response pathways including p53, MAPK, or JNK, and may enhance the sensitivity of tumor cells to chemotherapy and radiotherapy, thereby intensifying the effects of genotoxic agents [171,172]. Additionally, metformin inhibits the expression of NFE2L1, which further worsens ROS accumulation [173]. There is evidence that metformin can selectively modulate ROS levels in tumor cells. In one study using AsPC-1 pancreatic cancer cells, metformin increased ROS, triggering apoptosis, autophagy, and necroptosis, with minimal effects on normal cells. These effects involved the AMPK–FOXO3a–MnSOD pathway and were enhanced by co-treatment with apigenin [150]. In contrast, another study using MIA PaCa and Panc1 cells under physiological glucose conditions found that metformin reduced ROS by increasing MnSOD and decreasing NOX2/NOX4 expression, also leading to reduced tumor cell viability [174]. In hepatocellular carcinoma (HCC), metformin-loaded hyaluronic acid-derived carbon dots enhanced intracellular ROS generation by inhibiting glutaminase-1 and GLUT1, leading to AMPK activation, Akt inhibition, and selective induction of apoptosis [175]. As stated in Table 1, in cases of ovarian cancer, metformin promotes apoptosis and ferroptosis during energy stress by directly affecting the NDUF4 subunit of mitochondrial complex I. This interaction results in mitochondrial dysfunction and a rise in ROS production [130]. In non-small cell lung cancer (NSCLC) cells, the cytotoxic effects of metformin and mitomet were enhanced through both apoptosis and ferroptosis, likely due to an increase in mitochondrial reactive oxygen species (mROS) production and a reduction in glutathione (GSH) levels. These effects were particularly evident in A549 cells deficient in LKB1. Furthermore, in vivo studies demonstrated that both compounds significantly reduced tumor burden, with mitomet showing a potency that was 100 times greater than that of metformin [133]. Overall, these findings suggest that metformin selectively disrupts redox homeostasis in tumor cells, with effects depending on the metabolic context and tumor type.

Unlike rotenone, metformin inhibits mitochondrial complex I without increasing ROS production in certain contexts, such as colorectal cancer. This results in reduced oxidative stress and the activation of HIF-1 α triggered by hypoxia, highlighting the context-dependent effect of metformin on ROS modulation [146]. Moreover, mitochondria are essential in the regulation of ferroptosis by affecting iron metabolism, lipid peroxidation, and the production of ROS in ovarian cancer cells [130]. Furthermore, metformin has been associated with a decrease in the expression of antioxidant enzymes, such as glutathione peroxidase (GPx) and superoxide dismutase (SOD), which exacerbates the accumulation of ROS [176,177]. The exact mechanism through which metformin interacts with complex I of the mitochondrial respiratory chain is not fully understood [152].

The effect of metformin on the metabolism of ROS varies according to the tumor type, the intrinsic redox state of the cells, and the tumor microenvironment. However, the increase in ROS induced by metformin has been demonstrated to be selective for tumor cells, sparing normal cells, which have superior redox regulation [150,174]. Thus, the modulation of ROS metabolism by metformin constitutes one of the central mechanisms of its anticancer action, especially when combined with therapies that exploit oxidative stress as a pathway for cancer cell death.

3.2.2. The Role of PI3K/AKT/mTOR Pathway in Mediating the Anticancer Effects of Metformin

The effect of metformin on mitochondrial respiratory chain complex I is also associated with activation of the AMPK pathway, a central kinase in detecting the cellular energy status [178,179]. In tumor cells, the activation of AMPK and inhibition of the mTOR pathway, which results from AMPK activation, have been linked to metformin's antitumor potential, although the exact mechanism is not yet fully understood [132,143] (Figure 4).

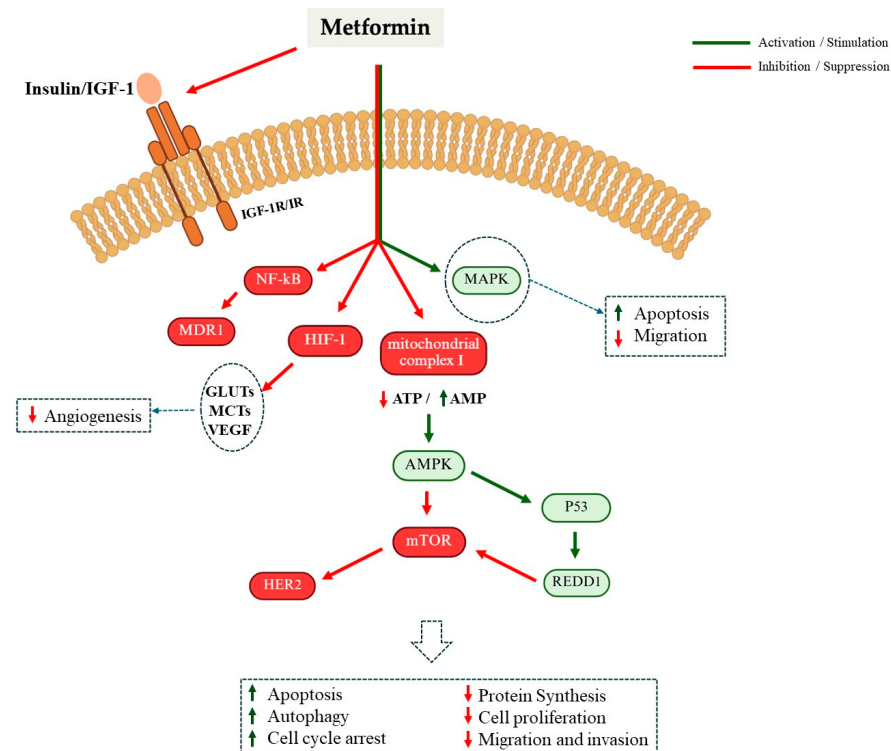


Figure 4. Schematic representation of the effects of metformin in cancer cells. Metformin inhibits mitochondrial complex I, increasing the AMP/ATP ratio and leading to activation of AMPK. AMPK activation suppresses mTOR, thereby promoting apoptosis, autophagy, cell cycle arrest and reducing protein synthesis, cell proliferation, migration and invasion. Metformin also inhibits MAPK, leading to increased apoptosis and decreased migration. Additionally, metformin suppresses NF-κB, resulting in reduced expression of drug resistance genes such as MDR1 (P-gp). Finally, metformin decreases HIF-1α stability/activation, inhibiting adaptive hypoxic responses (e.g., anaerobic glycolysis and angiogenesis). Black lines indicate activation and red lines indicate inhibition; arrows with ↑/↓ indicate increased/decreased processes.

While AMPK activation has been linked to metformin's antitumor potential, recent studies suggest that AMPK may actually attenuate metformin's toxicity by enhancing glycolysis [180]. Metformin inhibits hepatic mTORC1 signaling through dose-dependent mechanisms involving AMPK and the TSC complex [181]. It also suppresses mitochondrial-dependent biosynthesis, reducing cancer cell proliferation [182]. The AMPK pathway is one of the main sensors of cellular energy status and is involved in regulating multiple metabolic processes, including glucose homeostasis, lipid metabolism, autophagy, and cell proliferation. Under conditions of energy stress, such as low ATP levels, AMPK is activated to restore metabolic balance [183,184]. It also blocks lipogenesis by inhibiting acetyl-CoA carboxylase (ACC), reduces nucleotide and cholesterol biosynthesis, limiting precursors for highly proliferative cells, and promotes autophagy as a cellular recycling mechanism and, in some contexts, induces cell death [185].

Metformin activates this pathway indirectly through inhibition of mitochondrial complex I. This leads to decreased ATP production and increased levels of AMP and ADP. This imbalance in the AMP/ATP ratio promotes phosphorylation and activation of AMPK by the LKB1 kinase (liver kinase B1), particularly at the Thr172 residue of the catalytic subunit [136,184]. The AMPK has a multifaceted role in cancer, functioning as both a tumor suppressor and a promoter, based on the specific context [186]. AMPK inhibits mTORC1, which leads to a decrease in cell proliferation and tumor growth [187]. Conversely, mTORC1 can directly inhibit AMPK, thereby encouraging cell proliferation during periods of nutrient stress [188]. Before the onset of cancer, AMPK serves as a tumor suppressor; however,

once cancer is established, it may transition to promoting tumor survival [189]. Metformin plays a key role in modulating this pathway. For instance in HCC, metformin binds to the mitochondrial voltage-dependent anion channel 1 (VDAC1), disrupting the IP3R-GRP75-VDAC1 complex and leading to AMPK activation, which contributes to autophagy-mediated cell death and antiproliferative effects [136]. In breast cancer cells, metformin induces G0/G1 cell cycle arrest via AMPK activation and cyclin D1 downregulation. However, full growth inhibition also requires the presence of CDK inhibitors, highlighting the role of AMPK in modulating cell cycle machinery [138]. In pancreatic cancer, metformin activates AMPK at Thr172 and inhibits insulin/IGF-I signaling, promoting apoptosis under normoglycaemic conditions. Hyperglycaemia, however, impairs this effect by increasing Ser485 phosphorylation, which antagonizes Thr172 activation [142].

Besides metabolic effects associated with AMPK activation, it may also lead to reduced expression of cyclins and growth factors, causing cell cycle arrest in G1 phase, reducing invasion and metastasis, possibly by inhibiting epithelial–mesenchymal transition (EMT), and modifying the inflammatory and immune profile of the tumor microenvironment [190,191]. This effect is especially relevant in HNSCC, where metformin impairs tumor growth by AMPK-mediated mTOR inhibition. Notably, when HNSCC cells express the metformin-insensitive mitochondrial protein NDI1, metformin fails to activate AMPK or reduce tumor burden, suggesting its action depends on complex I inhibition and metabolic reprogramming [132]. Moreover, as previously mentioned, AMPK activation can inhibit aerobic glycolysis (the Warburg effect), redirecting cellular metabolism and creating an unfavorable environment for tumor growth. Many studies have linked AMPK activation by metformin directly to reduced tumor cell viability and clonogenicity, as well as increased sensitivity to chemotherapy [192,193].

In summary, by activating AMPK, metformin promotes an intracellular environment that is hostile to tumor survival, thereby limiting the growth, proliferation, and adaptive capacity of cancer cells [193,194]. The PI3K/Akt/mTOR pathway is a major pro-survival and pro-proliferative signaling route in tumor cells, frequently hyperactivated by oncogene mutations or loss of tumor suppressor genes such as PTEN [195,196]. This pathway regulates fundamental processes like growth, metabolism, cell survival, and therapy resistance, and is one of the most frequently deregulated pathways in cancer. Aberrant activation contributes to malignant transformation by promoting uncontrolled proliferation, apoptosis evasion, angiogenesis, and metastasis [196]. It stimulates increased protein synthesis, glycolysis, and lipid biosynthesis, inhibits apoptosis, and activates mTORC1 [197]. Metformin interferes with this pathway through two main mechanisms. First, it can indirectly inhibit it via AMPK activation. By activating AMPK, metformin inhibits the mTORC1 complex, a central regulator of cell growth. This inhibition primarily occurs through phosphorylation and activation of TSC2 and the protein raptor, both direct inhibitors of mTORC1 [181,194,198]. Consequently, protein synthesis is interrupted by inhibiting phosphorylation of key proteins like 4E-BP1 and S6K1, essential for protein translation [199,200]. This blockade reduces cell cycle progression and compromises tumor cell survival. This mechanism is especially relevant because it acts downstream of the PI3K/Akt pathway, which is often activated in many cancer types. Thus, metformin may exert antitumor effects even in the presence of mutations that activate Akt or PI3K, bypassing excessive activation and inhibiting tumor growth [198,201].

On the other hand, metformin may have direct effects on PI3K and Akt. Some studies have shown that metformin can also reduce Akt phosphorylation at key residues such as Ser473 and Thr308, interfere with PI3K binding to membrane receptors, reducing activation of pro-survival signaling, increase tumor cell sensitivity to apoptosis, and to therapies targeting the PI3K/Akt pathway [202–204]. However, these direct effects are highly de-

pendent on tumor type, presence of mutations in PTEN, LKB1, or p53, and the metabolic conditions of the cell [205]. For example, in metformin-sensitized cells such as HL60 and MOLM14, metformin treatment increased and sustained phosphorylation of Akt at Ser473, while in PTEN-null cells like U937, which exhibit high basal glycolysis and Akt phosphorylation, no further increase in Akt phosphorylation was observed upon metformin treatment. These metabolic differences contribute to these cells' resistance to metformin-induced cell death, likely due to their reduced mitochondrial dependence [144].

Metformin's impact also depends on nutrient conditions. Under low glucose, it is more cytotoxic by inhibiting Akt/mTOR and reducing ATP, without significantly changing AMPK phosphorylation, indicating AMPK-independent effects. Conversely, high glucose maintains glycolytic compensation, reducing metformin's efficacy [206]. For instance, in breast cancer cells, metformin induces apoptosis mainly under nutrient-poor conditions through AMPK activation and downregulation of PKM2. High glucose and amino acid levels abolish this effect, suggesting that nutrient availability critically modulates metformin's anticancer activity [207].

Metformin's interference with the PI3K/AKT/mTOR pathway reinforces its potential as an adjuvant agent in oncology, especially in combination with specific pathway inhibitors (e.g., rapamycin, everolimus), in tumors resistant to conventional chemotherapy, and in cancer models metabolically dependent on PI3K/Akt signaling [198]. Figure 4 summarizes the main metformin targets and the drug's effect on oncogenic characteristics.

3.2.3. Metformin-Based Strategies to Enhance Antitumor Therapy

To overcome the pharmacokinetic limitations of metformin and maximize its therapeutic potential in oncology, a range of innovative strategies has been developed. These include nanoparticle-based delivery systems and combination regimens with chemotherapeutic agents, immunotherapies, and molecularly targeted drugs [208]. These approaches aim to improve drug bioavailability, enable tumor-specific delivery, and exploit synergistic mechanisms of action. This section reviews the most promising advances in metformin-based antitumor strategies, with a particular focus on nanotechnology-driven delivery systems and combination therapies, highlighting their mechanistic rationale and translational relevance.

3.2.4. Metformin Encapsulation and Nanoparticle Delivery

Despite its well-documented antitumor properties, the clinical application of metformin in oncology has been hindered by its low bioavailability, rapid systemic clearance, and limited tumor tissue penetration [209,210]. To address these challenges, nanotechnology-based delivery systems have emerged as a powerful tool to enhance the pharmacokinetic and pharmacodynamic profiles of metformin. Encapsulation into nanocarriers, such as liposomes, polymeric nanoparticles, solid lipid nanoparticles, and functionalized hybrid systems, has demonstrated improved drug stability, controlled release, and preferential accumulation in tumor tissues via the enhanced permeability and retention effect [209–212]. Furthermore, surface functionalization with ligands such as folate or hyaluronic acid enables active targeting of tumor-specific receptors, thereby increasing therapeutic efficacy while minimizing off-target toxicity [211,213,214].

Table 2 summarizes the most relevant nanoparticle-based systems developed for metformin delivery in cancer therapy, highlighting their composition, target indications, therapeutic goals, and supporting studies.

Table 2. Overview of in vitro studies on nanoparticle-based systems for metformin delivery in cancer therapy, including the type of nanoparticle used, composition/carrier, target/indication, purpose, and mechanism/outcome.

Nanoparticle Type	Composition/Carrier	Target/Indication	Purpose	Mechanism/Outcome	References
Functionalized polymeric NPs	Folate-PLGA-PEG	Breast cancer (MDA-MB-231)	Targeted delivery, enhanced apoptosis and gene modulation	The effect of metformin was enhanced through increased cellular internalization, activation of the AMPK pathway, and inhibition of the mTOR pathway, promoting apoptosis and cell cycle arrest	[211]
Functionalized chitosan NPs	WZB117-OCMC	Breast cancer	Target GLUT1-overexpressing breast cancer cells and enhance metabolic disruption via dual delivery	Dual inhibition of GLUT1 and mTOR pathways enhances metformin uptake, reduces glycolysis, and promotes apoptosis and cell cycle arrest	[209]
	O-CMC (O-carboxymethyl chitosan)	Pancreatic cancer	Improve intracellular retention and controlled release of metformin	NPs enhance metformin's cellular uptake and retention, enabling sustained drug release and increased apoptosis in pancreatic cancer cells	[215]
Polymeric NPs	PLGA	Liver cancer (HepG2 cells)	Deliver metformin efficiently to hepatocellular carcinoma cells and enhance radiosensitization	NPs enhance metformin's antitumor effect by blocking autophagy, inhibiting mTOR/p53/HIF1A signaling, and inducing cell cycle arrest in HepG2 cells	[212]
Dual gold NP system	MET-GNPs + COL-GNPs	Breast cancer spheroids	Facilitate deep tumor penetration and selective delivery of metformin in dense tumor ECM	NPs enhance tumor penetration by degrading ECM and improve metformin delivery, leading to increased apoptosis in breast cancer spheroids	[210]
Hybrid NPs	Chitosan + Silver NPs	Breast cancer (radiation therapy)	Radiosensitization enhancement during radiotherapy	NPs enhance radiosensitization by increasing oxidative stress, impairing DNA repair, and promoting apoptosis in breast cancer cells	[216]
Carbon-based NPs	Activated Carbon NPs	Hepatocellular CSCs	Target hepatocellular CSCs with sustained metformin delivery and selective cytotoxicity	Activated carbon nanoparticles improve metformin delivery to hepatocellular CSCs, enhancing apoptosis and suppressing proliferation via AMPK activation and mTOR inhibition	[217]
Topical polymeric NPs	HA-coated chitosan/gelatin	Melanoma (topical)	Enable topical delivery of metformin to melanoma cells with enhanced skin penetration and retention	HA-coated chitosan/gelatin NPs enhance topical metformin delivery by improving skin penetration, cellular uptake, and cytotoxicity against melanoma cells	[213]
Functionalized mesoporous silica NPs	HA-coated MSNs	Lung cancer (A549)	Target CD44-positive lung cancer cells via HA-modified silica NPs for selective metformin delivery	HA-modified mesoporous silica NPs enhance metformin delivery to A549 cells via CD44 targeting, promoting AMPK activation, mTOR inhibition, and apoptosis	[214]
Lipid-based NPs	Lecithin	Colorectal cancer	Improve metformin bioavailability and enable epigenetic modulation	Lecithin NPs enhance metformin's cytotoxicity in colorectal cancer cells via epigenetic modulation of noncoding RNAs, leading to apoptosis and reduced cell viability	[218]
		Colon cancer	Enhance metformin stability and sustained release for colorectal cancer therapy	NPs improve metformin's stability and sustained release, enhancing cellular uptake and cytotoxicity against colorectal cancer cells.	[219]
Functionalized selenium NPs	TW80-SeNPs + Metformin	Breast cancer (MCF-7)	Combine selenium NPs with metformin to synergistically target breast cancer cells	NPs synergize with metformin by modulating selenoproteins, increasing oxidative stress, and promoting apoptosis in breast cancer cell	[220]
Gold NPs	AuNPs + Metformin	Breast and lung cancer (MCF-7, A549)	Evaluate synergistic antitumor effects of metformin and gold NPs	Nps synergize with metformin to enhance apoptosis, modulate BAX/BCL2 expression, and PI3K/Akt/mTOR pathway inhibition, reducing viability in breast and lung cancer cells	[208]

PLGA, Poly(lactic-co-glycolic acid); PEG, Polyethylene glycol; OCMC, O-carboxymethyl chitosan; NPs, nanoparticles; GNPs, Gold nanoparticles; MET-GNPs, Metformin-loaded gold nanoparticles; COL-GNPs, Collagen-coated gold nanoparticles; HA, Hyaluronic acid; MSNs, Mesoporous silica nanoparticles; CSCs, Cancer Stem Cells; TW80, Tween 80 (Polysorbate 80); SeNPs, Selenium nanoparticles; AuNPs, Gold nanoparticles; AMPK, AMP-activated protein kinase; mTOR, Mechanistic target of rapamycin; PI3K, Phosphoinositide 3-kinase; Akt, Protein kinase B; ROS, Reactive oxygen species; ECM, Extracellular matrix.

Table 3. Cont.

Chemotherapeutic Agent	Cancer Type	Observed Effect	Mechanism of Synergy	References
Doxorubicin	Gastric cancer	Metformin reduced viability and confluence of resistant cells; induced both autophagy and apoptosis	Induction of autophagy markers (Atg5, Atg12, LC3-II); increased caspase-3 and -7 activity; effect reversed by 3-MA, confirming autophagy involvement	[235]
		Increased chemosensitivity to cisplatin; reduced cell viability and metabolic activity; enhanced apoptosis and oxidative stress	Downregulation of Nrf2; activation of p53 and AMPK pathways; reversal of effects by Nrf2 overexpression or AMPK/p53 inhibition	[236]
	Bladder cancer (T24 cells)	Synergistic inhibition of metastatic potential; reduced migration and invasion	Likely via modulation of EMT and metabolic stress pathways	[223]
	Breast cancer (4T1 mouse model)	Enhanced antitumor efficacy; increased CD8 ⁺ T cell frequency in tumor microenvironment; reduced toxicity	Immunomodulation via increased CD8 ⁺ T cell infiltration; modulation of HIF-1 α and STAT3 expression; reduced doxorubicin toxicity	[237]
5-Fluorouracil	Breast cancer (DMBA-induced in rats)	Reduced tumor incidence and volume; improved survival; reduced organ toxicity	Enhanced antioxidant and anti-inflammatory activity; reduced Ki67 expression; decreased IL-6, IL-1 β , and NF- κ B levels	[224]
	Gastric cancer	Inhibition of tumor colony formation; reduced expression of Gli1 and TWIST1	Suppression of the Shh/Gli1 signaling pathway; EMT modulation	[222]
		Increased apoptosis; reduced chemotherapy-induced senescence	Loss of mitochondrial membrane potential; activation of caspase-dependent apoptosis pathways	[238]
		Reversal of 5-FU resistance; increased apoptosis and cytotoxicity	Downregulation of DKK1, WNT5A, ABCB1 (MDR1), P-gp, and CD44; inhibition of drug resistance pathways	[239]
	Colorectal cancer	Inhibition of proliferation and metastasis; enhanced anti-tumor effect in vivo	Synergistic suppression of tumor growth and invasion; possibly via modulation of cell cycle and apoptosis pathways	[240]
		Reversal of 5-FU resistance induced by radiotherapy; enhanced chemosensitivity	Restoration of folate metabolism via HM13-GGH-5,10-CH ₂ -THF axis; modulation of gene expression linked to drug resistance	[241]
	Hepatocellular carcinoma	Reversal of multidrug resistance; enhanced anti-proliferative and pro-apoptotic effects	Activation of AMPK/mTOR pathway; suppression of HIF-1 α ; downregulation of P-gp and MRP1	[242]
Docetaxel	Gastric cancer	Inhibition of tumor colony formation; reduced expression of Gli1 and TWIST1	Suppression of the Shh/Gli1 signaling pathway; EMT modulation	[222]
	Breast cancer (metastatic)	Enhanced antitumor efficacy; prolonged survival in vivo	Dual targeting of tumor bulk and cancer stem-like cells; improved delivery via PEGylated liposomes	[243]
	Prostate cancer	Reduced proliferation, motility, and viability in wild-type cells; partial reversal of resistance	Inactivation of ABC drug transporters; metabolic imbalance; Warburg effect induction; lineage-specific EMT responses	[244]
Paclitaxel	Ovarian cancer	Reversal of paclitaxel resistance; reduced cell viability, migration, and invasion	Inhibition of SNHG7/miR-3127-5p axis; suppression of autophagy; promotion of apoptosis	[245]
	NSCLC	Suppression of cancer stemness; reduced self-renewal and tumorigenicity in resistant cells	Activation of AMPK; upregulation of FOXO3a; inhibition of Akt and MEK pathways; downregulation of stemness markers (c-MYC, Oct4, Nanog, Notch)	[246]
	Breast cancer (T47D cells)	Significant reduction in IC ₅₀ of paclitaxel when combined with metformin; enhanced cytotoxicity	Enhanced cellular uptake via niosomes; metformin-induced sensitization; potential reduction in required paclitaxel dose	[247]
Temozolomide	Glioblastoma	Enhanced inhibition of proliferation and migration; increased apoptosis	Suppression of mitochondrial biogenesis, EMT, and MGMT expression; increased ROS production and mitochondrial dysfunction	[248]
		Well-tolerated combination; promising progression-free survival and overall survival	Activation of AMPK-FOXO3a pathway; differentiation of glioma stem-like cells; suppression of tumor formation	[249]

NSCLC, Non-small cell lung cancer; ORR, overall response rate; PFS, progression-free survival; OS, overall survival; AMPK, AMP-activated protein kinase; NPC, nasopharyngeal carcinoma; ROS, reactive oxygen species; HNSCC, head and neck squamous cell carcinoma; EMT, epithelial–mesenchymal transition; ATM/CHK2, ataxia telangiectasia mutated/checkpoint kinase 2; MDR1, multidrug resistance protein 1; P-gp, P-glycoprotein; MRP1, multidrug resistance-associated protein 1; STAT3, signal transducer and activator of transcription 3; HIF-1 α , hypoxia-inducible factor 1-alpha; shh, sonic hedgehog; Gli1, GLI family zinc finger 1; FOXO3a, forkhead box O3; MEK, mitogen-activated protein kinase.

4. Clinical Trials

Metformin is a well-established antidiabetic drug widely used as a first-line treatment for T2D. Its primary effect is the reduction in blood glucose levels, achieved mainly through the inhibition of hepatic gluconeogenesis and improvement of insulin sensitivity in peripheral tissues, particularly skeletal muscle. The use of already clinically approved and well-tolerated drugs in cancer treatment—an approach known as drug repurposing—is gaining increasing attention. This strategy offers several advantages, including reduced development time, cost effectiveness, and established safety profiles [250]. In particular, interest has grown around repurposing metabolic drugs, such as metformin, due to their potential to target cancer-related pathways beyond their original indications. The prolonged use of metformin has been associated with a decrease in tumor incidence and a lower rate of cancer-related mortality [251]. Based on these premises, several clinical trials have been conducted using metformin to assess its efficacy and safety profile in the combinatorial therapy of various types of cancer. The search of clinical trials clinicaltrials.gov, using “cancer” and “metformin” as keywords, gave 484 registered studies, and 54 at the EU Clinical Trials Register (<https://www.clinicaltrialsregister.eu>) (accessed on 25 July 2025). Table S1 of Supplementary Materials summarizes some selected clinical trials in different cancer types and with different drugs, highlighting cancer types, treatment protocols, outcomes, and safety profiles, based on the clinical trials that presented outcomes in their description and with published results [252–266]. Although numerous large randomized clinical trials across different tumor types have been conducted, the overall results have been disappointing, concerning the use of metformin in combination with anticancer therapies. Nevertheless, a few studies have yielded promising findings, like the NCT05351021 clinical trial. Furthermore, for many clinical trials, several limitations were found. Detailed trial results with clinical efficacy data are not yet publicly disclosed and, in many cases, the lack of adequate sample sizes prevents conclusive interpretation, and prospective studies are thus needed.

Breast cancer is among the cancer types with the highest number of clinical trials exploring the combination of metformin with chemotherapy, targeted therapies, or immunotherapies, with several phase II and III studies completed or currently ongoing. Nevertheless, for many cases, metformin, when added to standard breast cancer therapy, failed to show significant benefits in terms of disease-free survival or overall survival, compared to placebo. Still, different reports demonstrated that metformin improves metabolic factors that can be linked to breast cancer, namely in body weight, glucose, insulin, leptin or C-reactive protein [267]. Furthermore, several clinical and preclinical studies have demonstrated that metformin decreases ATP production, which is essential for the function of efflux pumps, lowers blood insulin levels, and can reverse the EMT [268]. Additionally, besides its potential use in combined anticancer therapies, there are clinical assays reporting the reduction in the risk of growing cancer after using metformin [269]. The lack of promising results in clinical trials may also be linked to glucose levels. Indeed, some studies have reported that the effects of metformin are particularly pronounced under conditions of glucose deprivation—a common feature of the tumor microenvironment due to the Warburg effect—, where its use has been associated with increased apoptosis. In contrast, under high-glucose conditions, this pro-apoptotic effect is reduced [268]. These potential benefits of metformin are further supported by *in vivo* assays. In different breast cancer mouse models, the combination of metformin with other agents, such as propranolol, curcumin, or glycolytic inhibitors, has shown promising therapeutic effects without associated toxicity [270–272]. In other clinical trials (NCT01929811, NCT00897884, NCT02472353), although metformin did not enhance pathological response or survival outcomes, it showed a beneficial effect in preventing increases in cholesterol levels during

treatment (NCT01929811), decreasing insulin and insulin receptors (NCT00897884), and reducing the tendency of significant change in left ventricle ejection fraction (NCT02472353). Additionally, a meta-analysis review of clinical trials indicated that metformin proliferation markers like Ki-67 decreased in three trials [273].

In other types of cancer, metformin has also shown encouraging signs in the fight against cancer. For instance, clinical trials in endometrial cancer patients reported significant reductions in proliferation markers following metformin monotherapy [274]. In ovarian cancer, patients treated with metformin demonstrated significantly longer overall survival compared to those who did not receive the drug [275]. The combination of metformin and irinotecan in patients with refractory colorectal cancer was positive concerning disease control, but further studies are necessary to validate these findings [276]. In oral cancer, a phase II trial of metformin provided evidence that metformin treatment leads to promising histological improvements and modulation of the mTOR signaling pathway [277], whereas in pancreatic cancer, metformin use decreased the activity of pro-tumoral M2 macrophages while enhancing the recruitment and function of tumor-resolving dendritic cells (DCs), thereby promoting antitumor immune responses and survival outcomes [278].

5. Conclusions

Drug repurposing refers to the strategy of using existing, approved medications for new therapeutic indications beyond their original clinical use. This approach can significantly reduce development time, costs, and adverse effects, as the safety profiles of these drugs are already well-established [279]. In the global emergency of the COVID-19 pandemic, drug repurposing emerged as one of the key strategies employed to rapidly identify effective treatments, but this approach is gaining particular importance in other diseases, particularly in cancer [280]. Recently, Khzem et al. described several classes of drugs being investigated for such effects, namely anti-platelet, anti-helminthic, anti-viral, cardiovascular, antibiotic, anti-malarial, antipsychotic, non-steroidal anti-inflammatory drug (NSAID), antirheumatic, anti-epileptic, anesthetic, or antidiabetic medications [281]. From this panacea of drugs, numerous preclinical and clinical studies have reported positive effects of anti-diabetic medication in the context of cancer treatment, namely the ones mentioned in this review. Diabetes and cancer share several risk factors, like chronic inflammation and obesity, as well as metabolic features, including altered glucose metabolism, all of which can influence tumor development and progression. These shared mechanisms have prompted growing interest in exploring anti-diabetic drugs, such as metformin, with the potential to improve cancer therapeutic outcomes. Metformin targets critical signaling pathways that regulate cell growth and survival, namely the AMPK/mTOR axis, involved in key biological functions such as metabolism, energy homeostasis, and cellular proliferation. All these processes are compromised in both diseases, making this signaling pathway a promising therapeutic target. Metformin exerts other beneficial effects on diabetes that can also be relevant to cancer, including modulation of the gut microbiome and the regulation of obesity, improving metabolic health, and reducing inflammation. Taking these premises, metformin is increasingly being explored as an adjuvant in cancer treatment, often in combination with different therapies, to enhance treatment effectiveness and overcome drug resistance. To improve its bioavailability and targeted delivery, novel approaches such as metformin encapsulation and nanoparticle-based delivery systems are being developed, aiming to maximize its anticancer potential while minimizing side effects. However, although preclinical and in vivo studies have demonstrated promising anticancer effects of metformin, the translation of these findings into clinical settings has been less consistent. Several clinical trials have yielded disappointing or inconclusive outcomes, particularly regarding survival benefits. These discrepancies may be due to differences in

patient populations, tumor types, dosing regimens, or metabolic conditions [282]. As such, further research is needed to better define the context in which metformin can be most effective in cancer therapy.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app152111576/s1>, Table S1: Summary of the clinical trials regarding metformin use in anticancer therapeutic strategies and their main findings.

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