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Metformin and anti-cancer effects: Systematic insights into its therapeutic potential

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ABSTRACT

Background: Metformin is a very widely prescribed antidiabetic drug that has recently received broad attention for its potential anticancer properties. New evidence suggests multifarious effects of metformin on cancer cell metabolism, growth, and survival.

Objective: The objective of this systematic review is to take a deeper look and synthesize available research into the anti-cancer effects of metformin, underlining its treatment potential across different types of cancers.

Methods: A systematic literature search up to June 2024 in PubMed, Scopus, Medline, and EMBASE has been done. The literature search primarily includes clinical trials, pre-clinical studies, and meta-analyses on the examination of anticancer mechanisms and efficacy of metformin. Extracted data have been analysed according to the criteria, most importantly on how metformin would influence cancer incidence and rate of progression besides its effects on patient outcomes.

Results: 1026 articles were identified, after inclusion and exclusion criteria only 28 research articles were included. Various antiproliferative mechanisms contributing to the antineoplastic effects of metformin have been described. Metformin exerts significant pro-apoptotic and antiproliferative effects in a variety of cancer cell lines, such as prostate, breast, and pancreatic cancers. Mechanistically, this suggests that metformin activates the AMP-Activated Protein Kinase pathway (AMPK), inhibits mTOR signalling, and impacts the insulin/insulin-like growth factor 1 axis to create a net antitumor effect. Clinical reports showed that the administration of metformin to diabetic and nondiabetic patients lowered cancer risk selectively.

Conclusion: Metformin in adjunct cancer therapy has some promise; this is so because many studies support its potential to improve cancer treatment outcomes. Further studies should focus on establishing optimal dosing regimens, understanding patient variation of response, and carrying out large-scale clinical trials that clearly define the role of metformin in oncology. The present systematic review underlines the need for integrative approaches to take full advantage of the therapeutic potential for the cure of cancer.

Keywords: metformin, anti-cancer, Activated Protein Kinase (AMPK), immunotherapy, mTOR signalling

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INTRODUCTION

One of the antidiabetic medications that is most frequently prescribed globally is metformin. Its origins began in 1918 when it was demonstrated that guanidine, a substance present in Galega Officinalis, a traditional herbal remedy used throughout Europe, may decrease glycemia [1]. Metformin's potential as a cancer treatment generated a lot of interest when a number of epidemiological studies revealed that it may lower the incidence of cancer in diabetic populations. Regarding its pharmacodynamic effects on tumours, there is still some disagreement, and up to this point, no cancer indication has shown any clinical benefit from recent randomized trials.

Metformin has been shown to "inhibit cell proliferation and colony formation in a variety of breast cancer cell lines, regardless of their p53, HER-2, progesterone, and estrogen receptor status [2-4]. Other cancer cell line types, including those from the prostate, colon, ovarian, endometrial, brain, and lung, have also been demonstrated to be inhibited in their proliferation. The results of in vitro research support those of animal studies that used mouse models of several malignancies, including lung and breast, showing that metformin treatment at a dose similar to that used in humans inhibited tumour growth [5-13].

With time, the advantages of using metformin for other difficult conditions, such as obesity, have been established. The main mechanism by which metformin exerts an anticancer effect is through the activation of the adenosine monophosphate-activated protein kinase/mammalian target of rapamycin pathway when complex I in the mitochondrial respiratory chain is inhibited [14-16]. On the other hand, the good success in a preclinical trial was in stark contrast to the ambiguous performance in a clinical study. Additionally, it has been demonstrated that metformin can reduce tumour growth by 25% in mice who are heterozygous for the tumour suppressor gene PTEN, which causes tumours to develop in a variety of organs [17]. The metformin dosage utilized in this investigation, however, was more than 10 times greater than the typical dosage in a clinical environment. In fact, a large number of these trials were carried out with supra pharmacological doses of metformin, which resulted in plasma levels that were 10–100 times greater than those that could be reached in humans. Because of this, conclusions drawn from these studies could not have the same impact on people [18, 19].

In certain clinical trials, metformin did not provide any help

in the treatment of cancer. Consequently, there are numerous difficulties in metformin's clinical translation. The relationship between metformin and cancer has been extensively reviewed from a variety of angles, including particular cancer types, diabetes, pharmacology, and molecular processes. Still, there is a lack of understanding regarding the therapeutic repurposing of metformin [20-28]. We know that metformin has protective effects against a variety of tumour types and an increasing number of subtypes based on the study of the literature [29, 30]. Therefore, the hallmarks of cancer, which have been suggested as a shared set of functional capacities essential to the transition from normalcy to malignancy, must be intimately associated with the mechanisms of action of metformin [31].

Metformin is known to decrease hepatic gluconeogenesis and increase skeletal muscle glucose uptake by activating AMPK, a cellular energy detecting enzyme. It does this by shifting the cellular energy status through the phosphorylation route to increased activity with a decrease in ATP and a rise in AMP. This shift in the ATP : AMP ratio acts as a signal for energy deficit [32]. This way, type II diabetes mellitus is prescribed; however, this has recently changed: in newly diagnosed patients with T2DM, metformin now constitutes 91.0% of first-line medication prescribed, and for patients already taking sulfonylureas," it is accounting for 79.9% of add-on therapy [33].

It has been demonstrated that the AMPK and mTOR signalling pathways are how metformin affects protein synthesis. The AMPK pathway, which is involved in protein synthesis and cell division, is activated more when "metformin inhibits the mitochondrial respiratory chain complex on tumour cells [34]. Fatty acid oxidation, glycolysis, and the suppression of protein and fatty acid synthesis are the results of the AMPK pathway being activated [35]. One of the primary routes for the development of human breast tumours is increased mTOR-dependent protein synthesis. Metformin may also have anticancer effects by reducing insulin resistance and glycemia, which in turn lowers insulin and insulin-like growth factor 1 (IGF-1), which may stop the proliferation of cancer cells [34, 36]. In fact, it has been demonstrated that growth factors and hormones like insulin contribute to carcinogenesis by triggering the phosphatidylinositol 3-kinase signalling pathway. Metformin has been demonstrated in both in vitro and in vivo studies to impact cancer cell proliferation by interfering with various pathways [37]. This study updated the clinical translation of metformin in cancer treatment and concentrated on the drug's anti-cancer properties concerning cancer's biomarkers.

METHODS

Search strategy

The current review has taken into consideration the guidelines on the PRISMA statement for reporting systematic reviews and meta-analyses of studies. In this study, a detailed search was undertaken in electronic databases such as PubMed, Scopus, Medline, and EMBASE for studies relevant to the anticancer effects of metformin. Some of the keywords used in the search process were "metformin," "anticancer," "AMPK," "mTOR," "insulin/IGF-1 axis," and types of cancer such as "breast cancer," "prostate cancer," "colorectal cancer," and "pancreatic cancer." The literature search was restricted to articles published from January 2000 through June 2024.

Study selection

It included cohort studies, randomized controlled trials, case-control studies, and laboratory-based preclinical research. In addition, meta-analyses taking data from several studies, which go on to calculate a general effect of metformin on outcomes of cancer, were also included.

Data extraction

Information was systematically extracted on the study design, sample size, cancer type, dose and duration of metformin treatment, and the outcomes measured. In this review, the key endpoints evaluated include cancer incidence, progression, survival rates, and mechanistic insights including AMPK activation, mTOR inhibition, and insulin/IGF-1 axis modulation.

Publication bias

The heterogeneity of the different study designs and dosing variation of metformin, with evidence mostly comprised of observational studies that might indicate confounding factors, are some key limitations. Another possible limitation could be how generalizable these results are, given the variability in patient populations and cancer types studied. Further large-scale randomized clinical trials are required to reach definite conclusions about the role of metformin in cancer therapy.

Inclusion and exclusion criteria

The inclusion criteria in this systematic review were clinical trials, pre-clinical studies, and meta-analyses to June 2024, published in English, whose primary interest was the anticancer mechanisms and effectiveness of metformin. In particular, studies were required that reported cancer incidence, progression, and survival rates, or provided mechanistic insights into the effects of metformin. On the other hand, reviews, case reports, editorials, and commentaries were excluded from the exclusion criteria. The exclusion criteria also included studies with insufficient data on the anticancer potentials of metformin or those not primarily related to the role of metformin in cancer treatment.

RESULTS

Database searching brought about 1026 records; no additional records were found from other sources. After removing 294 duplicates and other ineligible data, 674 unique records remained to be screened. Of these, 272 records were excluded. Afterward, 244 full-text articles were reviewed for eligibility, of which 216 were excluded due to irrelevant outcomes, insufficient data, review, and non-English language. Finally, 28 studies remained for qualitative synthesis. 28 studies were identified for the qualitative synthesis that had examined the anticancer effects of metformin. Figure 1 PRISMA flow diagram of the systematic review.

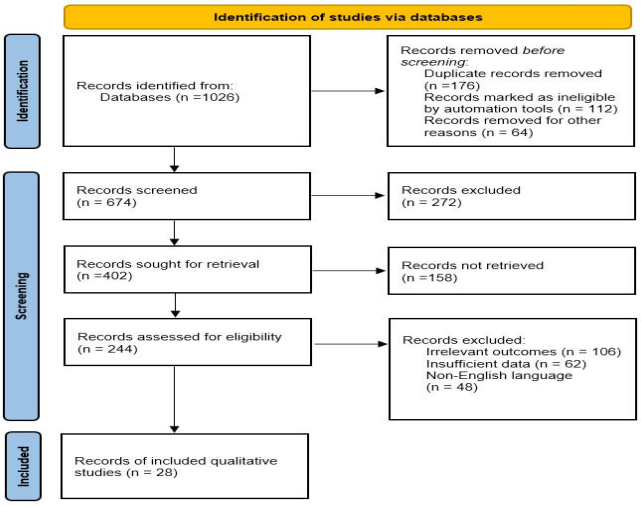

Fig. 1. PRISMA flow diagram of study selection

AMPK and mTOR signaling pathways

Metformin has been shown to exhibit anti-cancer effects through a variety of mechanisms. These routes entail the suppression of mTOR, which is essential for the growth of tumours and for blocking autophagy and apoptosis, both in an AMPK-dependent and independent manner. Metformin works by lowering systemic insulin levels to indirectly decrease mTOR, an effect that happens without the assistance of AMPK [38-40]. The activation of AMPK via LKB1 starts the AMPK-dependent anti-tumour actions of metformin. The downstream mTOR signalling axis is suppressed as a result of this activation [41]. ZN8 formulation corrected the imbalance between apoptosis and proliferation while producing a notable anti-tumorigenic impact and enhanced survival rates. Tumour suppressor genes P53 and miRNA-543 were upregulated in this effect, but cyclin D1 expression and miRNA-191-5p were downregulated [42]. Metformin and Phenformin are examples of AMPK activators that inhibit the proliferation and migration of HCT-116 and A549 cells by repressing p38MAPK activity. This is followed by an increase in the R1 repressor and a matching downregulation of MAO-A expression and activity, which lowers intracellular ROS. In LKB1 mutant A549 cells, SRT-1720 directly activates AMPK, either by itself or in conjunction with metformin, regulating the aggressiveness of cancer cells [43].

The biguanide metformin interacts with and inhibits complex I and III of the mitochondrial electron transport chain. This results in a slight decrease in ATP generation and an increase in cellular ROS due to a moderate leakage of electron transport. Reduced cellular ATP levels often activate AMPK, which in turn activates the transcription factor forkhead box O3a (FOXO3a) by directly phosphorylating it. This process occurs in the metformin route. This is noteworthy because FOXO3a regulates cell division and development, detoxifies ROS, regulates glucose metabolism, and extends life. When it comes to ROS detoxification, the FOXO3a protein lowers oxidative stress by raising the amounts of antioxidant enzymes in cells, such as Manganese Superoxide Dismutase (MnSOD), which breaks down superoxide, catalase and hydrogen peroxide. Metformin elevated the levels of AMPK, p-AMPK, MnSOD, p-FOXO3a, and AMPK in HDFs, but not in HCT116 cells. Metformin reduced the cellular ATP level solely in HDFs. Only in HCT116 cells did metformin elevate the amount of Reactive Oxygen Species (ROS) [44].

This review found that metformin inhibits the mTOR protein, downregulates the expression of p62/SQSTM1, blocks the cell cycle at the G0/G1 phase, and regulates important signalling pathways including Phosphoinositide 3-Kinase (PI3K), Beclin-1, p53, LC3-I and LC3-II, and the Autophagy-Related Gene (ATG). Additionally, metformin can promote autophagy via pathways linked to AMPK, which can impede the growth and advancement of a number of human malignancies, including osteosarcoma, myeloma, pancreatic cancer, non-small cell lung cancer, hepatocellular carcinoma and prostate cancer.

Modulation of Insulin/IGF-1 Axis

Metformin lowers insulin levels and may slow the development of cancer cells via modulating the insulin/IGF-1 axis. Metformin acts indirectly through the interaction of insulin, the receptor for IGF-1 and the type-1 Insulin-Like Growth Factor (IGF-1). The tyrosine protein kinase receptor family includes the IGF-1 receptor. It plays a significant role in preserving cells from apoptosis, encouraging the phenotypic of malignant cells, and promoting cell proliferation [45, 46]. The signalling pathway including Phosphoinositide 3-Kinase (PI3K) and Protein Kinase B (AKT) is also lowered when insulin levels drop. This leads to an increase in TSC2 activity, which in turn suppresses mTOR [47].

Cell development and proliferation are controlled by an array of signals, including growth factors, the availability of nutrients, and the presence of intracellular ATP. Mammals synthesise more glycogen, proteins, and lipids when the IGF1 signalling pathway is triggered in the presence of nutrition [48]. IGF1R (IGF1 Receptor) is linked to the development and occurrence of cardiovascular disease, inflammation, and diabetes. It also plays a role in neutrophil physiology and glucose metabolism [49, 50]. On the other hand, the activation of the AMPK pathway in response to energy starvation causes the inhibition of multiple biosynthetic pathways, including fatty acid and protein synthesis, gluconeogenesis, cholesterol biosynthesis, and the promotion of catabolic processes such as glycolysis and fatty acid beta-oxidation [51].

As a result, this thorough analysis offers a foundation for additional research that could evaluate metformin's anticancer capabilities and its potential for use in the treatment of cancer. In cancer cell lines from prostate, breast, pancreatic and colorectal, malignancies, metformin showed strong antiproliferative and pro-apoptotic actions. The list of papers that were part of the review and a

concise synopsis of the conclusions are displayed in table 1.

Tab. 1. Studies evaluating metformin and anti-cancer effects on tumour biology

References	Tumour Type	Design	Outcomes	Summary
Goodwin et al. [52]	Breast cancer	Randomized trial	Disease-free survival	Metformin significantly improves disease-free survival rates in patients with early-stage breast cancer
Xie et al. [53]	Endometrial cancer	Meta-analysis	Overall survival	Metformin users among endometrial cancer patients have better progression-free and overall survival
Lee et al. [54]	Metastatic prostate cancer	Retrospective Study	Overall Survival (OS), radiographic Progression Free Survival (rPFS) and Prostate-Specific Antigen (PSA) response.	The use of metformin did not show any significant association with improved OS in the multivariate analysis in metastatic prostate cancer patients
Tarhini et al. [55]	Colorectal cancer	Retrospective cohort study	Disease-free survival and overall survival	Metformin improves overall survival and disease-free survival in colorectal cancer patients with type 2 Diabetes Mellitus (DM)
Garcia et al. [56]	Ovarian cancer	Cohort Study	Overall survival	Metformin improves overall survival in ovarian cancer patients after 30 months of treatment
Fransgaard et al. [57]	Colorectal cancer	Randomized Trial	Overall survival	The use of metformin is associated with improved overall survival after colorectal cancer surgery
Park et al. [58]	Ovarian cancer	Cohort Study	Overall survival	There is a statistically significant association between metformin exposure and improved overall survival among ovarian cancer patients with DM
Seo et al. [59]	Gastric cancer	Randomized controlled trial	Overall survival	In patients undergoing chemotherapy for gastric cancer, metformin is associated with improved overall survival

DISCUSSION

Prospective research on metformin and cancer prevention is scarce. Epidemiological data support testing this idea in insulin-resistant or obese patients, and early clinical trial evidence supports it. Metformin is a well-tolerated, biguanide hypoglycaemic agent with a primarily involving activation of the AMPK signalling pathway, reducing hepatic glucose output and enhancing peripheral tissue glucose uptake. Other mechanisms include increasing sensitivity to insulin, inhibition of absorption of glucose into intestinal cells, promotion of GLP-1 secretion, and effects on gut microbiota [60, 61]. Compared to the placebo control group, metformin-treated individuals had thinner endometrium on transvaginal ultrasonography, suggesting it may prevent tamoxifen-induced endometrial hyperplasia [62]. With increased awareness of metformin on cancer, many studies evidenced that it can reduce cancer incidence and improve prognosis. Metformin reduced the risk of cancer by 30%-50% in diabetic patients, especially pancreatic, hepatocellular, and colon tumours [63].

A murine model of familial adenomatous polyposis coli showed that metformin suppressed intestinal polyp growth while a randomised clinical trial demonstrated reductions in metachronous adenomas or polyps following polypectomy after 12 months [64, 65]. Metformin's anti-tumour mechanism has been extensively studied. Metformin inhibits cyclin D1 expression and stimulates AMPK to stop the cell cycle in G1 [66]. AMPK activation increases p53 gene expression and fights cancer. AMPK activation by low metformin concentrations induces p53-dependent liver cancer cell senescence [67]. Metformin also inhibits tumour growth via AMPK-dependent and independent mTOR signalling [68]. Metformin decreases serum insulin and IGF-1, which can stimulate cell survival and mitogenesis [69, 70].

In tumour models and a clinical investigation of advanced cervical cancer patients utilising Fluoroazomycin Arabinoside (FAZA) PET-CT, metformin reduced hypoxia by reducing oxidative respiration and oxygen consumption [71, 72]. Hypoxia suppresses the anti-tumour immune response through many mechanisms, which may be a major immune checkpoint immunotherapy resistance mechanism [73]. Metformin may enhance anti-PD-1 immunotherapy by remodelling the hypoxic tumour microenvironment [74].

Metformin may improve tumour immunosurveillance beyond lowering tumour microenvironment hypoxia. Activation of AMPK in immune cells causes PD-L1 phosphorylation, glycosylation, and endoplasmic reticulum accumulation and destruction. Anti-CTLA-4 therapy was increased by metformin in syngeneic in vivo cancer models [75]. Metformin-induced "AMPK activation may reduce myeloid-derived suppressor cell-driven immunosuppression by downregulating CD39 and CD79 gene expression [76]. Through immunomodulatory cytokines, tumour-associated macrophages inhibit the immune system and promote tumour growth. Preclinical research indicates that metformin can change macrophage polarisation from M2 to M1-like, decreasing angiogenesis and tumour growth," possibly through AMPK/NF-κB signalling [77, 78].

A recent study demonstrated that metformin inhibits IGF1-mediated biological effects in prostate cancer cells through upregulation of IGF-IR [79]. While normal cells generate ATP through mitochondrial oxidative phosphorylation, the vast majority of tumour cells produce ATP through a cytoplasmic process called anaerobic glycolysis, the Warburg effect [80]. AMPK adjustment inhibits fatty acid synthetase and acetyl-CoA carboxylase, thus lowering tumour cell fatty acid synthesis and proliferation. Previous research has demonstrated that excessive glucose promotes tumour growth. However, metformin's hypoglycaemia action may disguise its tumour therapeutic route regulation. It's important to evaluate whether metformin works for hypoglycaemia, normal blood glucose, or pre-diabetes.

Elgendy et al. observed that metformin and intermittent fasting suppress tumour cell oxidative phosphorylation and glycolysis, making it the best anticancer medication for low blood glucose [81]. AMPK activity does not affect metformin's best anti-tumour action at hypoglycaemia. Zhuang et al. observed that low glucose increases metformin toxicity in ovarian and breast cancer cells and decreases intracellular ATP [82]. Considering the present literature, metformin for normal blood glucose patients is still debated. Clinicians should be cautious about metformin's adverse effects and blood glucose implications. Late-phase efficacy studies on metformin as a cancer treatment have yielded dismal results.

Some trials were developed without considering mechanism of action, patient selection, or combination in a rush to prove its efficacy. Well-designed clinical trials are needed to evaluate new combinations of immunotherapy and cancer prevention in selected groups. Many human clinical trials are investigating metformin's chemoprevention and therapeutic effects, inspired by preclinical investigations on various malignancies. Novel metformin analogs and nanotechnology-based targeting have increased metformin anticancer therapy's potential.

Strengths and limitations

This systematic review of the anticancer effects of metformin demonstrates several strengths and limitations. Among its strengths are a comprehensive search strategy across multiple databases, the inclusion of diverse study types (clinical trials, preclinical studies, and meta-analyses), and a focus on detailed mechanistic insights such as the activation of AMPK and inhibition of mTOR signalling. The review also covers a variety of cancer types and includes clinical studies reporting on cancer risk reduction and improved survival outcomes, adding practical relevance. However, limitations include potential variability in study quality, publication bias, language restrictions to English, heterogeneity in study designs, and the current lack of large-scale clinical trials to confirm optimal dosing and patient-specific responses.

CONCLUSION

Metformin exhibits considerable promise as an adjunctive therapy in cancer treatment. This systematic review highlights its multifaceted anticancer effects, including significant antiproliferative and pro-apoptotic activities in various cancer cell lines such as breast, prostate, colorectal, and pancreatic cancers. Mechanistic insights suggest that metformin's activation of the inhibition of mTOR signalling, AMP-Activated Protein Kinase (AMPK) pathway, and modulation of the insulin/IGF-1 axis contribute to its antitumor activity. Clinical studies also report a reduction in cancer risk and improved survival outcomes in patients treated with metformin.

FUTURE RECOMMENDATIONS

To fully harness the therapeutic potential of metformin in oncology, future research should focus on optimizing dosing regimens tailored to individual patient needs. There is a need for large-scale clinical trials to further establish the safety and efficacy of metformin in cancer therapy. Additionally, research should aim to understand patient-specific responses and identify biomarkers that predict response to metformin treatment. Integrative approaches combining metformin with other anticancer therapies could also be explored to enhance treatment outcomes. This review underscores the importance of continued investigation into metformin's role in cancer management, potentially paving the way for novel therapeutic strategies.

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