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Review Article

Metformin: moving the cheese for tumor?

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Abstract: Metformin, an insulin sensitizer, is a biguanide commonly used to treat type 2 diabetes mellitus. An increasing number of clinical studies on its anti-tumor effects have suggested that metformin not only reduces the risk of developing cancer but also decreases recurrence and mortality. It is reported that metformin can activate AMPK (AMP-activated protein kinase) and inhibit mTOR (mammalian target of rapamycin) signaling to suppress the growth of tumor cells. Moreover, emerging evidence suggests that metformin also exerts anti-tumor effects by inhibiting insulin-like growth factor (IGF) or human epidermal growth factor receptor 2 (HER2) signaling, targets cancer stem cells (CSCs), and regulates expression of tumor-related microRNAs. This review critically discusses the role and mechanism of metformin as a potential treatment for cancer.

Keywords: Metformin, anti-tumor effects, clinical trials, signaling pathway

Introduction

Metformin (1,1-dimethylbiguanide hydrochloride) is a biguanide commonly used to treat type 2 diabetes mellitus. It is frequently referred to as an "insulin sensitizer" because it lowers circulating insulin levels in scenarios of insulin resistance and hyperinsulinemia [1]. The primary actions of metformin are inhibition of hepatic glucose production and reduction of insulin resistance in peripheral tissue, leading to enhanced glucose uptake and utilization by skeletal muscle. The effect is a reduction in circulating glucose levels and in plasma insulin levels, both of which improve long-term glycemic control and decrease the incidence of diabetes-related complications [2, 3]. Use of metformin has been found to be generally safe, with mild gastrointestinal symptoms being the most common adverse effects [4].

Several retrospective epidemiologic surveys indicate that metformin not only can reduce the risk of cancer development but can also decrease the rates of recurrence and death [5]. Indeed, increasing evidence shows that metformin may be a potential agent for both preventing and treating neoplastic diseases with some potential anti-tumor effects. Type 2 diabetes mellitus, insulin resistance and a high insulin level might lead to tumorigenesis and tumor-related mortality, effects that are mainly associated with activation of the IGF, HER2, or estrogen receptor (ER) pathway. Therefore, it has been suggested that metformin may decrease insulin resistance and block the above signal transduction pathways and even affect the AMPK/mTOR pathway, inhibit the growth of tumor cells and reversetumor drug-resistant.In addition, metformin is associated with modulation of CSCs and microRNAs to ultimately suppress tumor growth [5].

This review discusses the current knowledge of metformin with regard to important anti-tumor clinical trials and the mechanism by which metformin may inhibit cancer growth and drug resistance by regulating certain signal pathways.

Clinical trials of metformin in cancer

Several observational and cohort studies indicate that metformin reduces the rates of cancer incidence and cancer-related mortality in diabetes patients (**Table 1**). In 2006, Bowker

Table 1. Primary clinical studies of metformin in anti-tumor

Reference/ Journal	Author	Year	Title of the paper	Study Type	Results	Conclusions
6/Diabetes Care	Bowker SL, et al	2006	Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin	A population-based cohort study	Cancer mortality over follow-up was 4.9% (162 of 3340) for sulfonylurea monotherapy users, 3.5% (245 of 6,969) for metformin users, and 5.8% (84 of 1443) for subjects who used insulin. After multivariate adjustment, the sulfonylurea cohort had greater cancer-related mortality compared with the metformin cohort (adjusted HR 1.3 95% CI 1.1-1.6; P = 0.012). Insulinuse was associated with an adjusted HR of cancer-related mortality of 1.9 (95% CI 1.5-2.4; P< 0.0001).	Patients with type 2 diabetes exposed to sulfonylureas and exogenous insulin hada significantly increased risk of cancer-related mortality compared with patients exposed to metformin.
7/Diabetes Care	Libby G, et al	2009	New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes	A population-based cohort study	Cancer was diagnosed among 7.3% of 4085 metformin users compared with 11.6% of 4085 comparators, with median times to cancer of 3.5 and 2.6 years, respectively (P< 0.001). The unadjusted hazard ratio (95% CI) for cancer was 0.46 (0.40-0.53). After adjusting for sex, age, BMI, A1C, deprivation, smoking, and other drug use, there was stilla significantly reduced risk of cancer-associated with metformin: 0.63 (0.53-0.75).	Metformin use may be associated with a reduced risk of cancer.
8/J Clin Oncol	Jiralerspong S, et al	2009	Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer	A population-based cohort study	The rate of pCR was 24% in the metformin group, 8.0% in the nonmetformin group, and 16% in the nondiabetic group (P = 0.02). Pairwise comparisons between the metformin and nonmetformin groups (P = 0.007) and the nonmetformin and nondiabetic groups (P = 0.04) were significant. Comparison of the pCR rates between the metformin and nondiabetic groups trended toward but did not meet significance (P = 0.10). Metformin use was independently predictive of pCR (odds ratio, 2.95; P = 0.04) after adjustment for diabetes, body mass index, age, stage, grade, receptor status, and neoadjuvant taxane use.	Diabetic patients with breast cancer receiving metformin and neoadjuvant chemotherapy have a higher pCR rate than do diabetics not receiving metformin.
9/Diabetes Care	Landman GW, et al	2010	Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16	A prospectively followed cohort study	In patients taking metformin compared with patients not taking metformin at baseline, the adjusted hazard ratio (HR) for cancer mortality was 0.43 (95% CI 0.23-0.80), and the HR with every increase of 1 g of metformin was 0.58 (95% CI 0.36-0.93).	Metformin use was associated with lower cancer mortality compared with nonuse of metformin.
10/Cancer Epidemiol	Zhang P, et al	2013	Association of metformin use with cancer incidence and mortality: a meta-analysis	A meta-analysis	Among metformin users compared with non-users, the summary relative risk (SRR) for overall-cancer incidence was 0.73 (95% CI 0.64-0.83) and that for mortality was 0.82 (95% CI 0.76-0.89). The risk reductions for liver, pancreatic, colorectal and breast cancer incidence were 78%, 46%, 23% and 6%, respectively. Also, metformin can reduce the mortality of liver cancer (SRR, 0.23; 95% CI 0.09-0.60) and breast cancer (SRR, 0.63; 95% CI 0.40-0.99). No statistically significant association between metformin and prostate cancer incidence was found.	Metformin can reduce the incidence of overall cancer, liver cancer, pancreatic cancer, colorectal cancer and breast cancer as well as the mortality of overall cancer, liver cancer and breast cancer.
11/Diabetes Metab Res Rev	Deng D, et al	2013	Association between metformin therapy and incidence, recurrence and mortality of prostate cancer: evidence from a meta-analysis	A meta-analysis	Compared with the control group, metformin therapy was associated with significantly decreased incidence of prostate cancer [RR = 0.88, 95% confidence interval (CI) [0.78, 0.99], P = 0.03, I(2) = 74.7%]. However, metformin therapy was not associated with decreased all-cause mortality (RR = 1.07, 95% CI [0.86, 1.32], P = 0.55, I(2) = 58.2%) or decreased recurrence of prostate cancer (RR = 0.90, 95% CI [0.75, 1.09], P = 0.27, I(2) = 0.0%).	Metformin therapy may decrease the incidence of prostate cancer but that there was no association between the treatment and all-cause mortality or recurrence.
12/PLoS One	Franciosi M, et al	2013	Metformin therapy and risk of cancer in patients with type 2 diabetes: systematic review	A systematic review	In observational studies there was a significant association of exposure to metformin with the risk of cancer death [6 studies, 24410 patients, OR:0.65, 95% CI 0.53-0.80], all malignancies [18 studies, 561,836 patients, OR: 0.73, 95% CI 0.61-0.88], liver [8 studies, 312742 patients, OR: 0.34; 95% CI 0.19-0.60] colorectal [12 studies, 871365 patients, OR: 0.83, 95% CI 0.74-0.92], pancreas [9 studies, 847248 patients, OR: 0.56, 95% CI 0.36-0.86], stomach [2 studies, 100701 patients, OR: 0.83, 95% CI 0.76-0.91], and esophagus cancer [2 studies, 100694 patients, OR: 0.90, 95% CI 0.83-0.98].	Metformin might be associated with a significant reduction in the risk of cancer and cancer-related mortality.
13/Sci Rep	Wu L	2015	Pharmacologic therapy of diabetes and overall cancer risk and mortality: A meta-analysis of 265 Studies	A meta-analysis	The use of metformin or thiazolidinediones was associated with a lower risk of cancer incidence (RR = 0.86, 95% CI 0.83-0.90, I(2) = 88.61%; RR = 0.93, 95% CI 0.91-0.96, I(2) = 0.00% respectively). On the other hand, insulin, sulfonylureas and alpha glucosidase inhibitor use was associated with an increased risk of cancer incidence (RR = 1.21, 95% CI 1.08-1.36, I(2) = 96.31%; RR = 1.20, 95% CI 1.13-1.27, I(2) = 95.02%; RR = 1.10, 95% CI 1.05-1.15, I(2) = 0.00% respectively).	Some anti-diabetic medications may modify the risk ofcancer in individuals with diabetes.

and colleagues compared diabetes patients treated with metformin alone versus diabetes patients treated with sulfonylurea or insulin alone, and the results suggested a lower tumor-related mortality rate in diabetes patients under metformin therapy [6]. In 2009, Libby and colleagues reported a cancer diagnosis of 7.3% among 4,085 metformin users compared with 11.6% for 4,085 comparators, with median times to cancer of 3.5 and 2.6 years, respectively [7]. In the same year, in an analysis of the impact of metformin in their series of 2592 patients who received neoadjuvant chemotherapy for early-stage breast cancer, Jiralerspong and colleagues found that the rate of pathologic complete response (pCR) was 24% in the metformin group (including 68 diabetic patients taking metformin), 8.0% in the non-metformin group (including 87 diabetic patients not taking metformin), and 16% in the non-diabetic group (including 2,374 nondiabetic patients) [8]. For patients taking metformin compared with patients not taking metformin at baseline, Landmanand colleagues reported in 2010 that the adjusted hazard ratio (HR) for cancer mortality was 0.43 (95% CI 0.23-0.80) and that the HR with every 1 g increase of metformin was 0.58 (95% CI 0.36-0.93) at a median follow-up time 9.6 years. It was concluded that metformin effectively decreases cancer mortality rates and that this effect was dose dependent [9]. In 2013, a meta-analysis analyzing the overall cancer incidence summary relative risk of 1,535,636 patients with or without metformin in 37 studies found that metformin can reduce the incidence of liver (78%, SRR, 0.22, 95% CI 0.11-0.46), breast (6%, SRR, 0.94; 95% CI 0.91-0.97), pancreatic (46%, SRR, 0.54; 95% CI 0.35-0.83) and colorectal (23%, SRR, 0.77; 95% CI 0.64-0.91) but not prostate cancer(RR, 0.93; 95% CI 0.82-1.05) [10]. However, another meta-analysis reported that patients using metformin have a reduced incidence of prostate cancer (RR = 0.88, 95% CI 0.78-0.99, P = 0.03) [11]. Moreover, in 2013, a systematic review of 35 studies reported that patients using metformin exhibited reduced risk of developing all cancer (OR = 0.73, 95% CI 0.61-0.88), liver cancer (OR = 0.34, 95% CI 0.19-0.60), colorectal cancer (OR = 0.83, 95% CI 0.74-0.92), pancreatic cancer (OR = 0.56, 95% CI 0.36-0.86), gastric cancer (OR = 0.83, 95% CI 0.76-0.91), and esophageal cancer (OR = 0.90, 95% CI 0.83-0.98) [12]. In 2015, a meta-analysis of approximately 265 studies showed a lower cancer incidence with metformin or thiazolidinediones use by diabetic patients (RR = 0.86, 95% CI 0.83-0.90 and RR = 0.93, 95% CI 0.91-0.96, respectively), whereas increased cancer incidence was associated with insulin, sulfonylureas, and alpha glucosidase inhibitor use (RR = 1.21, 95% CI 1.08-1.36; RR = 1.20, 95% CI 1.13-1.27 and RR = 1.10, 95% CI 1.05-1.15, respectively) [13].

Potential mechanisms of metformin in cancer

Based on preclinical studies, metformin possibly directly or indirectly regulates downstream targets through many molecular signaling pathways to reduce the growth and proliferation of tumor cells.

AMPK/mTOR signaling pathway

Metformin can exert its anti-tumor effects through the AMPK/mTOR pathway, critical and classic signaling, by activating AMPK and inhibiting mTORin breast cancer cells [14, 15]. Metformin is an activator of AMPK, which inhibits protein synthesis and gluconeogenesis during cellular stress. Tumor cell growth inhibition induced by metformin was reversed by knocking down the AMPK gene or applying an AMPK inhibitor [16]. mTOR is activated in gastric cancer cells and in colorectal cancer cells due to genetic alterations or aberrant activation of components of the PI3K/Akt pathway, which leads to phosphorylation of downstream signaling molecules and dysregulation of cell proliferation, growth, differentiation and angiogenesis [17, 18]. mTOR activation is often associated with a more aggressive, phenotype, poorer clinical outcomes and drug resistance [19]. Metformin-activated AMPK phosphorylates tuberous sclerosis complex/tuberin (TSC) to enhance its activity. TSC phosphorylation is required for mTOR to recruit regulatory factors, which includes oxygen level-dependent and growth factor signaling, such as the PI3K and MAPK pathways [20]. AMPK-mediated phosphorylation of TSC has been observed to increase the activity of TSC, leading to inactivation of mTOR [21]. In breast cancer cells, AMPK has also been described as directly inhibiting mTORC1 by phosphorylation of mTOR-binding raptor [22]. Comparing the effects of metformin with rapamycin, a direct mTOR inhibitor, metformin decreases AKT activation in addition

to AMPK-dependent mTOR inhibitionin pancreatic cancer cells and in gastric cancer cells [23, 24]. Thus, metformin results in a better anti-tumor response in cancer cells than rapamycin.

Insulin-like growth factor signaling pathway

IGF, a multifunctional cell proliferation regulation factor, plays key roles in human cell proliferation, differentiation and henogenesis ontogenesis [25]. Substantial evidence shows that IGF is involved in regulating pathways of proliferation of both normal and tumor cells [26]. IGF can bind to and activate insulin-like growth-1 receptor (IGF-1R), which is frequently overexpressed in cancer and is a key stimulator of cancer cell growth [27]. Metformin can reverse hyperinsulinemia in diabetic or non-diabetic patients by regulating the balance of glucose metabolism. Moreover, metformin can interfere with IGF signaling pathways and reduce the binding of insulin and IGF to IGF-1R in endometrial carcinoma cells [28]. This effect can result in cancer cell growth inhibition. In addition, recent studies suggest that AMPK activation induced by metformin also decreases tyrosine phosphorylation of insulin receptor substrates (IRSs) and disrupts crosstalk between the insulin/IGF-1R and G protein-coupled receptor signaling pathwaysin pancreatic cancer cells [29]. In a mouse model of tobacco carcinogen-induced lung cancer, inhibition of IGF-1R/IR by metformin decreased downstream signaling through the PI3K/Akt pathway [30].

HER2 signaling pathway

HER2 is a transmembrane receptor with tyrosine kinase activity. HER2 belongs to a family of four receptors (EGFR/HER1, HER2, HER3, HER4) that are involved in regulating cell growth, survival and differentiation through interlinked signal transduction via activation of the PI3K/Akt and Ras/Raf/MEK/MAPK pathways [31, 32]. Amplification of the *HER2* gene and/or overexpression at the messenger RNA or protein level occurs in approximately 20% of patients with early-stage breast cancer [31]. Metformin has been found to decrease HER2 expression in human breast cancer cells by directly inhibiting p70S6K1, a downstream effector of mTOR [33]. Interestingly, a low concentration of metformin can block activity of the HER2 protease and reverse drug resistance induced by HER2-targeted treatment in breast cancer cells [34]. Importantly, AMPK activation can protect cardiac cells from injury caused by HER2 treatment [35]. Thus, metformin may have a synergistic effect in HER2-targeted therapy.

Cancer stem cells

The wildly reported CSCs hypothesis of tumorigenesis was developed based on an understanding of the functional heterogeneity observed in human tumor cells [36]. In 2009, the first study of metformin and CSCs showed that the drug selectively targets cancer stem cells and acts together with chemotherapy to block tumor growth and prolong remission [37]. This study reported that doxorubicin, a standard component of breast cancer chemotherapy, produced a negligible effect on the proportion of $CD44^{+}/CD24^{low}$ CSCs among the remaining live cells, whereas metformin alone or in combination with doxorubicin significantly reduced the number of surviving CSCs. More important, doxorubicin plus metformin caused a durable regression of tumors in nude mice with tumor xenografts, even after cessation of therapy, similar to the results of rapamycin in a preclinical model of pancreatic cancer [38]. These results were subsequently extended to cancer cell lines including prostate and lung adenocarcinoma, and metformin similarly inhibits CSCs [39]. However, the specific mechanisms by which metformin inhibit CSCs remain unclear. Some studies have found that metformin treatment can decrease the mRNA levels of the transcription factors Nanog, Otc4 and Otc2, which were originally defined as components of the self-renewal/maintenance machinery in embryonic stem cells [40, 41]. Other studies have suggested that metformin also inhibits the mRNA expression of Notch1 and enhancer of zeste homolog 2 (EZH2) in tumor cells. Notch signaling is key for the regulation of CSCs [42], and EZH2 is methyltransferase component of the polycomb repressor complex 2, which modulates certain genes involved in CSC differentiation [43]. Despite ongoing research, we still have a very limited understanding of the molecular mechanisms underlying the effects of metformin in tumor suppression and CSC targeting.

MicroRNAs

MicroRNAs are key regulators of many biological processes, such as cell proliferation, differ-

entiation, apoptosis, stress response and angiogenesis, due to their ability to bind to the 3'UTR of multiple target mRNAs [44, 45]. miRNAs can behave as either oncogenes or tumor suppressor genes, thereby promoting or inhibiting cancer progression, and growing evidence shows that metformin can exert anticancer effects through miRNA modulation. One study showed that in pancreatic cancer cell lines, metformin can up-regulate expression of miR-26a, miR-192 and let-7c to inhibit cancer cell proliferation, invasion, migration and promote apoptosis through direct modulation of HMGA1 [46]. Interestingly, after metformin treatment, the pancreatic cancer cells re-expressed many miRNAs that are usually switched off during cancer progression, such as the miR-200 family, which plays a major role in the epithelial to mesenchymal transition and in maintaining the stem cell state [47]. Furthermore, metformin can inhibit tumor sphere formation by down-regulating several CSC markers such as CD44, EpCAM, EZH2, Notch1, Nanog and Oct4, partially through up-regulation of miRNAs [48, 49]. In human lung cancer cell lines A549 and NCI-H358, metformin inhibited growth and cell cycle progression by reducing miR-222 expression, which directly inhibited p27, p57 and PTEN [50]. In breast cancer cell lines, metformin decreased c-MYC expression to inhibit chemoresistance, possibly by up-regulating miR-33a levels [51].

Conclusion

In summary, there is an increasing amount of evidence from pre-clinical data and population-based studies of carcinogenesis that supports the potential efficacy of metformin as an anticancer agent. Metformin inhibits the growth of cancer cells by modulating AMPK/mTOR, IGF, and HER2 signaling pathways, reducing the number of surviving CSCs, and regulating the expression of tumor-related miRNAs. However, studies on the specific molecular mechanism of metformin are still in early stages. Moreover, it is very important to improve histology techniques and identify the appropriate tumor stages for utilizing metformin therapy. If the above limitations are resolved, metformin may prove to be a non-toxic, inexpensive anticancer drug in the future.

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Disclosure of conflict of interest

None.

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