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SHORT REVIEW

Expanding the therapeutic spectrum of metformin: from diabetes to cancer

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Abstract
Introduction Metformin, an oral hypoglycemic agent, was introduced in the clinical practice for the treatment of type 2 diabetes mellitus more than a half-century ago. Over the years, several studies demonstrated that diabetic patients treated with metformin have a lower incidence of cancer, raising the hypothesis that the spectrum of clinical applications of the drug could be expanded also to cancer therapy. Following these initial findings, a large number of studies were performed aimed at elucidating the effects of metformin on different types of tumor, at explaining its direct and indirect anti-cancer mechanisms and at identifying the molecular pathways targeted by the drug. Several clinical trials were also performed aimed at evaluating the potential anti-cancer effect of metformin among diabetic and non-diabetic patients affected by different types of cancer. While the results of several clinical studies are encouraging, a considerable number of other investigations do not support a role of metformin as an anti-cancer agent, and highlight variables possibly accounting for discrepancies.
Aim We hereby review the results of in vitro and in vivo studies addressing the issue of the anti-cancer effects of metformin.
Conclusions If in vitro data appear solid, the results provided by in vivo studies are somehow controversial. In this view, larger studies are needed to fully elucidate the role of metformin on cancer development and progression, as well as the specific clinical settings in which metformin could become an anti-cancer drug.

Keywords Metformin · Diabetes · Cancer · Therapy

Introduction

The history of Metformin (1,1-dimethylbiguanide) dates back to 1920s to the use of *Galega officinalis* (goat's rue or French Lilac) as a botanic medicine, which was already known as a remedy for polyuria in medieval Europe [1]. In the 1950s, biguanides were developed as therapeutic agents [2] and their derivates, such as metformin, were introduced in the clinical practice, initially in Europe and Canada (since the 1970s), and then in the United States where the Food and Drug Administration approved it in 1994. Metformin has a glucose-lowering effect and reduces hyper-insulinemia [3–5] by improving insulin sensitivity in peripheral tissues. Nowadays, metformin in its different formulations is the most widely used drug in the biguanide class for the treatment of Type 2 Diabetes Mellitus (T2DM) because of its high efficacy and safety and also for its relatively low cost [6]. Other metabolic disorders in which metformin was proven of therapeutic efficacy include PCOS [7, 8] and NAFLD [9] two often associated clinical conditions. More recently, identified actions of metformin are represented by its ability to reduce some [10] but not other circulating markers of inflammation and a TSH-lowering effect [11, 12].

The link between diabetes and cancer, and the role of metformin

Epidemiologic data demonstrate that T2DM is associated with a higher risk for developing several types of cancer

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[2]. Moreover, T2DM is often associated with obesity, which, in turn, is an independent risk factor for the occurrence of cancer [13]. T2DM is characterized by insulin resistance and hyper-insulinemia, the latter was shown to favor the development of breast cancer in experimental animal models [14]. Patients with T2DM also display high serum levels of insulin-like growth factor (IGF)-1, a potent mitogen that can contribute to carcinogenesis [15].

Interest in metformin as a potential anti-cancer agent was raised by studies reporting that diabetic patients given metformin had a lower incidence of cancer, as compared with diabetic patients treated with other drugs. Nearly, 10 years ago Evans et al. [16] first reported the unexpected finding of a lower prevalence of cancer in diabetic patients treated with metformin. In agreement with this study, Bowker et al. reported that, compared with other anti-diabetic treatments, diabetic patients receiving metformin had a lower cancer-specific mortality [17]. Further studies showed that metformin can inhibit the growth of cancer cells and reduce the risk of developing several solid tumors [18], such as colorectal, liver, pancreatic, stomach, esophagus and breast cancer [19, 20].

The mechanistic effects of metformin

The discovery that metformin has anti-cancer effects raised attention on the potentially involved mechanisms. The first step was directed at understanding whether the anti-diabetic and anti-cancer effects would involve the same or different pathways.

Mechanistic effects of metformin in diabetes

The primary mechanism of action of metformin in diabetes occurs through a reduced output of glucose from the liver. This effect is mediated by the AMP-activated serine–threonine protein kinase (AMPK), an intracellular sensor of energy and nutrient availability, and a regulator of energy homeostasis [21]. AMPK is a key therapeutic target in patients with diabetes, as it regulates lipid, cholesterol and glucose metabolism in specialized tissues such as liver, muscle, and adipose tissue [22]. Metformin targets the mitochondrion by inhibiting the respiratory chain complex I [23, 24] with subsequent decrease in the production of ATP and increase in the accumulation of ADP and AMP [21]. Both AMP and ADP regulate AMPK function by preventing its de-phosphorylation and inactivation [25]. Activated AMPK switches off ATP-consuming pathways and switches on pathways for ATP generation. The phosphorylation of the AMPK catalytic subunit is mediated by LKB1 (a protein called after liver kinase B1, the product of the corresponding tumor suppressor gene). Thus, metformin, by activating AMPK, induces a decrease in energy-consuming processes, such as gluconeogenesis, protein and fatty acid synthesis. In patients with T2DM, the final result of these metabolic changes is a reduction of blood glucose levels and of insulin resistance [26, 27].

Mechanistic effects of metformin in cancer

Since the first description of the anti-cancer effect of metformin, it became evident that more than one mechanism was involved. In particular, the observation that the anti-cancer effect of metformin also occurs in non-diabetic patients [28, 29] supported the hypothesis that, besides an indirect effect via its insulin lowering action, the drug influences other signaling pathways. [22, 30–33]. The direct and indirect mechanistic effects of metformin on tumor cells are summarized in Fig. 1.

The AMPK-dependent pathway

The inhibition of the LKB1-mediated-mTOR signaling pathway is a major candidate for the anti-cancer effect of the drug. The activation of the LKB1–AMPK pathway inhibits the mammalian target of rapamycin complex

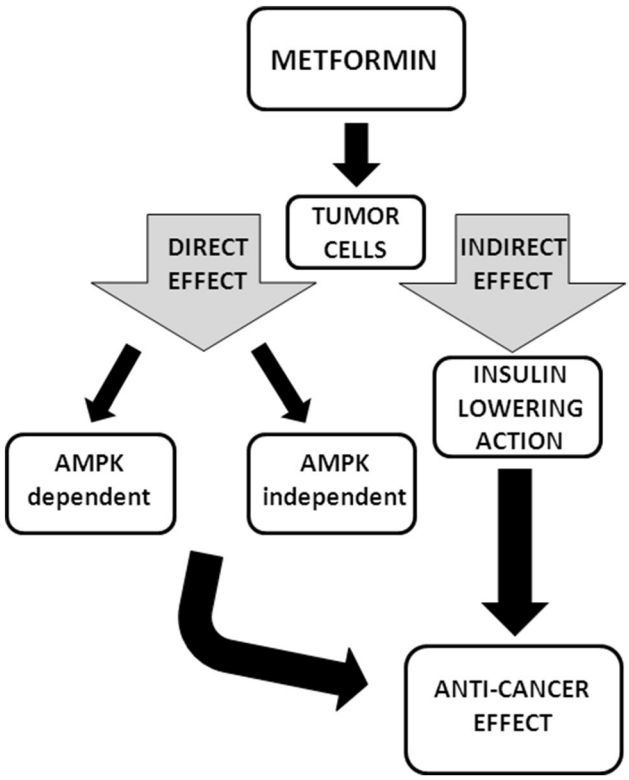

Fig. 1 Metformin anti-cancer effects. The direct and indirect action of Metformin on tumor cells by AMPK-dependent and AMPK-independent mechanisms leads to obtain the anti-cancer effects of the drug

1 (mTORC1), a kinase being activated in the majority of human cancers [34]. The sequence of events is as follows: AMPK phosphorylates the tuberous sclerosis complex protein 2 (TSC2), which results in the accumulation of Rheb-GDP (a protein with GTPase activity) and the subsequent inhibition of mTORC1 [35]. Although the LKB1, AMPK, and mTOR pathway is considered a potential pharmaceutical target in tumors, due to its crucial role in the regulation of cancer cell metabolism, growth, and proliferation [34], other mechanisms are also involved in the anti-cancer effect of metformin.

The AMPK-independent pathways

Several experimental data support the concept that AMPK-independent pathways are involved in the anti-cancer effect of metformin [36]. The drug inhibits hepatic gluconeogenesis by a mechanism, which is independent from the LKB1–AMPK pathway and involves a decrease in the hepatic energy state [37]. Metformin also inhibits the proliferation of human leukemia cells by inducing a cell cycle arrest in the G0/G1 or S-G2/M phase, and promotes the apoptosis of these neoplastic cells [38]. In prostate cancer cell lines, the drug exerts an anti-proliferation effect by an AMPK-independent pathway, which involves the induction of a p53 target gene (REDD1) [39]. In breast cancer cells, metformin interferes with purine/pyrimidine and glutathione synthesis, a pathway which is upstream and AMPK independent [40]. Taken together, these findings indicate that multiple molecular pathways account for the anti-proliferative effect of metformin on cancer cells.

The NF-kB pathway

The attention on the role of nuclear-factor-κB (NF-κB) in human cancer stems from the growing interest for tumor-related inflammation. NF-κB is involved in innate and adaptive immune responses, but it is also constitutively activated in human tumors [41, 42]. NF-κB activation results both in enhanced proliferation and invasiveness of cancer cells, and in their resistance to apoptosis induced by tumor surveillance mechanisms [41]. In vascular endothelial cells, metformin inhibits the activation of NF-κB induced by TNF-α [43]. Further to this observation, it was shown that in smooth muscle cells metformin suppresses the phosphorylation of three MAP kinases (p38, JNK, and Erk) and of Akt, all involved in the NF-κB pathway [44].

In vitro and in vivo experiments supporting the anti-cancer effect of metformin

A consistent number of in vitro studies evaluated the effect of metformin on cell cycle, cell proliferation, apoptosis and metastatic potential. Zakikhani et al. demonstrated that metformin exerts an anti-mitogenic effect in breast cancer cells [45] by activating AMPK and by inhibiting the MAPK pathway, a major mediator of the insulin-IGF signaling, which promotes cell growth. Carmignani et al. showed that the drug exerts an anti-cancer effect in glioblastoma cells by blocking the LKB1–AMPK–mTOR–S6K1 pathway [46]. Metformin exhibits a dose- and time-dependent anti-proliferation effect on intrahepatic cholangiocarcinoma cell lines, which appear to be mediated by different mechanisms including induction of apoptosis and cell cycle arrest. The in vitro anti-proliferative effect of metformin, although by different mechanisms, was consistently reported in several cancer cell lines derived from breast [47], colon [48], ovary, [49] pancreas [50], lung [51], and prostate [52] tumors.

Pre-clinical in vivo experiments confirmed the anti-cancer effect of metformin in mouse models of tumor xenografts [53] and chemically induced cancers. Cancer stem cells (CSC) were also identified as a target of the anti-cancer effect of metformin. Bao et al. showed that metformin attenuates the CSC phenotypes, functions and mediators [54]. The drug reduces the expansions of CSC clones by inducing apoptosis and by inhibiting CSC mediators and markers. By these mechanisms, metformin attenuates the growth of tumors in animal models.

Other lines of research showed that metformin regulates the epithelial–mesenchymal transition (EMT) status, an essential differentiation program in early embryonic development that is modified in cancer to mediate acquisition of malignant properties including stemness [55–57]. Metformin decreases the expression of key drivers of EMT including the transcription factors ZEB1, TWIST1 and SNAI2 (Slug), and the pleiotropic cytokines TGFβs [58, 59] in several cell types. The inhibition of these components of EMT by metformin causes an inhibition of cell invasiveness without affecting cell migration [60]. Metformin was also shown to reduce the expression of miR-34a and its direct EMT targets Notch, Slug, and Snail [61].

A recent line of research investigates the inhibition of NFκB activity by metformin. The available studies addressing this issue may be summarized as follows: (1) In vitro experiments by Tan et al. demonstrated that the invasiveness of endometrial cancer cells was significantly attenuated by sera from women with polycystic ovary syndrome in treatment with metformin [62], (2) Metformin inhibits the NFκB inflammatory signaling in mouse pancreatic tumors, as evidenced by the blocking effect on NFκB phosphorylation and by the decreased mRNA expression of the downstream genes MCP-1, TGF-β1, TNF-α, and IL-1β [63], (3) Metformin inhibits the TNF-α-induced secretion of CXCL8, a chemokine with well-established pro-tumorigenic actions [64, 65].

In vivo studies showing an anti-cancer effect of metformin

As reviewed in the previous section, over the past two decades experimental data raised interest on the potential benefits of metformin treatment in human malignancies. If in vitro data appear solid and encouraging, the results provided by in vivo studies are somehow controversial. Table 1 summarizes the studies performed both in diabetic and in non-diabetic patients with various types of cancer, evaluating the possible anti-cancer effect of metformin.

Table 1 Summary of previous studies evaluating the role of metformin as an anti-cancer agent performed in diabetic and non-diabetic patients with different types of cancer

References	Years	Diabetes	Type of cancer	Anti-cancer effect
Evans [9]	2005	Yes	Any	Yes
Bowker [10]	2006	Yes	Any	Yes
Decensi [86]	2010	Yes	Hepatocellular	Yes
			Pancreatic	Yes
			Colon	No
			Breast	No
			Prostate	No
Hadad [21]	2011	No	Breast	Yes
Bosco [59]	2011	Yes	Breast	Yes
Tan [61]	2011	Yes	Nonsmall cell lung	Yes
Baur [62]	2011	Yes	Any	Yes
Monami [63]	2011	Yes	Any	Yes
Lee [60]	2012	Yes	Colorectal	Yes
Campagnoli [22]	2012	No	Breast	Yes
Niraula [71]	2012	No	Breast	Yes
Yin [65]	2013	Yes	Any	Yes
Klubo-Gwiezdzinska [68]	2013	Yes	Thyroid	Yes
Cazzaniga [73]	2013	No	Breast	Yes
Sehdev [66]	2014	Yes	Colorectal	Yes
Tseng [69]	2014	Yes	Thyroid	Yes
Decensi [74]	2014	No	Breast	Yes
Oppong [75]	2014	Yes	Breast	No
Luo [77]	2014	Yes	Endometrial	No
Suissa [81]	2014	Yes	Any	No
Gandini [82]	2014	Yes	Any	No
Tsilidis [83]	2014	Yes	Any	No
Lee [67]	2015	Yes	Gastric	Yes
Goodwin [70]	2015	No	Breast	Yes
Dowling [72]	2015	No	Breast	Yes
Ko [76]	2015	Yes	Endometrial	No
Sakoda [78]	2015	Yes	Lung	No
Merrick [79]	2015	Yes	Prostate	No
Soffer [80]	2015	Yes	Breast	No
Kowall [84]	2015	Yes	Any	No
Vissers [85]	2015	Yes	Breast	No

Studies in diabetic patients with cancer

As previously discussed, in 2005 Evans et al. first reported that the overall incidence of cancer was lower in diabetic patients given metformin compared with patients treated with other anti-diabetic drugs [16]. They also found that a longer duration of metformin treatment and a larger number of prescriptions correlated with a lower incidence of cancer. The anti-cancer effect of metformin, in terms of reduced cancer incidence and increased survival, was subsequently reported in diabetic patients affected by other types of cancer [18, 66–69]. The beneficial effect of metformin was found to be dose dependent and persisted when the biguanide was administered in combination with insulin. The latter observation suggested that the anti-cancer effect of metformin was, at least in part, insulin independent [17, 70–72]. A recent meta-analysis evaluating 13,008 subjects with cancer and concurrent T2DM showed that metformin administration, compared to treatment with other glucose-lowering drugs, has beneficial effects in terms of overall survival and cancer-specific survival [73].

A few studies focused their attention on tumors of the gastric-intestinal tract and of the thyroid gland. In a population survey performed in North America, Sehdev et al. showed that metformin reduces the risk of colorectal cancer in patients with diabetes [74]. Furthermore, it was recently reported that an increased cumulative duration of metformin treatment decreases recurrence rate, all-cause mortality, and cancer-specific mortality in diabetic patients affected by gastric cancer [75]. In a retrospective study, Klubo-Gwiezdzinska et al. found that the size of thyroid tumors was significantly smaller in patients with diabetes treated with metformin as compared with those not receiving the drug [76]. In a large series of diabetic Taiwanese patients, treatment with metformin was shown to reduce the risk of thyroid cancer [77]. The protective effect of metformin was maintained after disregarding age and sex of patients, and did not change after excluding users of insulin, sulfonylurea, or insulin plus sulfonylurea. A previous diagnosis of other types of cancer that might introduce a searching bias did not affect the results [77].

Studies in non-diabetic patients with cancer

Several clinical trials were performed in non-diabetic patients with breast cancer. The first pilot randomized trial demonstrated a reduction of Ki67 and of other cancer-specific biomarkers in women with breast cancer treated with metformin [28]. Further support to the anti-cancer

effect of metformin derived from the observation that non-diabetic women with breast cancer treated with a higher dose of metformin (1.5 g/day) experienced a more profound decrease of biochemical prognostic markers (insulin level, HOMA-IR index, testosterone level, and free androgen index) compared with those taking a lower dose of the drug (1 g/day) [29]. In a recent trial, non-diabetic women with breast cancer (T1–3, N0–3, M0) were randomized to receive metformin vs. placebo after surgical treatment [78]. The results showed that a 6-month metformin treatment significantly improved several metabolic factors associated with poor breast cancer outcomes (weight, insulin, glucose, leptin, and C-reactive protein). This observation is in agreement with previous data indicating that metformin treatment in newly diagnosed, untreated, non-diabetic breast cancer patients results in clinical and cellular changes consistent with beneficial anti-cancer effects. In particular, the treatment with metformin before surgery resulted in a decrease of weight, BMI, HOMA index and breast cancer cell proliferation index (Ki67) [79].

Two recent studies specifically evaluated the anti-tumor effect of metformin in non-diabetic women with breast cancer starting from the diagnostic biopsy until surgery. In the first study, metformin treatment significantly reduced the insulin receptor expression in tumor cell. The phosphorylation status of protein kinase B (PKB)/Akt (S473), the extracellular signal-regulated kinase 1/2 (ERK1/2, T202/Y204), AMPK (T172) and acetyl coenzyme [80] were also reduced. In the same clinical setting, Cazzaniga et al. compared, after 4 weeks of preoperative metformin, the apoptosis levels in core biopsies and surgical specimens of non-diabetic women with breast cancer. They found a heterogeneous effect of metformin on the modulation of apoptosis, which was dependent upon the degree of individual insulin resistance [81]. This finding would suggest for the first time that metformin exerts a dual effect of on breast cancer growth according to the insulin resistance status [81]. Moving from this observation, the same group of investigators evaluated the effect of metformin according to several biomarkers of insulin resistance (HOMA index, BMI, C-peptide, IGF-I, IGFBP-1, IGFBP-3, free IGF-I, C-reactive protein, adiponectin) and to the tumor subtype. They found that the effect of metformin on the tumor proliferation index Ki67 varies according to both host and tumor characteristics [82].

In vivo studies showing no anti-cancer effect of metformin

Overall, the above reported data suggested that metformin could be the drug of choice in the treatment of patients with cancer and concurrent T2DM, with promising results also for the treatment of oncologic non-diabetic patients. However, a beneficial effect of metformin on tumor progression was not consistently reported in all studies.

In 2014, Oppong et al. analyzed a cohort of patients with T2DM receiving systemic chemotherapy for invasive breast cancer. After a follow-up of 87 months, there were no differences in terms of recurrence-free survival, overall survival and contralateral breast cancer in metformin users vs. nonusers [83]. Ko et al. evaluated the potential benefit of metformin treatment in women who were new users of the drug compared with a control group of new users of sulfonylureas. They found no association with a decreased risk of developing endometrial cancer in women treated with metformin [84]. Negative results, indicating no beneficial effect on cancer, were also reported in recent studies performed in patients with endometrial, lung, prostate, and breast cancer [85–88].

Factors possibly accounting for the discrepant in vivo results

In 2012, Suissa et al. first reported that time-related biases (immortal time bias, time-window bias, and time-lag bias), which potentially lead to an overestimation of the protective effects of metformin, were present in several observational studies reporting a decreased incidence or of cancer mortality in patients taking metformin [89]. This observation may explain the discrepant results obtained in different studies. Time-related biases were taken into account in four recent studies. The results of these more controlled investigations are summarized as follows: (1) the reduction in cancer incidence and cancer mortality observed in diabetic patients taking metformin is of modest relevance and does not affect all populations equally [90], (2) in a cohort of patients with a new diagnosis of diabetes, there was no evidence that metformin use is associated with reduced cancer risk in comparison to sulfonylurea use [91], (3) in a retrospective observational study considering a total of 22,556 diabetic patients, the authors failed to found a reduced cancer risk in metformin users [92], (4) among 1057 patients with T2DM before the diagnosis of breast cancer, the cancer-specific mortality was inversely related to the duration of metformin use, supporting an inverse association between long-term metformin treatment and breast cancer-specific mortality [93]. Thus, it would appear that the discrepancy among different studies is not solved even when time-related biases are taken into account.

The study by Decensi et al. might shed light on the controversy. These authors reported a significant correlation between decreased cancer risk and metformin treatment for hepatocellular and pancreatic cancers, whereas no significant relationship was found for colon, breast or prostate

cancers [94]. This finding suggests that metformin elicits cancer site-specific effects. In other words, the effect of metformin could be tumor specific (which would be in line with the results of some in vitro studies) and/or the effect of metformin could be more or less relevant in relation with a given stadium of the malignancy. This appears at the moment the most likely explanation for the discrepant results obtained by in vivo studies evaluating the anti-tumor effect of metformin.

Conclusions

During the past decade, we witnessed a progressive transition of the pharmacological effects of metformin from a well-established anti-diabetic drug to a potential anti-cancer drug. Current knowledge supports the concept that metformin is able to modify the clinical course of human cancer.

The anti-tumor activity of metformin appears to be elicited by several indirect (through lowering insulin levels) and direct mechanisms acting on tumor cells. The latter encompass the more extensively characterized actions of metformin on both AMPK-dependent (mTOR) and AMPK-independent mechanisms. Alternative actions include an effect of metformin on tumor stem cells and the Nf-Kb pathway.

Following initial enthusiasm derived from experimental and early clinical studies, it is now clear that the field of clinical applications of metformin in cancer therapy is more limited than previously believed. Indeed, while in vitro studies consistently point toward anti-cancer effects of metformin, discrepant results are reported by in vivo data. Several studies indicate that the therapeutic benefit of metformin treatment in cancer patients is not universally obtained, but rather they may be more or less evident in specific clinical settings. The latter include both disease-related (i.e., type of tumor, clinical stage) and patient-related (i.e., insulin resistance, age, sex) factors. The retrospective design, the limited number of patients enrolled, the length of the follow-up period and the presence of time-related biases of most currently available studies should be taken into account for interpreting the discrepancy. The most recent advance in this field is represented by clinical trials in which metformin was prescribed to non-diabetic patients with cancer.

Future large-series longitudinal studies in both diabetic and non-diabetic patients with cancer will be required for fully elucidating the role of metformin on cancer development and progression, as well as the specific clinical settings in which metformin could become a prescribed anti-cancer drug.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study formal consent is not required.

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