Chapter

Preventive and (Neo)Adjuvant Therapeutic Effects of Metformin on Cancer

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Abstract

Metformin, the first-line antidiabetic drug, has become an attractive candidate in cancer therapy since retrospective clinical investigations reported that patients with type 2 diabetes receiving metformin had lower incidence of cancer than those with other glucose lowering drugs. In line with this, preclinical studies have demonstrated that the antitumor activity of metformin could proceed through several mechanisms. Thus far, metformin has been used in cancer prevention with reduced risk as consequence and treatment of various cancers as an adjuvant or neoadjuvant drug. Thus, existing data support the beneficial effects of metformin on many types of cancers such as reducing metastasis and mortality and improving pathological responses and survival rates. However, some reports do not support this and even show adverse effects. The discrepancy may be attributed to expression levels of its transporters or genetic background. Hence, this chapter briefly reviews information on the mechanism of metformin action and summarizes both completed and ongoing clinical trials in an attempt to evaluate the value of metformin in prevention and treatment of various cancer types.

Keywords: metformin, AMPK, mTORC1, diabetes, lipogenesis, cancer prevention and therapy, clinical trials

1. Introduction

Metformin is derived from *Galega officinalis*, a natural herbal medicine. The herb was first used to relieve polyuria, a symptom of diabetes in ancient Egypt and medieval Europe [1]. Metformin is a widely used frontline drug for type 2 diabetes mellitus (T2DM). The major function of metformin is to decrease hepatic gluconeogenesis and enhance insulin sensitivity by increasing glucose uptake in muscle and adipose [2]. In addition to antidiabetes, metformin has proved to be beneficial to metabolic syndrome and nonalcoholic fatty liver disease [3, 4]. Cancer is characteristic of a metabolic disorder, inasmuch as metabolism is reprogrammed by switching oxidative phosphorylation into aerobic glycolysis, and thus, many of key molecules in these two routes are altered in their expression or posttranslational modification [5]. The incidence of cancer is higher in patients with T2DM than those without diabetes, indicating that diabetes is a risk factor of cancer [6]. Since Evan et al. reported in 2005 lower cancer incidence in patients with T2DM taking metformin than those with other antidiabetic drugs, great efforts have been made to

elucidate the antitumor activity of metformin [7]. A considerable number of preclinical and clinical investigations support the beneficial effects of metformin on both prevention and treatment of various cancers. At the same time, some of mechanisms underlying metformin action on cancer cells have been unraveled, although much of them is still incomplete. Thus far, more than 300 clinical trials using metformin as a single or adjuvant agent in combination with other chemotherapies have been initiated in the treatment of various types of cancer in the world (www.clinicaltrials.gov).

2. Targets of metformin

Many functions of metformin are mediated by adenosine monophosphate-activated protein kinase (AMPK). Metformin at high doses leads to elevation of AMP, which binds to and allosterically activates AMPK, while at low doses, it engages lysosomes in the absence of AMP [8, 9]. The upstream kinases that phosphorylate AMPK α subunits at Thr172 include liver kinase B1 (LKB1), calmodulin-dependent kinase beta, and TGF-β-activated protein kinase [10–12].

AMPK plays important roles in regulating lipid and protein metabolisms by phosphorylating a series of target proteins. Thus, LKB1-AMPK pathway is critically important for metabolic adaption under stress condition, which aims to protect cells in the beginning [13]. However, persistent activation of AMPK by metformin can also cause cytostatic and even cytotoxic effects. Mounting evidence shows that metabolic syndrome and diabetes increase the risk of cancer, and correction of metabolic abnormalities alleviates cancer burdens and improves survival [14–16]. Drugs that target AMPK or downstream molecules are research focus nowadays for cancer prevention and treatment. Some of pathways downstream of AMPK essential for tumorigenesis and cancer progression are depicted in **Figure 1**.

PI3K-protein kinase B (AKT)-mammalian target of rapamycin (mTOR) pathway is well received as the target of AMPK. Mammalian target of rapamycin complex 1 (mTORC1) consists of mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein 8, proline-rich AKT substrate 40 kDa, and DEP domain-containing mTOR-interacting protein [17]. Tuberous sclerosis complex 2 (TSC2) is a GTPase-activating protein that forms a complex with TSC1 to stimulate GTPase activity of Ras homolog enriched in brain (Rheb) and thus inhibits mammalian target of rapamycin complex 1 (mTORC1) activation. TSC2 is subjected to inhibition by AKT and activation by AMPK via phosphorylation at different sites. In addition, AMPK phosphorylates and inhibits Raptor, a scaffold of mTORC1. A plethora of cellular events, such as protein translation, lipogenesis, cell cycle progression, and autophagy, are regulated by the activated mTOR pathway, which are counteracted by AMPK [18]. Thus, control of mTORC1 activity is crucial for prevention and treatment of cancer.

Cancer cells always require large amount of building blocks for dividing progenitor cells. Thus, synthesis of fatty acid and cholesterol is very active [19]. Acutely, AMPK inhibits acetyl CoA carboxylase (ACC) and HMG-CoA reductase (HMGCR), which are rate-limiting enzymes for de novo synthesis of fatty acid and cholesterol, respectively [20]. In addition, AMPK activates malonyl-CoA decarboxylase (MAD) that converts malonyl-CoA to acetyl CoA. As cytosolic malonyl-CoA decreases, fatty acid synthesis is attenuated [17, 21]. AMPK also influences de novo synthesis of glycerolipid by inhibiting the rate-limiting enzyme glycerol phosphate acyltransferase (PAT) [17, 22]. Chronically, AMPK phosphorylates sterol regulatory element-binding protein-1c (SREBP-1c) and its related protein carbohydrate-response-element-binding protein (ChREBP), restricting the nuclear localization of

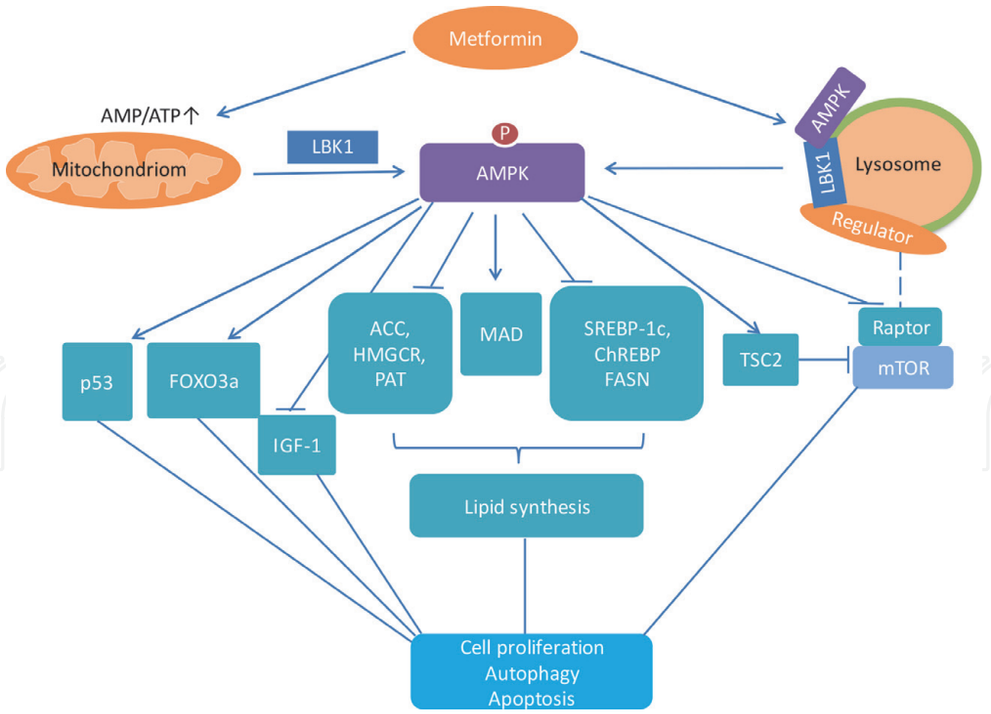

Figure 1.
AMPK activation and its biological functions. AMPK is activated by increased AMP:ATP ratio induced by metabolic stress and metformin. In addition, metformin can activate AMPK through lysosomal pathway, where v-ATPase-regulator-AXIN/LKB1-AMPK complex is formed. After activation, AMPK acts on multiple molecules/pathways, including inhibition of mTORC1, lipogenesis and IGF-1 expression, and activation of p53 and FOXO3a [17, 22, 87–89]. As such, AMPK regulates cell proliferation, autophagy, and apoptosis of cancer cells.

these transcription factors, so as to inhibit transcription of target genes for lipogenesis, including those encoding ACC and fatty acid synthase (FASN) [23].

3. Clinical investigations

Decreases from 20 to 94% in cancer risk among patients with T2DM after the use of metformin have been reported since 2005 [24]. A large population study conducted by Taiwan National Health Insurance Data Survey evaluated 16,602 individuals treated with metformin or other antidiabetic drug between 2000 and 2007 and concluded a 88% reduction in the risk of various cancer types after metformin treatment [25, 26]. In line with this, numerous investigations provided supporting data that metformin reduced incidence of various cancers. For example, DeCenci et al. have found a 30% decrease in cancer incidence in patients with T2DM treated with metformin compared to those with other drugs [27, 28]. Currie et al. conducted a large cohort study with around 60,000 patients from the UK database and revealed that metformin alone decreased the incidence of colorectal and pancreatic cancer compared with insulin and sulfonylureas monotherapy after the adjustment of confound bias, but this was not seen in breast cancer (BC) and prostate cancer [29]. It is noteworthy that metformin plus insulin could alleviate the progression of cancer [hazard ratio (HR) = 0.54, 95% confidence interval (CI) 0.43–0.66] [29]. With respect to mortality, ZODIAC trial with a 10-year follow up has indicated a lower death rate of cancer among metformin users with T2DM [30]. According to Noto et al. meta-analysis, diabetic patients taking metformin showed significant reduction of incidence of multiple types of cancer [risk ratio (RR) = 0.67, 0.53–0.85], including colorectal cancer (CRC) (RR = 0.68, 0.53–0.88) and cancer

mortality (RR = 0.66, 95% CI = 0.49–0.88) [31]. A study of Bowker et al. reported that metformin decreased cancer mortality in T2DM, as compared with insulin and/or sulfonylurea groups [32]. After 1-year observation, the cancer death rate of metformin, insulin, and sulfonylurea users is 3.5, 8.8, and 4.9 per 1000 patients, respectively.

Regarding tumor types, dosage of metformin, study setting, and period of intervention associated with the treatment outcomes, examples are listed in **Table 1**.

Cancer type	Intervention	Outcome
Breast cancer		
Bodmer et al. [39]	Metformin or other antidiabetic drugs	Diabetic patients treated with metformin ≧ 5 years had a lower incidence of cancer, compared with nonusers or short-term (<5 years treatment) metformin users
Jiralerspong et al. [45]	Metformin + chemotherapy	The pCR rate in 68 diabetic patients treated with metformin, 87 diabetic patients without metformin, and 2374 nondiabetic patients was 24, 8, and 16% (P = 0.02)
Niraula et al. [46]	Metformin	Reduction of cancer cell proliferation (Ki67) by 3% (P = 0.016) and increases in apoptosis by 0.49% (P = 0.004) was compared between pre- and post-surgery, despite minor change of fasting insulin level
Hou et al. [51]	Metformin + chemotherapy	1013 BC patients with diabetes and 4621 BC patients without diabetes were analyzed. Nondiabetic group had higher 5-year survival rate than diabetic group (82 vs. 79%, $P < 0.001$). In diabetic subgroup, metformin-treated group had significant higher 5-year survival rates than nonmetformin-treated group (88 vs. 73%, $P < 0.001$)
El-Haggar et al. [42]	Metformin + chemotherapy or +hormone therapy, tamoxifen	Non-diabetic women with newly diagnosed BC (68/129) were prescribed with metformin (860 mg b.i.d.) along with chemotherapy or hormone therapy compared to nonmetformin-treated control arm over 6 or 12 months. A 3.27-fold decrease (P = 0.023, 95% CI 1.17–9.06) at the time of developing metastasis and an increase in average DFS by 2.137 (P = 0.044) in the metformin-treated group. Also, the levels of IGF-1, the ratio of IGF-1 to IGFBP-3, insulin, fasting blood glucose, HOMA-IR index notably decease, while IGFBP-3 levels significantly increase after using of metformin
He et al. [53]	Metformin or other antidiabetic drugs	A cohort study evaluated a total of 1983 women with stage ≧ 2 Her2 positive BC. Among 154/1983 diabetic patients who had already responded to previous chemotherapy. Metformin users had prolonged OS (HR = 0.52, 95% CI 0.28–0.97, P = 0.041) and reduced cancer-specific mortality of BC (HR = 0.47, 95% CI 0.24–0.90, P = 0.023), compared with nonusers
Colon cancer		
Coyle et al. [33]	Metformin	Significant benefit of RFS (n = 623 patients in two studies), OS (n = 1936 patients in five studies), and CSS (n = 535 patients in two studies) was observed in metformin-treated patients from 3094 patients with early stage CRC in nine studies, compared with that in nonmetformin using group

	Cancer type	Intervention	Outcome
	Rokkas and Portincasa [55]	Metformin	A significant decrease in the risk of developing colon neoplasia [RRs (95% CI) = 0.75 (0.65–0.87), $Z = -3.95$, $P < 0.001$], including the reduction of colon cancer [0.79 (0.69–0.91), $Z = -3.34$, $P < 0.001$] and colon polyps [0.58 (0.42–0.80), $Z = -3.30$, $P < 0.001$] among patients with T2DB after metformin treatment
	Garrett et al. [58]	Metformin or other antidiabetic drugs	After adjustment of cofound variates, a 30% increase in OS was demonstrated among 424/4758 patients who were diagnosed of T2DM and CRC and administrated to metformin as compared with that in other antidiabetics users
	Higurashi et al. [59]	Metformin	A total of 151 nondiabetic patients with CRC after polypectomy was randomized to metformin-treated arm (250 mg daily over 1 year) or placebo control arm with 1-year endoscopy reports. The incidence of total polyps and adenomas decreased in metformin-treated group by 18.5% [RR = 0.67, 95% CI (0.47–0.97), $P = 0.034$] and 21% [RR = 0.60, 95% CI (0.39–0.92), $P = 0.16$], compared with that in control group
	Endometrial cancer		
	Sivalingam et al. [60]	Metformin	A total of 40 women with atypical endometrial hyperplasia (AEH) or EC was assigned to receive metformin 850 mg b.i.d. over average 20 day, or no treatment before hysterectomy. Ki67 was reduced by 17.2% (95% CI 27.4–7.0, $P < 0.002$) in metformin-treated group
	Schuler et al. [61]	Metformin	20 nondiabetic women with EC and obesity (BMI ≥ 30) were administrated with metformin 850 mg daily for 1–4 weeks before surgery. The levels of Ki67 and p-S6 were reduced between pretreatment and postsurgery by 11.75% ($P = 0.008$) and 51.2% ($P = 0.0002$), respectively. Besides, the levels of p-AMPK ($P = 0.00001$), p-Akt ($P = 0.0002$), p-4EBP1 ($P = 0.001$), and ER ($P = 0.0002$) also decreased after surgery
	Mitsuhashi et al. [63]	Metformin + MPA	17 AEH and 19 noninvasive EC patients received metformin (escalating from 750 to 2250 mg daily) after complete response treated by MPA and other drugs. Relapse rate among patients was 10%, and estimated 3-year RFS rate was 89%
	Nevadunsky et al. [66]	Adjuvant metformin	Metformin significantly improved OS (HR = 0.54, 95% CI 0.30–0.97, $P < 0.04$) in diabetic patients with nonendometrioid EC when compared with that in nonusers with EC
	Acute lymphoblastic leukemia		
	Ramos-Peñafiel et al. [67]	Metformin + prednisone	A total of 102 nondiabetic patients with ALL was enrolled, 26 received metformin (850 mg t.i.d.) for 6 days during preinduction stage, and 76 were treated with traditional chemotherapy without metformin. The use of metformin prevented therapy failure and early relapse ($P = 0.025$) in patients bearing relative to high levels of ABCB1
	Esophageal Cancer		
	Skinner et al. [68]	Neoadjunvant metformin + CRT	Metformin users along with CRT resulted in higher pCR (34.5%) than nonmetformin cohort (4.8%,

Cancer type	Intervention	Outcome
		P = 0.01) and nondiabetic patients (19.6%, P = 0.05). Higher pCR rate was found to be associated with higher metformin dose (≥1500 mg/d). Post-CRT maximum SUV decreased significantly in patients taking metformin (P = 0.05)
Lee et al. [25]	Adjuvant metformin	Reduction of total CID and incidence of some gastroenterological cancers including CRC, HCC, and so on, among which the CID of esophageal cancer decreased in diabetic groups taking adjuvant metformin in comparison to non-DM groups. Metformin dosage giving rise to a significant decrease in cancer incidence was ≤500 mg/day
Leamm et al. [69]	Metformin + neoadjuvant chemo(radio)therapy	No statistically significant difference between metformin users and nonmetformin users for median overall survival (43.6 vs. 42.8 months, P = 0.66) or for median DFS (31.1 vs.47.0 months, P = 0.68)
Prostate cancer		
Wright et al. [70]	Metformin	A reduced risk of prostate cancer was showed among white men at age of 35–74 after the use of metformin, as reported by a case-control study
Rothermundt et al. [74]	Metformin	A total of 44 men with castration-resistant prostate cancer was assigned to receive metformin 500 mg b.i.d. until progression. After initial metformin treatment, changes in IGF and IGBP3 and improvement of insulin sensitivity from baseline were observed but without correlation with progression. At week 4, only four patients did not have progression (95% CI, 3–22). Average PFS was 2.8 months (95% CI, 2.8–3.2) and PSA double time declined in 23 patients but not significant
Joshua et al. [75]	Metformin	Metformin 500 mg t.i.d. was prescribed to 24 men with operable prostate cancer before prostatectomy. In a per patient and per tumor analyses, Ki67 was reduced by 29.5% (P = 0.0064) and 28.6% (P = 0.0042) in comparison with the initial biopsy and postprostatectomy sections
Rieken et al. [77]	Metformin	Metformin users with prostate cancer exhibited a minor improvement of RFS after prostatectomy
Spratt et al. [78]	Metformin	A retrospective study examined 2901 noninvasive prostate cancer patients through radiation therapy. In 157 patients treated with metformin, PSA-RFS and DMFS were improved and the castration-resistant prostate cancer progression was alleviated

Table 1.
Examples of clinical investigations of metformin used as a neoadjuvant and adjuvant agent in cancer therapy.

3.1 The role of metformin in radiotherapy and chemotherapy

Metformin has been reported to be a useful adjuvant drug to radiotherapy or chemotherapy for different cancers, especially prostate and colon cancers [33]. The effects of metformin on overall survival (OS), relapse-free survival (RFS), and cancer-specific survival (CSS) after concurrent chemotherapy and/or radiotherapy vary on cancer types.

A previous study has shown that metformin increases radiosensitivity of luminal BC by influencing expression of thioredoxin and intracellular redox homeostasis [34]. A high level of AMPKα expression correlates with the increased radiosensitivity and better prognosis. A systemic review and meta-analysis conducted in 2018 summarized the impact of metformin on the efficacy of radiotherapy in 17 studies, including prostate cancer, head and neck cancer, rectal cancer, lung cancer, esophageal cancer, and liver cancer [35]. The study compared diabetic patients with metformin (D + M) and diabetic or nondiabetic cohort without metformin (D − M or N − M) after radiotherapy. An improved pathologic complete response (pCR), 2y-OS, and 5y-OS vary in different cancer types when analyzing D + M and D − M groups, supporting that metformin is beneficial to OS of diabetic patients while distant metastasis-free survival (DMFS) and 5-year OS were not significantly different between D + M and N − M groups. With respect to the possible mechanisms by which metformin enhances radiosensitivity, studies have indicated that p53 and AMPKα are involved [36, 37]. Despite the increased sensitivity to radiotherapy and chemotherapy, cumulative side effects and toxicity concur with the use of metformin. For example, a study has shown that combination of metformin with radiochemotherapy can lead to less tolerance to cisplatin and radiotherapy and exacerbate gastrointestinal adverse effects such as grade ≥ 3 nausea/vomiting [38].

3.2 Breast cancer

Several lines of clinical investigations have been conducted to assess the beneficial effects of metformin on BC [39–52]. Two retrospective studies revealed that long-term use of metformin (>5 years) reduced the risk of BC in T2DM women as compared with other antidiabetic drugs [39, 40]. However, Currie et al. reported that metformin use did not affect risk of breast and prostate cancer, but the reduced risk was found in colon and pancreas cancer [29].

He et al. have shown improvement of disease-free survival (DFS), DMFS, and OS in diabetic women who well-responded to previous hormone therapy and then received metformin treatment. The results demonstrated that metformin synergizes with hormone therapy [53, 54].

Metformin was used as neoadjuvant chemotherapy of BC to improve pathological conditions prior to surgery [45–48]. The increased pCR in 2529 women with BC has been demonstrated in metformin-treated diabetic patients, compared to nonmetformin-treated patients with or without diabetes [45]. Another study by Niraula et al. evaluated the effect of metformin on serum biomarkers in nondiabetic BC patients before surgery [46]. The patients were treated with metformin for 2 weeks, and serum biomarkers were assessed. A notably reduction of Ki67 and elevation of apoptosis were observed in invasive tumor after the use of metformin. The significant decrease of homeostatic model assessment of insulin resistance (-HOMA-IR) was also observed, while insulin and leptin displayed a modest change. However, a study showed that metformin increased phospho-AMPK (p-AMPK) and decreased p-Akt and Ki67 without induction of apoptosis, suggesting a cytostatic effect [47].

The long-term use of metformin has been shown to reduce risk of distant metastasis and mortality of BC patients with type 2 diabetes [49–51]. Furthermore, metformin use as adjuvant therapy can also improve outcomes of BC in nondiabetic patients [41, 42, 52]. For example, a single-arm phase II trial enrolled nondiabetic women with M0 stage BC. After receiving metformin of 500 mg t.i.d. for 6 months, the result showed that fasting insulin level and HOMA-IR were significantly reduced. Total cholesterol, low density lipoprotein, and leptin also similarly declined [52]. Another study focused on the optimal dose of metformin that

achieves favorable effects on BC by comparing dose between 1500 and 1000 mg daily [41]. For postmenopausal women with basal testosterone levels≧0.28 ng/mL, it seemed that metformin of 1500 mg/d was better than 1000 mg/d in reduction of insulin and testosterone levels, which were associated with cancer incidence and prognosis. Combination of metformin with other chemotherapy usually generates better outcomes in nondiabetic BC patients with the higher HOMA-IR (>2.8), and HOMA-IR can be improved by metformin [42–44, 48].

In summary, studies showing beneficial effects of metformin are more than those without effects. Metformin as an adjuvant agent can suppress BC at various doses ranging from 500 to 1500 mg. The outcomes mainly include reduced risk of BC, decreases in cancer-promoting markers and metastatic events, increases in apoptotic markers, and improvement of progression-free survival (PFS) and OS.

3.3 Colon cancer

The role of metformin in preventing colon cancer has been documented in the following studies conducted in both diabetic and nondiabetic patients. A meta-analysis was carried out in 709,980 individuals with T2DM from 17 studies showing a significant decrease in the risk of colon neoplasia among metformin-treated patients compared to those without metformin, with respective reduction for either cancer or polys [55]. A randomized study enrolled a total of 26 nondiabetic individuals with aberrant crypt foci (ACF) (biomarker of CRC development) and assigned them to either receive metformin 250 mg daily for 1 month or control group [56]. Significant decreases in the average number of ACF by a 3.67-fold (P = 0.007) and in proliferating cell nuclear antigen index were discovered in metformin arm. This indicates that metformin prevents CRC by attenuating cell proliferation and ACF development.

Metformin has been used as an adjuvant agent in the treatment of CRC. First, a single-arm study has demonstrated a median PFS of 1.8 months and an OS of 7.9 months in metastatic CRC with combination of metformin (850 mg b.i.d.) and 5-fluorouracil treatment. Surprisingly, the improvement in median survival was more obvious in obese patients [57]. Second, Coyle et al. have evaluated 3092 patients with early stage of CRC [33]. It was found that the use of metformin significantly improved RFS (HR = 0.63, 95% CI 0.47–0.85), OS (0.69, 95% CI 0.58–0.83), and CSS (0.58, 95% CI 0.39–0.86) in patients with T2DM, compared with other antidiabetic drugs. Likewise, progression of CRC is also inhibited by metformin. A similar study showed prolonged OS in patients with T2DM with CRC receiving metformin, as compared with nonmetformin users (79.6 vs. 56.9 months, P = 0.048) [58]. The last randomized trial used metformin (250 mg daily) for a year in nondiabetic patients with high-risk adenoma recurrence and no colorectal polyps after polypectomy [59]. The results showed that polyps and adenomas are noticeably fewer in the metformin arm than in the control arm. The study also showed that average HOMA-IR status was significantly reduced in nonrecurrent patients by metformin, while the value remained stable in recurrent patients, indicating that insulin resistance is associated with chemoprevention outcome.

3.4 Endometrial cancer

Clinical investigations support that metformin could serve as a potential drug for protection against endometrial cancer (EC) [60–65]. Several studies have evaluated the effects of short-term use of metformin as a neoadjuvant therapy between initial recruitment and hysterectomy surgery in nondiabetic women with EC [60–62]. The first nonrandomized trial has examined the change of Ki67 and

shown a remarkable reduction after metformin use at 850 mg b.i.d. for average 20 days [60]. A significant reduction in phospho-4E-binding protein 1 (p-4EFBP1) downstream of mTOR was also observed by immunohistochemistry, while indirect serum markers of insulin resistance (fasting glucose, insulin, and HOMA-IR) and leptin only showed a decrease trend but not significant after adjusting difference. Another preoperative clinical trial was done in nondiabetic women with body mass index (BMI) ≧ 30 [61]. After taking metformin 850 mg daily for 1–4 weeks prior to surgery, Ki67, p-AMPK, p-Akt, phospho-S6 Ribosomal Protein (p-S6), and p-4EBP were significantly lower in resected specimens than in pretreatment. The reduction of p-AMPK is inconsistent with purported positive effect of metformin. This study also showed a decrease in estrogen receptor (ER) but not progesterone receptor.

According to a study evaluating the effect of metformin on EC of diabetic patients (n = 114) as compared with diabetic (n = 136) and nondiabetic (n = 735) patients without metformin from 1999 to 2009, metformin-treated group exhibits prolonged OS than nonusers before and after the adjustment of confound bias [66]. A phase II study has examined the effects of long-term metformin (2250 mg daily until recurrence) on RFS after a complete response to medroxyprogesterone acetate (MPA) in 17 individuals with atypical endometrial hyperplasia and 19 with EC [63]. The 3-year estimated RFS was 89%, and the 3-year recurrence rate showed a 4.7-fold decrease in this study compared with a previous study [64]. In contrast to short-term treatment, the other randomized factorial study does not have a significant change in PFS/OS after metformin treatment (1700 mg/d for 16 weeks and 1-year follow up) [65].

3.5 Acute lymphoid leukemia

A single study randomized to assign 102 patients with nondiabetic acute lymphoid leukemia (ALL) into a group of 26 with metformin at 850 mg t.i.d. for 10 days and the rest to the group without metformin before remission therapy [67]. Metformin displayed a beneficial effect on OS in the patients with high levels of ABCB1 expression, the gene encoding multidrug resistant protein-1. The failure rate of therapy was significantly reduced and early relapse after remission prevented by metformin, as compared with nonusers.

3.6 Oesophagal cancer

Oesophagal cancer is deadly cancer with poor prognosis, and patients usually do survive or die no longer than 30 months after chemoradiation and surgery [68]. A prospective cohort study by Taiwan National Health Insurance revealed a positive effect of metformin as an adjunct to standard chemotherapies on the cancer incidence density (CID) of gastroenterological cancers [25]. In this study, a decrease in total CID including esophageal cancer was found in diabetic groups taking adjuvant metformin in comparison to nondiabetic groups. Another study reported that metformin enhanced the efficacy of radiochemotherapy in patients with T2DM resulting in superior pCR and low postconcurrent chemoradiation (CRT) maximum SUV compared to patients with T2DM without metformin and non-DM patients [68]. Additionally, higher pCR rate was correlated with higher metformin dose (≥1500 mg/d). However, a report in 2015 demonstrated inconsistent results, in which no difference in pCR was found between metformin users and nonmetformin users [69]. Furthermore, it was shown that together with neoadjunvant chemoradiation, metformin did not improve the median OS or median DFS in diabetic patients with esophageal cancer.

3.7 Prostate cancer

The effect of metformin on prostate cancer is ambiguous. Studies of Wright and Stanford have provided a 44% decrease in the risk of prostate cancer among Caucasian men with diabetes [70]. However, investigations by others could not obtain the same conclusion on the incidence of prostate cancer in diabetic patients treated with metformin, but the mortality might be reduced [71–73]. A single-arm clinical trial has revealed a decrease in insulin-like growth factor-1 (IGF-1) and an increase in insulin-like growth factor-binding protein-3 (IGFBP-3), alongside lowering prostate-specific antigen (PSA), after giving metformin 500 mg b.i.d. over 12 weeks to patients with castration-resistant prostate cancer [74]. In a single-arm study on men with biopsy-proven localized prostate cancer, 22 patients were selected to receive metformin at 500 mg/d or b.i.d., followed by t.i.d. for 28–84 days preceding their prostatectomy. The results revealed that Ki67 index was reduced by comparing the initial biopsy with postprostatectomy sections [75]. However, the changes were not recapitulated by another study, although metformin in the prostate tissue was detected after a median of 34 days prior to prostatectomy [76]. In a retrospective study, metformin-treated diabetic individuals gained the improvement of RFS among 6863 patients after radical prostatectomy [77]. Study of Spratt et al. also demonstrated the significantly elevated PSA-RFS, DFS, and lower cancer mortality in localized prostate cancer with metformin treatment compared with that of nonusers [78].

4. Ongoing clinical trials

Previous studies of metformin use as neoadjuvant or adjuvant therapy for various types of cancer provide strong rationale of clinical trials in more vigorous settings. Thus far, more than 300 clinical trials have initiated in the world despite some are somehow either terminated or withdrawn. **Table 2** lists some of them. For example, NCT02065687 is a randomized, metformin-placebo, phase II/III study that enrolls a total of 540 participants and examines the effect of adjuvant metformin together with paclitaxel and carboplatin in treatment of stages III–IV or recurrent EC. Patients receive metformin twice a day in a 5-year follow up until disease progression or undesirable adverse effects appear. According to this trial, prolonged PFS and OS will be observed after the use of metformin together with other chemotherapeutic drugs. One of the ongoing phase II trials carrying out in 151 premenopausal BC patients with BMI $\geqq$ 25 kg/m^2 evaluates treatment effect with 850 mg metformin b.i.d. vs. placebo for a year, by examining the primary outcome changes of breast density at time points of 6 and 12 months. This study spanning from March 7, 2014 to June 30, 2020 also identifies biomarkers associated with metabolic effects of metformin and attempts to find prediction factors of BC risk (NCT02028221). Also, a trial (NCT02614339) is undergoing to follow-up 3-year DFS and 5-year OS in nondiabetic patients with stage II high-risk/III CRC treated with metformin (1000 mg/day) for 48 months. This study has enrolled 593 participants and is still recruiting and expected to complete in July 2021.

The trial of double-blinded 2 $\times$ 2 factorial (aspirin $\times$ metformin) design registers 160 patients with stages I–III colon cancer who undertake a completed polypectomy within recent 24 months (NCT03047837). After randomized allocation, patients will receive metformin at 850 mg b.i.d. or aspirin at 100 mg daily or two drugs together vs. placebo over 1 year. Immunohistochemistry for NF-κB, glucose metabolism, pS6K, and other biomarker will be compared pre- and postintervention (ClinicalTrials.gov Identifier: NCT03047837).

NCT number	Status	Participants	Period	Intervention	Cancer type
NCT02581137 https://ClinicalTrials.gov/show/NCT02581137	Active	(a) 26, (b)18 years and older (adult, older adult), (c) all sex	June 10, 2016 to not indicated	Drug: metformin hydrochloride	Oral cancer
NCT02028221 https://ClinicalTrials.gov/show/NCT02028221	Active	(a) 151, (b) 21 years to 54 years (adult), (c) female	March 7, 2014 to June 30, 2020	Drug: metformin & placebo	BC
NCT02431676 https://ClinicalTrials.gov/show/NCT02431676	Active	(a) 100, (b) 50 years to 65 years (adult, older adult), (c) female	May 1, 2013 to September 1, 2022	Drug: metformin & placebo	EC
NCT01697566 https://ClinicalTrials.gov/show/NCT01697566	Active	(a) 100, (b) 50 years to 65 years (adult, older adult), (c) female	May 1, 2013 to September 1, 2022	Drug: metformin & placebo	EC
NCT01797523 https://ClinicalTrials.gov/show/NCT01797523	Active	(a) 62, (b) 18 years and older (adult, older adult), (c) all sex	May 1, 2013 to October 1, 2020	Drug: metformin, letrozole, & everolimus	EC
NCT02065687 https://ClinicalTrials.gov/show/NCT02065687	Active	(a) 540, (b) 18 years to older (adult, older adult), (c) female	Match 17, 2014 to	Drug: carboplatin, metformin hydrochloride, paclitaxel, & placebo	EC
NCT03047837 https://ClinicalTrials.gov/show/NCT03047837	Recruiting	(a) 160, (b) 18 years to 80 years (adult, older adult), (c) all sex	March 15, 2017 to March 15, 2020	Drug: aspirin (ASA) + metformin (MET)|Drug: ASA|Drug: MET|Drug: placebos	Tertiary prevention in colon cancer
NCT01905046 https://ClinicalTrials.gov/show/NCT01905046	Recruiting	(a) 128, (b) 25 years to 55 years (adult), (c) female	August 2013 to	Drug: metformin hydrochloride & placebo	BC
NCT02614339 https://ClinicalTrials.gov/show/NCT02614339	Recruiting	(a) 593, (b) 20 years to 80 years (adult, older adult), (c) all sex	December 2015 to July 2021	Drug: metformin & placebo	CRC
NCT03378297 https://ClinicalTrials.gov/show/NCT03378297	Recruiting	(a) 143, (b) 18 years and older (adult, older adult), (c) female	May 4, 2018 to June 1, 2020	Drug: metformin & acetylsalicylic acid & drug: olaparib & drug: letrozole	Ovarian cancer
NCT03685409 https://ClinicalTrials.gov/show/NCT03685409	Recruiting	(a) 62, (b)20 years to 70 years (adult, older adult), (c) all sex	October 1, 2018 to September 30, 2020	Drug: metformin hydrochloride & placebo	Oral cancer

NCT number	Status	Participants	Period	Intervention	Cancer type
NCT01864096 https://ClinicalTrials.gov/show/NCT01864096	Recruiting	(a) 408, (b) 18 years to 79 years (adult, older adult), (c) male	October 1, 2013 to August 1, 2024	Drug: metformin & placebo	Prostate cancer

Table 2.
Summary of ongoing clinical trials approved by FDA.

5. Cautions to be considered

5.1 Cancer type-specific effects

Whether a cancer type is sensitive to metformin depends on expression level of OCT1 in the cell membrane. Thus far, majority of previous studies have demonstrated that metformin exerts beneficial effects on different types of cancer, while some do not respond. On contrary, in some cases, for example, in glioma and leukemia cancer cells, metformin reduces cisplatin-induced apoptosis, suggesting that metformin exerts a protective effect on cytotoxic agents in some cells [79]. Hence, before going to clinical trials, preclinical tests should be undertaken to ascertain if metformin enhances the inhibitory effect of other drugs. This is feasible when PDX animal models or organoid culture techniques are available.

5.2 Genetic background of cancer

Responses of cancer cells with and without LKB1 to metformin are different. Metformin exerts cytostatic effect on cancer cells with wild-type LKB1, while it causes cytotoxicity in cells lacking LKB1. If metformin is used together with most of chemotherapeutic drugs that are cytotoxic in cancer containing wild-type LKB1, the cooperative effects might not be achieved. The reason is that more rapidly dividing cells are more sensitive to cytotoxic drugs, while cytostatic drugs slow down speed of cell growth, which might compromise the efficacy of cytotoxic chemotherapy. In this scenario, it might be a good idea to take metformin and cytotoxic drug alternately. For example, patients take a couple of cycles of cytotoxic chemotherapy and then have rest for period of time during which metformin is alternately used. The purpose is to restrain cancer in dormancy and allow the patients to restore healthy condition. In addition, Birsoy et al. have delineated that the most metformin-sensitive cells contain mutations of genes responsible for upregulation of mitochondrial oxidative phosphorylation, for example, complex I components, or glucose utilization [80]. Thus, these genes may serve as biomarkers for metformin use. Altogether, these studies point to importance of personalized medicine to determine the efficacy of metformin in cancer therapy.

5.3 Sensitivity of cancer stem cells

Cancer stem cells (CSCs) are refractory to chemotherapy, leading to the relapse of cancer. These cells metastasize to distant organs after flowing in circulation, resulting in poor prognosis. Thus, CSCs have become an important target for anticancer therapies. Hirsch et al. have reported that the CSCs derived from BC are preferentially sensitive to metformin that is used from 10 to 100 times less dosage

than nonstem cancer cells [81]. This finding suggests that metformin could effectively prevent metastasis. It is especially meaningful in the case of surgically resected cancer when local metastasis in lymph nodes is cytologically tested negative, but a few CSCs may escape to circulation. At this time, metformin can be used as preventive measure.

Previous studies have demonstrated that metformin selectively targets CSCs via regulation of different pathways in various cancer types including breast, pancreatic, prostate, and colon cancer [82, 83]. For example, Zhu et al. have shown that metformin inhibits $CD61^{high}/CD49f^{high}$ subpopulation, markers of tumor initiating cells, by inactivating epidermal growth factor receptor/ErbB2 signaling. Similarly, CD133+, aldehyde dehydrogenases 1+, and other molecules are inhibited in pancreatic and colon cancer through inhibition of the Akt/mTOR pathway [84, 85]. However, a recent study using head and neck squamous cell carcinoma has shown that metformin protects CSCs against the cisplatin-induced cell death when combining these two, which discord with previous studies [86]. Thus, it should be cautious to ascertain if metformin exerts inhibitory or protective effects on specifically originated CSCs.

6. Conclusion

Metformin is a cheap and nontoxic first-line antidiabetic medicine. It is an attractive drug that is being repurposed for multiple usages in treatment of other diseases in addition to diabetes. Metformin implements its function through AMPK-dependent and independent mechanisms. Preclinical and retrospective clinical investigations have inspired clinical trials of metformin use in various cancer therapies. It is a promising drug in neoadjuvant and adjuvant therapies. We hope these trials will come to end with positive or negative results in the next few years. In considering genetic heterogeneity of cancer, responses of different cancer types and subpopulations in the same cancer might be different. Therefore, we still have long way to go and loads of questions to be addressed.

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Conflict of interest

The authors declare no conflict of interest.

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