Metformin: A candidate for the treatment of gynecological tumors based on drug repositioning (Review)

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Abstract. Metformin is a first-line drug used for the treatment of type 2 diabetes. Recently, metformin has been reported to reduce the carcinogenic risk and inhibit tumor cell growth in glioma and breast cancer. The anticancer action of metformin involves the enhancement of phosphorylation of liver kinase B1, activation of adenosine monophosphate-activated protein kinase and inhibition of mammalian target of rapamycin, which reduces cell growth. Metformin is anticipated to exert antitumor effects in gynecological cancer, and its efficacy for the treatment of endometrial, breast and ovarian cancer has been suggested in preclinical studies and clinical trials. Although the effect of metformin on cervical cancer remains to be examined in clinical trials, its antitumor effects have been reported in preclinical studies. Thus, the use of metformin for the treatment of gynecological cancer may become a successful example of drug repositioning, following establishment of the drug's antitumor effects, risk evaluation, screening and validation of efficacy.

Contents

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1. Introduction

Metformin is an oral biguanide that is used worldwide for the treatment of type 2 diabetes (1). Previous studies have provided evidence that long-term administration of metformin may reduce the carcinogenic risk in various organs, and may have an inhibitory effect on cell growth in breast and colon cancer, as well as glioma (2,3). The mechanism underlying the antitumor effect of metformin is considered to involve the activation of adenosine monophosphate-activated protein kinase (AMPK) and inhibition of mammalian target of rapamycin (mTOR), which reduces cell growth (4). Focusing on gynecological cancer, as carcinogenesis in endometrial cancer appears to be associated with obesity, type 2 diabetes and hyperestrogenic conditions, metformin may be effective for prevention and improvement of prognosis in endometrial cancer (5). Thus, the effect of metformin on gynecological tumors, particularly endometrial cancer, is currently under investigation.

The aim of drug repositioning is to identify novel pharmacological effects for conventional drugs, in which human safety and pharmacokinetics are already established, and to expand the application of the drug for the treatment of additional diseases (6). As the adverse reactions of the repositioned drugs are known from previous clinical trials, safety is guaranteed, and the time and cost of drug discovery are considerably alleviated (6). Despite recent efforts, the efficacy of the existing antitumor drugs requires improvement, since they frequently cause adverse reactions, including nausea, vomiting, hair loss, nephrotoxicity and myelosuppression, which may limit their use. We hypothesize that by combining traditional antitumor drugs with novel antitumor agents identified by drug repositioning, improved therapeutic efficacy and reduced adverse reactions may be achieved. In the present review, the clinical application of metformin for the treatment of different types of gynecological cancer is evaluated from the perspective of drug repositioning.

2. Metformin in the treatment of type 2 diabetes

Metformin is an oral biguanide that is safe and cost-effective for the treatment of type 2 diabetes (1). Structurally, metformin contains two conjugated guanidine groups and an additional

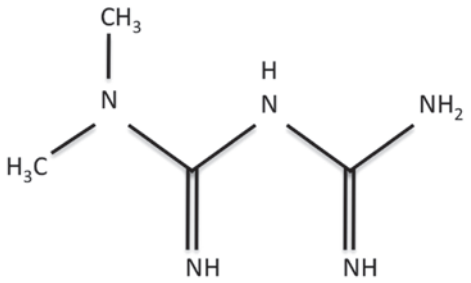

Figure 1. Structure of metformin. Metformin contains two conjugated guanidine groups and an additional amine.

amine (7) (Fig. 1). Metformin is one of the first-line agents prescribed worldwide for the treatment of type 2 diabetes (1,8), based on its inhibition of insulin-dependent hepatic gluconeogenesis, promotion of glucose uptake into surrounding cells by improvement of insulin resistance and reduction of free fatty acids by inhibition of lipolysis (9-11). Metformin additionally inhibits the development of macroangiopathy to a greater extent than sulfonylureas do, which may be utilized for the treatment of type 2 diabetes (12).

Metformin enhances glucose consumption in the intestine and produces lactic acid, which is used in hepatic gluconeogenesis (13). This causes adverse reactions, including lactic acidosis, intestinal symptoms such as diarrhea and abdominal pain, and vitamin B12 deficiency (14). Lactic acidosis increases the risk of impaired hemodynamics due to ischemia and shock, nephropathy, hepatic dysfunction, alcoholism and heart failure (15-18). Therefore, it is clinically important to consider the balance between the therapeutic effects and the risks of adverse reactions when using metformin (17). Nevertheless, the incidence of lactic acidosis with metformin is 9/100,000 patients/year, whereas with phenformin, an alternative drug used for the treatment of type 2 diabetes, 40-64/100,000 patients/year experience lactic acidosis (19). Therefore, metformin is generally considered to be safe, compared with alternative antidiabetic drugs (19).

3. Effect of metformin on carcinogenic risk

Type 2 diabetes and insulin resistance increase the carcinogenic risk in the large intestine, lung, breast, prostate gland and pancreas (20-25). A number of studies have evaluated the effects of metformin on cancer prevention. In a population-based study including 11,876 patients with type 2 diabetes, Evans *et al* (26) reported a reduced incidence of cancer in patients treated with metformin, compared with patients not treated with metformin [odds ratio (OR), 0.79; 95% confidence interval (CI), 0.67-0.93]. Bowker *et al* (27) compared patients with type 2 diabetes in the metformin (monotherapy or combined) group and sulfonylurea monotherapy group, and reported that the cancer mortality rate was significantly decreased in the metformin group [hazard ratio (HR), 0.80; 95% CI, 0.65-0.98; P=0.03), compared with the sulfonylurea group. In a study of 4,085 patients exhibiting type 2 diabetes, Libby *et al* (28) identified that the incidence of cancer in patients treated with metformin (7.3%) was significantly lower than that observed in patients treated with alternative drugs (11.6%). Following adjustment for confounding factors, the authors observed that the use of metformin significantly reduced the risk of cancer (HR, 0.63; 95% CI, 0.53-0.75). The results of the aforementioned studies suggest that metformin is able to reduce the carcinogenic risk in patients with type 2 diabetes.

4. The antitumor effect of metformin

Multiple pathways are considered to be involved in the antitumor activity of metformin (4,29-32) (Fig. 2). The primary action of metformin occurs via activation of AMPK (33). Metabolically, AMPK inhibits the expression of certain enzymes involved in hepatic gluconeogenesis, enhances glucose uptake into muscle and fat cells, and increases insulin sensitivity in cells, resulting in decreased insulin levels (33,34). Furthermore, the fact that the metformin-induced activation of AMPK is mediated by the tumor suppressor liver kinase B1 (LKB1) suggests the antitumor potential of metformin (29). AMPK inhibits the activity of mTOR in the phosphoinositide 3-kinase/Akt/mTOR signal transduction pathway, which stimulates cellular proliferation (30,31). AMPK is also known to inhibit cell cycle progression via the activation of tumor protein p53 (4).

Additional mechanisms of metformin that do not involve AMPK have been reported. Metformin inhibits cell cycle progression by decreasing cyclin D1 expression (35), and Cantrell *et al* (32) identified that telomerase activity was inhibited by metformin. However, the mechanism of action of metformin remains to be fully elucidated, for which further studies are required.

5. Antitumor effect of metformin in endometrial cancer

Metformin may be an effective adjuvant for the treatment of endometrial cancer, based on the observation that type 2 diabetes and obesity are risk factors of endometrial cancer (5). Epidemiological data has demonstrated that obese individuals possess a significantly increased risk of developing endometrial cancer in comparison with non-obese individuals (risk ratio, 6.25; 95% CI, 3.75-10.42; P<0.001) (36). An additional risk factor for endometrial cancer is polycystic ovarian syndrome (PCOS), in which hyperinsulinemia and hyperandrogenism are the two central pathological conditions (37). Metformin has a therapeutic effect on the anovulatory cycle in PCOS (38), and is expected to reduce the carcinogenic risk in endometrial cancer (39).

Metformin is additionally likely to have a significant role in the prevention of endometrial cancer via cell cycle arrest and induction of apoptosis (32). In preclinical studies, Cantrell *et al* (32) observed that metformin caused G1 arrest at a low dose of 1 mM, and apoptosis via activation of caspase-3 at a high dose of 2-5 mM *in vitro*.

Telomere maintenance by telomerase has a significant role in tumor growth, and the messenger (m)RNA levels of human telomerase reverse transcriptase (hTERT) are used as an index for telomerase activity and cell growth (32). Metformin suppresses the mRNA expression of hTERT in endometrial cancer cells in a dose-dependent manner, leading to the inhibition of telomerase activity (35). The suppression of hTERT mRNA may be a direct effect of metformin or

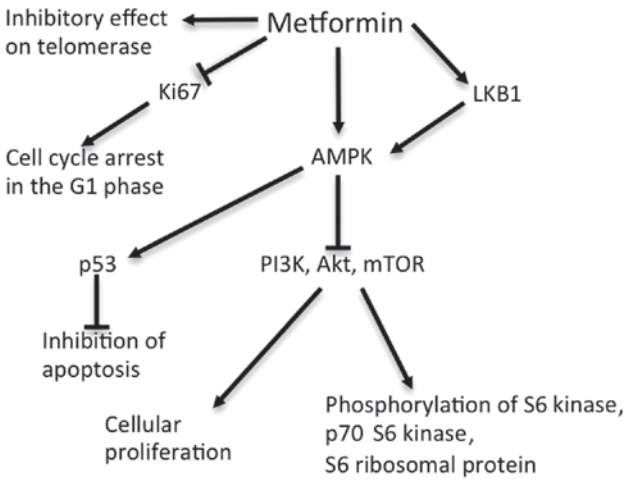

Figure 2. Antitumor mechanism of metformin. The primary pathway is considered to be the AMPK/PI3K/Akt/mTOR signal transduction pathway. Recent studies (4,29-32,35) have revealed that multiple signaling pathways contribute to the antitumor mechanism of metformin. AMPK, adenosine monophosphate-activated protein kinase; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; LKB1, liver kinase B1.

a secondary effect due to cell cycle arrest, as endometrial, ovarian and cervical cancer cell growth is additionally inhibited by rapamycin and accompanied by a decrease in hTERT mRNA (35). A direct inhibition caused by metformin has been suggested, due to the observation that rapamycin suppressed hTERT mRNA without cell growth inhibition or cell cycle arrest in cell lines that were resistant to rapamycin, indicating that a reduction in hTERT mRNA is able to occur independently from cell cycle arrest (40).

Progesterone is utilized for the treatment of early endometrial cancer. However, the therapeutic effect of progesterone in endometrial cancer cells is insufficient, due to the down-regulation of progesterone receptor (PR) in these cells (41). Xie *et al* (41) identified that metformin and progesterone had a synergistic effect in the treatment of endometrial cancer. Metformin inhibits the phosphorylation of S6 ribosomal protein (S6RP), increases PR expression and inhibits mTOR via AMPK phosphorylation, which enhances the efficacy of medroxyprogesterone acetate (MPA) in the treatment of endometrial cancer (42).

Ko *et al* (43) investigated the efficacy of metformin in 1,495 patients exhibiting endometrial cancer, including 363 (24%) patients with diabetes. Patients treated with metformin (54% of diabetic patients in the study) demonstrated significantly improved recurrence-free survival (RFS) and overall survival (OS) rates in comparison with patients who were not administered metformin. RFS in the non-metformin group was reduced by 1.8-fold (95% CI, 1.1-2.9; P=0.02), while OS was reduced by 2.3-fold (95% CI, 1.3-4.2; P=0.005). However, there was no association between metformin treatment and time to recurrence, indicating that metformin has a survival benefit for mortality, but does not prolong the time to recurrence, for reasons that remain to be elucidated (43). Thus, additional studies are required to confirm if adjuvant therapy with metformin is effective for patients exhibiting endometrial cancer, regardless of the complication of diabetes.

6. Antitumor effect of metformin in breast cancer

Breast cancer is associated with type 2 diabetes (36), and a previous epidemiological study demonstrated that type 2 diabetes increased the risk of developing breast cancer by 10-20% (44).

Triple-negative (TN) breast cancer refers to breast cancer cases that do not express the genes for estrogen receptor (ER), PR and human epidermal growth factor receptor 2 (HER-2) (45). TN breast cancer develops in perimenopausal women possessing a high body mass index (BMI) and over-expression of epidermal growth factor receptor, and has been identified to be highly sensitive to metformin (45). A previous study revealed that metformin was able to inhibit cell growth of TN breast cancer at a similar dose to that utilized for the treatment of type 2 diabetes by suppressing Ki67, arresting the cell cycle in G1 phase, and inducing intrinsic and extrinsic apoptosis via caspase-8 and -9 (45). The efficacy of metformin for the treatment of common subtypes of breast cancer, including luminal A and B and HER-2$^+$, has additionally been demonstrated. Colonization and tumor growth were simultaneously inhibited by metformin, and these effects occurred through a non-apoptotic mechanism, in which cyclin D1 and E2F transcription factor 1 (E2F1), which promote the transition from G1 to S phase, were implicated (46). In addition, metformin was able to alter tyrosine kinase signaling, downregulates HER-2 and activates mitogen-activated protein kinase at an identical dose to that utilized for the treatment of type 2 diabetes (46).

Overexpression of the insulin and insulin-like growth factor (IGF)-1 receptors is involved in the carcinogenesis of breast cancer, and breast cancer cell lines such as MCF-7 are responsive to insulin and IGF-1 (47). The absence of an inhibitory effect of metformin on cell growth following small interfering RNA inhibition of AMPK suggested that the effect of metformin on breast cancer cells occurs via AMPK (48).

The efficacy of metformin has been demonstrated in diabetic women exhibiting breast cancer in a retrospective study (49). Of the 155 diabetic patients included in the study, 68 received metformin and 87 did not, along with anthracycline-based chemotherapy regimens (49). The pathological complete response rate was 24% in the metformin-treated group, compared with 8% in the non-metformin-treated group (P=0.07) (49). Additional phase II and phase III studies are ongoing. The METEOR study is a phase II randomized trial of metformin plus letrozole vs. placebo plus letrozole, which aimed to assess the antitumor effects of metformin in postmenopausal non-diabetic patients exhibiting ER$^+$ breast cancer (50). An ongoing phase III clinical trial termed NCIC CTG MA.32, which aimed to study the effects of metformin on non-diabetic patients with breast cancer, requires a follow-up period of several years in order to evaluate the effects of metformin on mortality and define an optimal dose of metformin for the treatment of early breast cancer (51).

The optimal dose of metformin for the treatment of breast cancer remains to be elucidated. However, 1,500-2,250 mg/day metformin was observed to be required in order to reduce tumor size in xenograft models (50,52),

and in the NCIC CTG MA.32 trial, the metformin group was designed to receive 1,700 mg/day of this drug (51). These doses are tolerated in the treatment of type 2 diabetes (8).

7. Antitumor effect of metformin in ovarian cancer

The potential pharmacological effects of metformin in ovarian cancer are of interest. Obesity potentially contributes to the onset of ovarian cancer, and may additionally be stimulated by androgens, as in PCOS (36,39). Hyperandrogenism is caused by hyperinsulinemia, inhibition of IGF binding protein 1 (IGFBP1) and increased IGF-1 activity (39). Based on the risk reduction for ovarian cancer exhibited by oral contraceptives with anti-androgen activity (53) and the effects of metformin on PCOS, obesity and other tumors, we hypothesize that metformin may demonstrate efficacy for the treatment of ovarian cancer. Metformin inhibits tumor growth and induces apoptosis in ovarian cancer cells *in vitro*, as reported by Gotlieb *et al* (54), who identified that metformin inhibited cell growth in OVCAR-3 and OVCAR-4 cells in a dose-dependent manner, and administration of metformin in combination with cisplatin enhanced this pharmacological effect. These effects were induced by decreased phosphorylation of p70 S6 kinase (p70S6K) and S6K via AMPK phosphorylation (54).

A number of epidemiological studies have investigated the effects of metformin in ovarian cancer. In a case-control study of 1,611 diabetic patients, Bodmer *et al* (55) identified that the carcinogenic risk in the metformin-treated group was significantly lower (OR, 0.61; 95% CI, 0.30-1.25) than in the sulfonylurea-treated (OR, 1.26; 95% CI, 0.65-2.44) and insulin-treated groups (OR, 2.29; 95% CI, 1.13-4.65). In a study including 1,454 diabetic patients treated with metformin and 2,897 diabetic patients who were not administered metformin for a median duration of 4.0 years, Home *et al* (56) observed that none of the patients in the metformin group developed ovarian cancer, whereas 3 patients in the non-metformin group did. In an analysis with a median duration of 5.5 years, 6/3,344 patients treated with metformin and 3/1,103 patients who were not treated with metformin developed ovarian cancer (56).

In a systematic review of 28 studies, Zhang and Li (57) identified that metformin decreased mortality associated with ovarian cancer (relative risk (RR), 0.44; 95% CI, 0.30-0.64; P<0.001). In an epithelial ovarian cancer study, the effect of metformin on survival rate was examined in 61 metformin-treated diabetic patients and 178 non-diabetic controls (58). The 5-year disease-specific survival (DSS) rate in the metformin group was significantly increased, compared with the control group (67 vs. 47%; P=0.007) (58). Following adjustment for background factors including BMI, tumor grade, histology and chemotherapy, metformin remained an independent predictor of survival (58). In the same study, the 5-year DSS rate was compared between the metformin-treated diabetic group and the diabetic control group (patients on diabetic treatment other than metformin). The 5-year DSS rate was significantly reduced in the insulin (43%) and alternative antidiabetic medication group (34%; P=0.004), compared with the metformin-treated group. There were a limited number of patients exhibiting diabetes and ovarian cancer, and the small number of cases (n=61) is a limitation of that study (58). However, the results clearly demonstrated the overall efficacy of metformin for the treatment of ovarian cancer (58). As diabetes itself is known to be a poor prognostic factor for ovarian cancer, and diabetic patients are also likely to exhibit other poor prognostic factors, including cardiovascular disease and surgical history, the therapeutic effects of metformin may be overestimated when compared with non-metformin treated diabetic patients (58). Thus, it is a matter of discussion whether the antitumor effects of metformin should be compared with diabetic or non-diabetic controls in future studies.

8. Antitumor effect of metformin in cervical cancer

There have been few studies discussing the efficacy of metformin for the treatment of cervical cancer. However, based on its effects on tumor inhibition, metformin is likely to inhibit cervical cancer cell growth (59,60). Xiao *et al* (61) investigated the kinetics of metformin in cervical cancer cells and evaluated LKB1 activity in these cells. The authors observed that metformin inhibited the growth of the C33A, ME180 and CaSki cervical cancer cell lines, but exhibited reduced efficacy against HeLa, HT-3 and MS751 cells (61). Following analysis of LKB1/AMPK/mTOR signaling, metformin-sensitive cervical cancer cells were identified to activate AMPK via LKB1 and inhibit mTOR (61). In contrast, sensitivity to metformin was lost in LKB1-knockdown cells, whereas in cervical cancer cells expressing LKB1, metformin induced apoptosis and autophagy (61). These results suggest that metformin may be a promising drug for the treatment of cervical cancer, particularly in tumor cells expressing LKB1, by increasing LKB1 activity and activating AMPK (61).

9. Clinical studies of metformin in gynecological cancer

In antitumor mechanisms *in vitro* (Fig. 3), metformin arrests the cell cycle in endometrial cancer cells, decreases hTERT mRNA and inhibits phosphorylation of S6RP, resulting in inhibition of signaling downstream of the mTOR pathway (35,42). Metformin additionally antagonizes IGF-2, enhances expression of PR and improves the antitumor effect of MPA in cancer cells (41). The antitumor effect of metformin in breast, cervical and ovarian cancer also involves the inhibition of mTOR via AMPK activity (48,54,61).

Based on these findings, the efficacy of metformin for the treatment of gynecological cancer has been examined in clinical studies, and the results have been analyzed using RR in systematic reviews (57). Two studies have demonstrated the efficacy of metformin for the treatment of endometrial cancer (43,62). In these studies, the RR of treatment with metformin was 0.49 (95% CI, 0.32-0.73; P=0.001), with no difference in the results in meta-analysis and no heterogeneity (I^2=0%) (58). A total of four studies have demonstrated that metformin improves overall mortality in breast cancer (63-66), contrarily to three other studies (67-69), which did not observe any efficacy for this drug. The RR of treatment with metformin in the aforementioned studies was 0.70 (95% CI, 0.55-0.88; P=0.003) (58). High heterogeneity was identified in these studies (I^2=75%), but no publication bias. He *et al* (63) demonstrated that administration of metformin markedly decreased mortality specific to breast cancer (63), although

Table I. Effects of metformin in gynecological cancer prevention.

Type of cancer	Relative risk for all-cause mortality	95% Confidence interval	P-value	Heterogeneity, I^2, %
Endometrial	0.49	0.32-0.73	0.001	0
Breast	0.70	0.55-0.88	0.003	75
Ovarian	0.44	0.30-0.64	<0.001	0

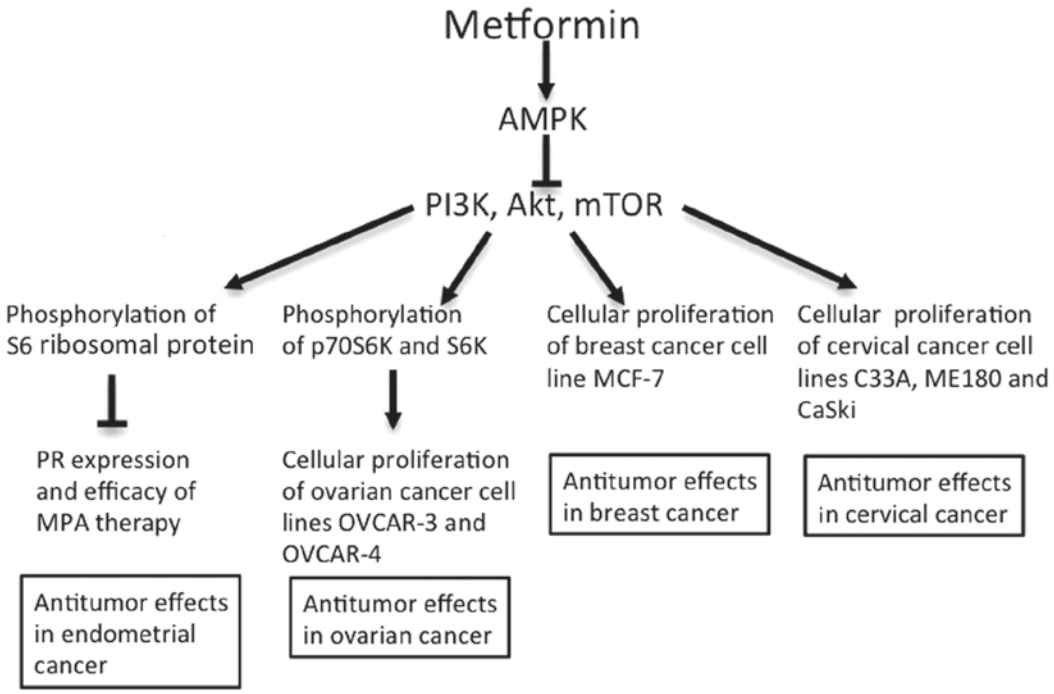

Figure 3. Antitumor effects of metformin in gynecological cancer. Antitumor effects of metformin have been demonstrated in endometrial, ovarian, breast and cervical cancer *in vitro* (41,46-48,54,61). AMPK, adenosine monophosphate-activated protein kinase; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; PR, progesterone receptor; MPA, medroxyprogesterone acetate; S6K, S6 kinase.

their findings did not correlate with the results of two other studies (67,68). The RR in these studies was 0.83 (95% CI, 0.63-1.08; P=0.16), and moderate heterogeneity was apparent (I^2=47%) (57). The high I^2 values in the analyses of trials of metformin for breast cancer reflected the varying results among the studies. In ovarian cancer, an association between the use of metformin and overall mortality has been identified in three studies (58,69,70). The RR in these studies was 0.44 (95% CI, 0.30-0.64; P<0.001), and there was no heterogeneity (I^2=0%) (57). A previous study demonstrated that metformin improved progression-free survival, with a relapse HR of 0.38 (95% CI, 0.16-0.90; P=0.03) (57). Overall, the results of the above studies indicate that metformin significantly increases survival in endometrial, breast and ovarian cancer (Table I). The efficacy of metformin in the treatment of cervical cancer has not been examined clinically to date.

Concurrent antitumor therapies used alongside metformin, dose adjustment, cancer stage, tumor size and histology are significant prognostic factors. However, they are not described in the majority of studies (57). We hypothesize that failure to adjust for these confounding factors may cause high heterogeneity. Ethnicity, education level and access to medical care may additionally influence survival, and these factors may bias estimates of the efficacy of metformin (57). Future studies should include clinical trials that consider these factors in order to increase the cohort size, reduce bias and evaluate the effect of metformin more accurately. It is additionally important to identify the optimum dose of metformin based on adverse reactions (57).

10. Conclusion

Metformin is a first-line drug that is used for the treatment of type 2 diabetes, and has additionally been identified to decrease carcinogenic risk and inhibit cancer cell growth (2,3). The antitumor mechanism of metformin involves the inhibition of the mTOR pathway through AMPK activation, as demonstrated in a number of studies on gynecological cancer (42,48,54,61). However, additional details of the mechanism responsible for the antitumor effects of metformin remain to be elucidated. In endometrial cancer, cell cycle arrest by metformin has been observed *in vitro,* and inhibition of telomerase activity may be an important mechanism to explain the antitumor activity of this drug (35). Metformin additionally has an increased antitumor effect when administered in combination with MPA therapy (41). Clinical studies of metformin have demonstrated

efficacy and safety in breast, endometrial and ovarian cancer (43,49,50,55-58). The effect in cervical cancer has not been examined in clinical studies thus far, although efficacy of metformin *in vitro* has been observed (61).

In conclusion, drug repositioning allows rapid clinical application of a drug with high safety and low cost (6). For drug repositioning of metformin, it will be particularly important to understand its antitumor mechanism, evaluate its adverse reactions and risks in clinical application, and determine the optimum dose required for the treatment of gynecological cancer.

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