



Metformin and ovarian cancer: the evidence

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Abstract: In recent decades, great interest in the off-label use of metformin has arisen as a result of its broad effects on different signaling pathways, with only a few side effects, and low cost. Metformin has been shown to have multiple, dose-dependent preclinical anticancer effects, which can be roughly divided into either direct effects via inhibition of mitochondrial respiratory chain complex I, or indirect effects through lowered glucose, insulin and insulin-like growth factor levels. Further details on *in vitro* and *in vivo* anticancer effects specifically in ovarian cancer are continuously reported. Preclinically metformin has clear chemosensitizing effects in ovarian cancer and it is an effective negative regulator of angiogenesis. There are also some epidemiological studies on metformin use in ovarian cancer, but the results of these studies are not as promising as those preclinical studies would indicate. Most preclinical studies have involved metformin concentrations that are many times higher than the pharmacological doses used in patients, which might confound the clinical use of metformin as regards the above-mentioned aspects. In this review we evaluate preclinical and clinical evidence concerning metformin in ovarian cancer treatment.

Keywords: Diabetes; epidemiology; incidence; survival

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Introduction

Galega officinalis, i.e., goat's rue or French lilac has been used to treat polyuria and other symptoms of diabetes for centuries (1). Metformin (1,1-dimethylbiguanide hydrochloride) was later extracted from this plant and it was found to have glucose-lowering activity more than 100 years ago (2). Metformin was officially introduced to diabetes treatment in 1957 (3). With few contraindications and strong evidence from clinical studies, along with usually manageable side effects, metformin is still the most widely recommended first-line medical treatment for type 2 diabetes (T2D) (4).

Drug repositioning or repurposing are defined as a method of finding novel diseases and target molecules for drugs with already existing indications (5). When successful, this may drastically save time and other resources compared with the traditional drug development. Some examples in the field of oncology are disulfiram and probably the most prominent example is aspirin in the prevention of colorectal cancer in patients with inflammatory bowel disease (6,7). Metformin is increasingly used in off-label indications, such as polycystic ovarian syndrome, obesity and metabolic syndrome (8). In the mid 1970s it was shown for the first time that biguanides inhibit mammary carcinogenesis in female rats (9). Suggested anticancer effects of metformin

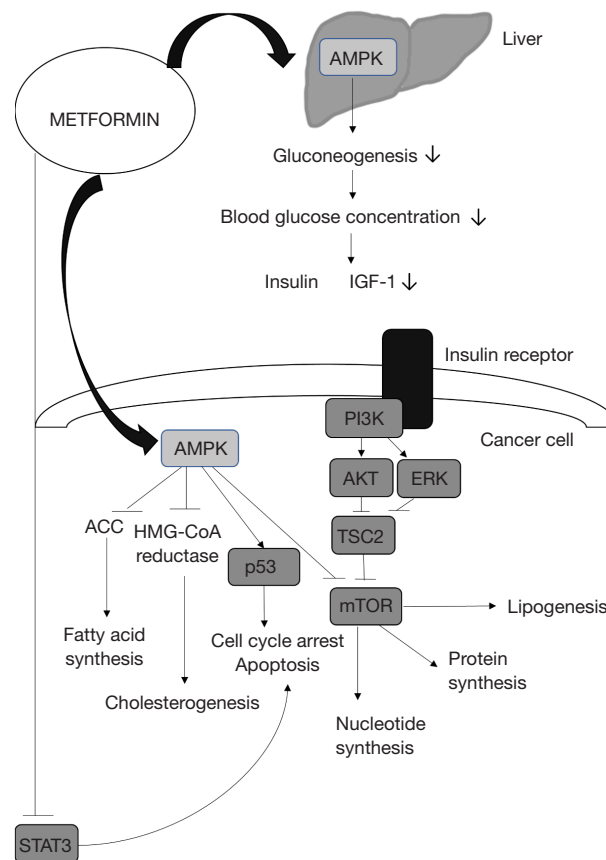


Figure 1 Metformin affects cancer cells both directly and indirectly. It activates AMP-activated protein kinase (AMPK), which leads, among other things, to inhibition of mammalian target of rapamycin (mTOR). It also sensitizes tissues to insulin, reduces hepatic gluconeogenesis and decreases circulating insulin levels. This leads indirectly to reduced phosphatidylinositol-3-kinase (PI3K) signaling. In addition, metformin deactivates the downstream signaling molecules ERK and STAT3, which have effects on cell growth and apoptosis (8,12,13). IGF-1, insulin-like growth factor 1; ACC, acetyl-CoA carboxylase; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; p53, tumor protein p53; AKT, serine/threonine-specific protein kinase; ERK, extracellular signal-regulated kinase; TSC2, tuberous sclerosis complex 2; STAT3, signal transducer and activator of transcription 3.

have drawn increasing attention during the last decade, mostly in breast cancer, but also considerably in the context of ovarian cancer (8). In the field of epidemiology, great interest in the possible role of metformin as a cancer-preventive agent arose in 2005 when an observational study from Scotland was published in which metformin use was related to a lower risk of cancer in general (10). At present, ovarian cancer patients are being recruited to take part in multiple randomized studies to evaluate the clinical efficacy of metformin (11). In this review, we will address the up-to-date preclinical and clinical evidence concerning metformin in the context of ovarian cancer.

Preclinical studies

AMPK pathway

In theory, metformin may have great potential as a drug to use in cancer prevention. Its anticancer effects have been traditionally divided into direct and indirect effects, although novel mechanisms of action are continuously emerging (*Figure 1*) (14). As a direct action against cancer growth, metformin reduces the energy consumption of (cancer) cells by inhibiting mitochondrial respiratory chain complex I (15). As an indirect anticancer effect, metformin reduces fasting plasma insulin levels and attenuates insulin resistance by

Table 1 Studies concerning the effects of metformin on ovarian cancer growth in preclinical models

Authors, year	Setting	Main result
Shank <i>et al.</i> 2012 (22)	A2780 and SKOV3 cells and xenografts treated with metformin	Metformin reduced cancer stem-cell growth, angiogenesis and proliferation
Kim <i>et al.</i> 2015 (23)	SKOV3 cells treated with metformin	Axl and Tyro3 were suppressed and Erk and STAT3 activated after metformin treatment
Patel <i>et al.</i> 2015 (24)	Metformin-treated primary human ovarian carcinoma cells	Metformin induced cell-cycle arrest and apoptosis, in which the Bcl-2 family had an essential role
Huo <i>et al.</i> 2017 (25)	The effect of metformin in SKOV3 and A2780 cells	Metformin decreased cell viability and clone formation in both cell lines.
Fu <i>et al.</i> 2017 (26)	SKOV3 cells treated with metformin	Metformin suppressed PI3K/AKT/mTOR signaling and triggered cell-cycle arrest. The IC50 value was 20 mmol/L
Erices <i>et al.</i> 2017 (27)	<i>In vitro</i> study on the relation of metformin and platelets	Metformin had anti-platelet effects, which led to decreased angiogenesis. Micromolar concentrations of metformin had no impact on proliferation
Zhang <i>et al.</i> 2018 (28)	Apoptosis and invasion were studied after metformin treatment of OVCAR-3 cells. siRNA against Cyr61 was used	Metformin improved chemosensitivity by targeting the Cyr61/PI3K/Akt/mTOR axis
Ma <i>et al.</i> 2019 (29)	Three ovarian cancer-cell lines and xenografts treated with metformin. Special interest in the role of ASK1	Metformin cytotoxicity was dependent on glucose levels and was mediated by ASK-1
Zou <i>et al.</i> 2019 (30)	SKOV-3 cells treated with metformin	Metformin suppressed invasion and migration capabilities and attenuated proliferation

Erk, extracellular-signal-regulated kinase; STAT3, signal transducer and activator of transcription 3; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; Cyr61, cysteine-rich angiogenic inducer 61; siRNA, small interfering RNA; ASK1, apoptosis signal-regulating kinase 1.

increasing skeletal-muscle glucose uptake. This leads not only to metformin's actual therapeutic effect, decreased blood glucose levels, but also cuts hyperinsulinemia. This may have effects on malignant cell mitogenesis and survival, since the association between insulin and insulin-like growth factor (IGF) in carcinogenesis has been repeatedly demonstrated (16), and cancer cells often show high-level expression of insulin receptors (14). Both indirect and direct routes are mediated by the cellular energy-status monitor adenosine monophosphate-activated protein kinase (AMPK), which under energy depletion is activated, switching the cell from an anabolic to a catabolic state (14). Metformin also very effectively attenuates p53 and mTOR signaling routes via AMPK (8,17). In the end, this leads to various growth-inhibiting properties including anti-mitotic and anti-inflammatory effects (18). AMPK activation has been suggested to play a most prominent role in the tumor-suppressing effects of metformin (19,20). LKB1 is a tumor suppressor gene with relevance to epithelial neoplasia.

Beyond AMPK, the metformin-stimulated activation of LKB1 as well takes place in hepatocytes, and loss of its function is associated with Peutz-Jeghers syndrome (21).

PI3K/AKT/mTOR signaling

Of all the preclinically studied tumor types, one of the strongest indications supporting metformin's anticancer effects comes from ovarian cancer studies (*Table 1*). Metformin effectively suppresses PI3K/AKT/mTOR signalling, e.g., in OVCAR-3 and SKOV3 cells, and triggers cell cycle arrest at the G2/M checkpoint (26,28). However, under hyperglycemic conditions metformin seems to have little or no anticancer effect in preclinical models, while low glucose levels enhance its cytotoxicity as a result of induction of ASK1-mediated mitochondrial dysregulation (29). mRNA and protein levels of the pro-survival and anti-apoptotic receptor tyrosine kinases Axl and Tyro3 are suppressed at transcriptional level when ovarian

cancer cells are treated with metformin, and this leads to subsequent deactivation of downstream signaling molecules such as Erk and STAT3 (23). Indeed, STAT3 inhibition has been proposed as one of the most significant effects of the anticancer action of metformin in at least pancreatic and triple-negative breast cancers, although its dependence on mTOR signaling is still under debate (8). Metformin inhibits the AMPK-dependent growth of multiple ovarian cancer cell lines and inhibits several vital receptor tyrosine kinases of ovarian cancer, such as HER4, epidermal growth factor and platelet-derived growth factor receptor (31).

Angiogenesis and stem cells

Angiogenesis is a crucial hallmark of ovarian carcinogenesis and also important from the therapeutic point of view. Decreased angiogenesis in metastatic tissues, attenuated ovarian cancer-cell adhesion and dampened macrophage infiltration were observed under metformin treatment *in vitro* and *in vivo* in murine experiments (32,33). Two other ovarian cancer studies showed that dampened neovascularization after metformin treatment can be driven either by blockage of the mTOR signaling pathway, or metformin's ability to reduce the angiogenic and other carcinogenic properties of platelets (27,34). Intriguingly, from a clinical point of view, reduced angiogenesis was demonstrated using only micromolar concentrations of metformin. In other reports these near-pharmacological concentrations of metformin did not actually have any impact on proliferation in SKOV3, UCI101 or A2780 cell lines (35,36). Metformin treatment *in vitro* has also effects on stem cell differentiation and growth. Metformin targets ALDH+ ovarian cancer stem cell populations *in vitro* and in xenograft models, leading to suppressed angiogenesis and proliferation (22). A relatively low dose of metformin selectively inhibited CD44+CD117+ ovarian cancer stem-cells through downregulation of epithelial-to-mesenchymal transition, without affecting the cancer-cell proliferation rate (36).

Apoptosis and p53

Increased Bcl-2-protein family dependent apoptosis has been linked in many preclinical ovarian cancer studies to metformin's chemosensitizing effects (24,32,37). Recently, additional mechanisms have also been revealed. Metformin may impede chemoresistance by targeting cancer stem cell populations and reducing the migratory properties of cancer

via upregulation of taurine (38). On the other hand, the chemosensitizing effect of metformin seems to be crucially dependent on p53 function (39). In the presence of p53, metformin suppresses a major glycolytic enzyme, hexokinase II, and pyruvate dehydrogenase kinase, which is an anti-apoptotic serine/threonine kinase of the same pathway. This leads to sensitization of ovarian cancer cells to metformin, which does not occur in the case of mutated p53. However, mutation of p53 is considered to be a driver mutation in high grade serous ovarian carcinoma and is present in more than 96% of cases (40). Thus, other pathways for apoptosis are likely to be involved in these cancers. On the other hand, metformin also seems to have capability to re-sensitize cisplatin-resistant or paclitaxel-resistant ovarian cancer cells to chemosensitive cells, possibly by induction of autophagy or via the anti-inflammatory properties of metformin (27,41).

Efficacy of metformin in cell-line and animal studies

Although more than 70% of ovarian cancers respond to first-line chemotherapy (42), the majority of cases relapse and develop chemoresistance eventually (43). Multiple lines of evidence suggest that metformin can be used to overcome chemoresistance, especially cisplatin resistance, in ovarian cancer *in vitro* (Table 2). In one of the earliest studies, metformin was reported to enhance cisplatin activity in OVCAR-3 and OVCAR-4 cells. Metformin as a single agent was also demonstrated to reduce cancer cell proliferation in this work (44). The same group later showed that metformin induces cell cycle arrest and apoptosis in the same ovarian cancer cell lines by means of a mechanism that was independent of AMPK but dependent on Bcl-2-family proteins (37). These observations were later repeated in primary human ovarian carcinoma cells, isolated from ascitic fluid or omental metastases (24,47). Metformin also induces growth inhibition and shows synergy with cisplatin in a time- and dose-dependent manner in more invasive SKOV3 ovarian cancer cells (30). In cisplatin-resistant ovarian cancer patient-derived xenograft models, *in vivo* treatment with metformin partially reversed platinum resistance, although at a suprapharmacological dose of 400 mg/kg (48). Metformin also sensitizes ovarian cancers to paclitaxel in mouse models and induces cell death in SKOV3 cells or ovarian cancer primary cultures when treated with paclitaxel and carboplatin (20,31).

It has to be noted that in most of preclinical studies the used metformin concentrations have been millimolar

Table 2 Studies concerning the effect of metformin on ovarian cancer chemoresistance in pre-clinical models

Authors, year	Setting	Main result
Gotlieb <i>et al.</i> 2008 (44)	OVCAR-3 and OVCAR-4 cell lines treated with metformin and/or cisplatin	Metformin enhanced cisplatin activity and itself inhibited proliferation
Yasmeen <i>et al.</i> 2011 (37)	OVCAR-3 and OVCAR-4 cell lines treated with metformin and/or cisplatin	Metformin induced cell-cycle arrest and apoptosis, in which the Bcl-2 family had an essential role
Zheng <i>et al.</i> 2018 (45)	SKOV-3 cells treated with metformin	Metformin decreased ovarian cancer (and metastatic) growth and invasion and had synergy with cisplatin
Yang <i>et al.</i> 2019 (41)	Cisplatin-resistant SKOV3 cells treated with metformin, methotrexate and/or cisplatin	Metformin re-sensitized cells to cisplatin and enhanced autophagy
Erices <i>et al.</i> 2013 (35)	A2780 and SKOV3 cell lines and primary cultured cells treated with low micromolar concentrations of metformin	Pharmacological doses of metformin had synergy with carboplatin, but not single-agent activity
dos Santos Guimaraes <i>et al.</i> 2018 (46)	Metformin and/or chemotherapy in paclitaxel-resistant A2780-PR cells and cisplatin-resistant ACRP cell lines	Metformin re-sensitized cell lines to chemotherapy, suppressed the NF- κ B pathway and decreased levels of inflammatory cytokines
Lengyel <i>et al.</i> 2015 (31)	The effect of metformin <i>in vitro</i> and in multiple cell lines	Metformin inhibited receptor tyrosine kinases, inhibited cell-line growth and sensitized tumors to paclitaxel in mouse models
Liu <i>et al.</i> 2018 (47)	Primary cultures from omental metastases were treated with metformin and various chemotherapeutic drugs	Metformin sensitized cancer cells to chemotherapy
Ricci <i>et al.</i> 2019 (48)	Cisplatin-resistant ovarian cancer patient-derived xenograft models	<i>In vivo</i> treatment with metformin partially reversed platinum resistance
Han <i>et al.</i> 2019 (39)	Cisplatin-sensitive and resistant cells treated with metformin	Metformin re-sensitized cell lines to chemotherapy, but only with functioning p53

NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells.

or at least clearly above 2.7 μ g/L (10 μ M range), which has been reported to represent mean metformin plasma levels in metformin users (49). For example, in widely used SKOV3 ovarian cancer cells an IC₅₀ value of 20 mmol/l has been reported for metformin (26). There is some evidence that even micromolar concentrations of metformin show synergy with chemotherapy in ovarian cancer cells, but only limited single-agent activity (35). On the other hand, metformin is not metabolized and relatively high metformin concentrations have also been found in tissues *in vivo*, especially in mitochondria. It may be argued that to achieve these such a concentrations and to study effects of metformin when administered over long period of treatments, millimolar metformin concentrations of metformin should be used in *in vitro* experiments (24). As a cation, metformin has a tendency to accumulate, especially within mitochondria, showing 100- to 500-fold concentrations (50). Nano-encapsulation

of metformin into polymeric nanoparticles has been very recently developed in order to overcome metformin's poor cell-membrane permeability. This approach was shown to induce metformin cytotoxicity by reducing cell viability, and bringing cells to G2/M arrest, apoptotic morphological changes and alteration in the expression levels of several apoptotic genes and tumor suppressors p53 and hTERT in SKOV3 cells (51).

Epidemiological studies

Incidence

There are few register-based epidemiological studies focusing on the incidence of ovarian cancer in metformin users (Table 3). In the study by Tseng, a lower incidence of ovarian cancer was reported in women with type 2 diabetes (T2D) who were metformin ever-users [adjusted hazard ratio (aHR) 0.66, 95% confidence interval (CI): 0.59–0.73] (53).

Table 3 Incidence of ovarian cancer in metformin users among women with type 2 diabetes

Authors, year	Design	Region and period	Patients	Reference group	Main results
Bodmer <i>et al.</i> 2011 (52)	Register-based case-control	UK 1995–2009	1,611 ovarian cancers of which 85 were in women with T2D and 41 in metformin users	Women with T2D and no prior metformin use	OR 0.38 (95% CI: 0.18–0.81) when ≥ 10 prescriptions of metformin OR 0.59 (95% CI: 0.25–1.41) when < 10 prescriptions of metformin
Tseng 2015 (53)	Register-based cohort	Taiwan 1998–2009	Cohort size 479,475 with 3,201 ovarian cancers of which 601 were in metformin users	Women with T2D and no use of metformin	aHR 0.66 (95% CI: 0.59–0.73)
Urpilainen <i>et al.</i> 2018 (54)	Register-based cohort and case-control	Finland 1996–2011	Cohort size 137,643 with 303 ovarian cancer cases	Women with T2D using other oral ADM	Full cohort aHR 1.02 (95% CI 0.72–1.45) Case-control aHR 0.91 (95% CI: 0.61–1.34)

T2D, type 2 diabetes; ADM, antidiabetic medication; OR, odds ratio; CI, confidence interval; aHR, adjusted hazard ratio.

However, in the study by Bodmer *et al.*, only long-term (more than 10 prescriptions) metformin use seemed to have an association with a lower incidence of ovarian cancer, although this result was based on only 25 ovarian cancer cases (52). In publications that have reported the incidence of several types of cancer, in the ovarian cancer subgroup no association was found between metformin use and ovarian cancer incidence (55,56). One of our own studies revealed no evidence of an association between metformin use and ovarian cancer incidence in women with T2D, not even with a cumulative metformin dose (54).

Prognosis

A few studies have shown a better prognosis of ovarian cancer in metformin users (57–59). In one study, favorable results were limited only to progression-free survival, and not seen in overall survival (60) (Table 4). However, the numbers of metformin users in these studies have been low, varying from 12 to 61 women. The more recent register-based study on ovarian cancer prognosis did not reveal an association between prediagnostic metformin use and overall survival (62). In one of our own studies, findings were also inconclusive as regards an association between metformin use and survival after ovarian cancer in women with T2D (63).

Clinical trials

There are few clinical trials designed to evaluate metformin

as therapeutic agent in ovarian cancer. At the moment, there are two recruiting and two completed clinical trials on ovarian cancer and metformin use (11). In a pilot study by Zheng *et al.*, there were 20 ovarian cancer patients without a diagnosis of T2D receiving metformin along with adjuvant chemotherapy (paclitaxel and carboplatin) in first-line treatment of ovarian cancer (64). In this study, metformin use did not improve progression-free survival (PFS). However, modulation of the IGF-1 signaling axis was seen in the metformin group. Khawaja *et al.* have published a study in which patients with advanced or refractory cancer types were treated with an mTOR inhibitor, temsirolimus, in combination with metformin (65). With combination therapy a promising response with few side effects was achieved when assessing all cancers together. However, there were only two ovarian cancer cases in this study.

Conclusions

In preclinical studies, metformin has been found to have several direct and indirect mechanisms which make it anti-mitotic, anti-angiogenic, anti-inflammatory and a cellular-energy-state modifier. These cancer preventing assets are well-studied in ovarian cancer cells. However, the results of epidemiological studies are not as well defined. There is not enough evidence to verify the role of metformin as an ovarian cancer preventive drug. In addition, association between metformin and survival after ovarian cancer is complex. Thus, it is unlikely that metformin has an

Table 4 Prognosis of ovarian cancer in metformin users among women with type 2 diabetes

Authors, year	Design	Country and period	Patients	Reference group	Main results
Romero <i>et al.</i> 2012 (60)	Hospital-based cohort	USA 1992–2010	341 ovarian cancers of which 44 were in women with T2D and 16 in metformin users	Women with T2D without metformin use	PFS: HR 0.38 (95% CI: 0.16–0.90), OS: HR 0.43 (95% CI: 0.16–1.19)
Kumar <i>et al.</i> 2013 (58)	Hospital-based cohort	USA 1995–2010	239 ovarian cancers of which 103 were in women with T2D and 61 in metformin users	Women without metformin use, also including women without T2D	OS: HR 0.45 (95% CI: 0.26–0.83)
Shah <i>et al.</i> 2014 (61)	Hospital-based cohort	USA 2004–2009	305 women without DM and 62 women with T2D of which 27 were metformin users	Women with T2D and no use of metformin	PFS: metformin use 10.1 months vs. non-use 10.3 months OS: metformin use 23.9 months vs. non-use 26.1 months
Bar <i>et al.</i> 2016 (57)	Hospital-based cohort	Israel 2000–2012	143 ovarian cancers of which 22 were in women with T2D and 12 in metformin users	Women with T2D without metformin use	RFS: HR 0.14 (95% CI: 0.00–0.52)
Wang <i>et al.</i> 2017 (59)	Hospital-based cohort	China 2011–2014	568 ovarian cancer cases of which 48 in metformin users	Women with T2D without metformin use	PFS HR 0.34 (95% CI: 0.27–0.67) OS HR 0.29 (95% CI: 0.13–0.58)
Garcia <i>et al.</i> 2017 (62)	Register-based cohort and nested case-control	USA 2007–2011	2,291 ovarian cancers of which 552 were in women with T2D and 172 in metformin users	Women without metformin use, also including women without T2D	OS: HR 0.96 (95% CI: 0.75–1.23)
Urpilainen <i>et al.</i> 2018 (63)	Register-based cohort	Finland 1998–2013	421 ovarian cancer cases of which 77 were in women who used only metformin and 100 were in women who used metformin combined with some other oral ADM	Women with T2D who used other oral ADM	OC mortality HR 1.15 95% CI: 0.74–1.79 Mortality from other causes HR 1.85 (95% CI: 0.44–7.73)

T2D, type 2 diabetes; DM, diabetes mellitus; ADM, antidiabetic medication; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; OS, overall survival; RFS, recurrence-free survival; OC, ovarian cancer.

anticancer role as monotherapy. Hopefully, ongoing clinical trials will more specifically clarify the role of metformin in ovarian cancer treatment in the near future.

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