Chapter

Antitumoral Effects of Metformin in Ovarian Cancer

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Abstract

In the last years, the antidiabetic drug metformin has received considerable attention in pursuing new drugs for anticancer treatments. Several reports have shown that metformin would have antitumor effects, not only attributable to its systemic effects but also due to direct effects on tumor cells. It has been proposed that metformin could be a suitable alternative for the treatment of gynecological cancers, such as ovarian cancer. This disease is characterized by high cell proliferation and angiogenesis potential, because ovarian cancer cells overexpress most oncogenic molecules including growth factors. The aim of the present chapter is to discuss the molecular mechanism by which metformin would affect tumor cells, with focus on epithelial ovarian cancer.

Keywords: metformin, ovarian cancer, cell proliferation, angiogenesis, growth factors, AMPK

1. Introduction

Metformin or 1,1-dimethylbiguanide is a derivate of isoamylene guanidine, a substance found in the plant *Galega officinalis* [1]. This drug is widely used in metabolic disorders as type 2 diabetes mellitus, metabolic syndrome, and gestational diabetes [2, 3]. Besides, metformin is used as a treatment for polycystic ovarian syndrome [4], which is characterized by the dysfunction of reproductive tissues such as the ovary and endometrium. In this context, metformin improves ovarian follicle dynamics and frequency of ovulation [5, 6], and it increases the expression of endometrial GLUT4 (insulin-regulated glucose transporter), which may improve endometrial physiology in these patients [7].

In the last decades, metformin has been studied in the context of cancer, especially after an initial report by Evans et al., performed with a Scottish database, who found that metformin intake reduces the risk of cancer in type 2 diabetic patients [8].

Type 2 diabetes and obesity affect a significant percentage of the world population [9, 10] whose food habits and lifestyle have been changing in the last decades. Both obesity and type 2 diabetes are pathologies associated with increased incidence and poor prognosis of ovarian cancer by several authors [11–13]. These observations could be explained because obesity and type 2 diabetes are characterized by molecular changes that could encourage tumoral transformation and progression, such as hyperinsulinemia, hyperglycemia, dyslipidemia, increased insulin-like growth factors (IGF), adipose tissue factors, and inflammatory components [14–19].

By its chemical nature, metformin gets into the cell through organic cation transporters (OCTs) and multidrug and toxin extrusion transporters [20]. Because metformin cannot be metabolized, almost its entirety is excreted by the kidneys; the plasmatic levels of this drug do not reflect its intracellular concentration, mainly by its high apparent volume of distribution and prolonged half-life [21, 22]. Therefore, metformin is accumulated in tissues, and its plasmatic concentration is probably lower than of organs that express OCT transporters. This observation supports most *in vitro* studies that use high concentrations of metformin to study its antitumoral properties. Importantly, these transporters are present in the ovary [23, 24], so ovarian cancer cells could be a target for metformin action.

2. Indirect antitumoral effects of metformin in cancer

It is discussed that metformin could display direct and indirect antitumoral effects. The systemic effects of this drug include the decrease of blood glucose and insulin levels by action in its classical target organs: liver, muscle, and fat tissues. In humans, metformin decreases the hepatic gluconeogenesis and the release of glucose from hepatic reserves, which produces an increase in the peripheral uptake of glucose and its metabolism, decreasing patients' hyperglycemia and hyperinsulinemia [1, 2, 25]. These conditions (hyperglycemia and hyperinsulinemia) favor tumoral growth and are associated with cancer incidence, by two possible mechanisms: (1) high availability of glucose for cancer cells and (2) high levels of insulin, which could act in insulin-like growth factor (IGF) receptors [14–16]. IGF/IGF receptors display an important role in the ovary, because 100% of the ovarian carcinomas express IGF receptors [26].

In fat tissue, metformin decreases the activity of lipogenic enzymes such as HMG-CoA reductase, acetyl-CoA carboxylase (ACC), and fatty acid synthase, decreasing the endogen production of cholesterol and the fatty acid synthesis [1, 27, 28]. This produces a decrease in the plasma levels of lipids in patients using metformin [29–32], which in addition to metformin-hypoglycemic properties, decreases the readiness of energy substrates of tumoral cells.

All these metformin-mediated changes impair survival and mitogenic signaling and decrease nutrient availability for ovarian cancer cells.

3. Effects of metformin in ovarian cancer

3.1 Direct effects of metformin in ovarian cancer cells: role of AMPK

Several studies have shown that metformin displays direct antitumoral effects. Most of these studies have been performed in ovarian cancer cell lines, where metformin impairs cell proliferation, migration, and angiogenesis potential and enhances the chemotherapy sensibility [33–36].

The direct antitumoral effects of metformin are commanded by metabolic changes in cancer cells. Because metformin is a drug with pleiotropic effects, several molecular targets at different levels of the tumoral cell have been described. One of the most studied targets for metformin is the adenosine monophosphate-activated protein kinase (AMPK), a key sensor of the energetic status of the cell [37], and it was described that metformin treatment can activate AMPK in *in vitro* and *in vivo* experiments of ovarian cancer models [33, 38]. The activation of AMPK occurs by increasing the AMP/ATP ratio [39] which exposes the activation loop of AMPK to be phosphorylated in the residue threonine 172 by serine/threonine kinases such as

liver kinase B1 (LKB1) [40]. Activated AMPK phosphorylates several proteins; the phosphorylation can either activate or repress protein function at the cellular level [41, 42]. Despite that an important part of the studies indicates that the antitumoral effect of metformin could be AMPK-dependent; in the absence of AMPK, metformin preserves most of its antitumoral effects [43], indicating that the mechanism of this drug is more complex.

3.2 Antiproliferative mechanism of metformin in ovarian cancer cells

One of the characteristic hallmarks of cancer cells is an increased cell proliferation. To do so, ovarian cancer cells overexpress several growth factors and its receptors, which produce an enhanced cell signaling related with survival and proliferation in these cells [44–46].

In ovarian cancer, growth factors can activate protein kinase B (AKT) and the extracellular signal-regulated kinase (ERK) signaling pathways, among others [47–49]. These signaling pathways are associated with an increase of cell proliferation in most kinds of cancer cells [50, 51]. Some studies have shown that metformin treatment decreases IGF-1 and insulin levels, in a mice model with ovarian cancer [51], and also metformin treatment blocks the pro-tumoral effects of the nerve growth factor (NGF) in epithelial ovarian cancer cells [35] or the insulin/IGF-I signaling in uterine serous carcinoma [52].

The activation by growth factors of AKT and ERK signaling in ovarian cancer cells induces the activation of mechanistic target of rapamycin complex 1 (mTORC1), which controls protein translation and cell growth [53–55]. It is described that metformin-activated AMPK inhibits mTORC1 signaling in ovarian cancer cells [56, 57], which could impair its cell potential to proliferate and fend it in unfavorable conditions. Additionally, one key point in the antitumoral effect of metformin is that AMPK decreases the signaling pathways mediated by AKT and ERK in several types of cells, including cancer cells [38, 57, 58]. These signaling pathways are associated with the increase of most oncoproteins, for example, the transcription factor c-MYC and the inhibitory apoptotic protein survivin (BIRC5) [59–62]. c-MYC is a proto-oncogene that controls several genes related with cell growth and cell proliferation, and some reports show that metformin decreases c-MYC protein levels in ovarian cancer cell lines [63, 64]. In addition, metformin decreases the mRNA levels of survivin in metastatic ovarian cancer cells [65].

According to current evidences, c-MYC controls the transcription and cell cycle inhibitors [66]. In agreement with the metformin-depending decrease of c-MYC in ovarian cancer cells, metformin induces the degradation of cyclin D1 [33, 38], a protein required for progression from G1 to S phase of the cell cycle, and increases p21 expression (a negative regulator of cell cycle) [67]. These results are consistent with experiments performed in primary ovarian cancer cell cultures and ovarian cancer cell lines, which show that metformin induces cell cycle arrest in the G0/G1 phase and decreases the percentage of cells in S phase of the cellular cycle [35, 68, 69]. These findings highly suggest that metformin decreases the progression of the cell cycle in ovarian cancer cells.

Even more, several authors have shown that metformin can elicit cytostatic or cytotoxic effects in ovarian cancer cells. A key point for a better understanding of these differences is that metformin inhibits tumor cell proliferation in the presence of glucose (with a cytostatic effect) but induces apoptosis in low-glucose conditions [70]. For example, ovarian cancer cells are more sensitive to metformin at concentrations of 2.5 millimolar than in 25 millimolar of glucose (found in culture conditions). This is a consequence of reactive oxygen species accumulation, which

increase cell apoptosis and endoplasmic reticulum stress and decrease of c-MYC protein levels [63, 70].

3.3 Effect of metformin in lipid metabolism of ovarian cancer cells

For cell proliferation, the cancer cell has high requirements of substrates for synthesis of structural components and signaling. One target of AMPK is the sterol regulatory element-binding protein 1 (SREBP1), a lipogenic transcription factor [71], which increases cellular biosynthesis of fatty acids and cholesterol by transcription of the enzymes ACC, HMG-CoA reductase, and fatty acid synthase [72], not only in fat tissue but also in ovarian cancer cells [73]. Because ACC is involved in the taxol-mediated cytotoxic effect of ovarian cancer cells [74], besides the fact that the inhibition of ACC suppresses ovarian cancer cell growth *in vivo* and *in vitro* [75], it is possible to conclude that ACC inhibition could contribute to an important part of the antitumoral effects of metformin.

3.4 Anti-angiogenic activity of metformin in ovarian cancer

Angiogenesis, defined as the generation of new blood vessels from preexisting ones [76], is an essential process to supply oxygen and nutrients to normal and tumoral ovarian cells. Unfortunately, this process is exacerbated in ovarian cancer cells, which overexpress some growth factors, such as vascular endothelial growth factor (VEGF) or NGF [77, 78] which promotes angiogenesis.

The relevance of metformin in the vascular context is recognized; however, its action depends on the cell type, metabolic status, and nutrient availability. For example, some pro-angiogenic properties have been attributed to metformin under hypoxia and hyperglycemia, similar characteristics to myocardial infarction in diabetic patients. In this context, metformin enhances endothelial cell survival, migration, and apoptosis inhibition [79, 80]; this strongly suggests that the use of metformin could be beneficial in the context of cardiovascular diseases in diabetic patients. On the other hand, metformin could have an opposite effect in endothelial cells under hypoglycemic conditions (as tumor endothelial cells), where metformin produces an inhibition of its cell proliferation and angiogenesis potential, as will be discussed later.

In the ovary, the correct formation and regression of blood vessels during each ovarian cycle is indispensable for proper follicular development, ovulation, and corpus luteum formation, so that angiogenesis displays a key role in ovarian homeostasis and pathogenesis [81]. In patients with polycystic ovary syndrome, an increased expression of VEGF is described, and it is hypothesized that part of the beneficial metformin-associated effects will be mediated by a decrease or normalization of its VEGF levels. For example, it is described that in a rat model with dehydroepiandrosterone-induced polycystic ovaries, metformin administration restores the ovarian-increased levels of VEGF and angiopoietin 1, both angiogenic factors [82]. In addition, women with polycystic ovarian syndrome who take metformin have decreased their levels of plasmatic endothelin 1 and plasminogen activator inhibitor-1 [83, 84], molecules that also promote angiogenesis.

The angioprotection is an antitumoral mechanism that has been explored in ovarian cancer. Considering that the most studied angiogenic factor is VEGF, a monoclonal antibody against VEGF called bevacizumab has been developed and was approved for the use in advanced stages of ovarian cancer [85, 86]. In ovarian cancer models, the main knowledge of anti-angiogenic characteristics of metformin comes from VEGF modulation. Several *in vitro* models have shown that metformin

decreases both VEGF mRNA and protein levels in ovarian cancer cell lines and then, its angiogenic potential [33, 64]. In a mice model with ovarian cancer, metformin decreases VEGF levels in plasma and ascitic fluid, with a consistent decrease of the ovarian tumor growth [51]. Interestingly, metformin reduces the vascular density (showed by CD31 staining) of ovarian cancer xenografts in mice, and metformin-/cisplatin-treated mice have significantly less vascular density than either metformin or cisplatin alone [33]. Because cisplatin/carboplatin and paclitaxel are drugs used in the first-line chemotherapy in ovarian cancer [87, 88], these results suggest that metformin could potentiate the anti-angiogenic effects of chemotherapy during ovarian cancer treatment.

On the other hand, metformin treatment (in millimolar concentrations) displays direct effects in the endothelial cells, by reducing cell proliferation in human umbilical vein endothelial cells (HUVEC) and endothelial progenitor cells [89, 90]. Similar results were replicated by our group where metformin decreases cell proliferation of the endothelial cell line EA.hy926, in a dose-dependent manner [35], as well as, the endothelial cell differentiation (**Figure 1**). These results suggest that metformin affects in a direct manner the angiogenesis potential of endothelial cells.

3.5 Posttranscriptional regulation by metformin in ovarian cancer cells

In the ovarian cell, posttranscriptional regulations control gene expression at RNA level [91]. The micro-RNAs (miRs) are short non-codificant RNAs that regulate the expression of approximately 60% of protein-coding genes of the human genome [92]. miRs bind to a messenger RNA target, producing its degradation or translational repression depending of complementary degree [93]. The machinery for expression, processing, and exportation of miRs depends on several proteins as RNAse III DICER and exportins [93]. It is described that DICER downregulation is an oncogenic event that enhances epithelial-mesenchymal transition (EMT) and metastatic dissemination in cancer cells [94]. An important antecedent is that metformin elicits anticancer effects through the sequential modulation of DICER and c-MYC in breast cancer cells, increasing oncosuppressor miRs [95]. These mechanisms have not been investigated in ovarian cancer cells; nevertheless, preliminary results from our group show that metformin increases the oncosuppressor miRs 23-b and miR-145 in the epithelial ovarian cells [96].

As already mentioned in point 3.3, the activation of AMPK by metformin produces an inhibitory phosphorylation of acetyl-CoA carboxylase, an enzyme that regulates lipid metabolism. Importantly, intermediaries of lipid metabolism participate in cell signaling and chromatin structure, modulating processes as cell histone acetylation that depends on cytosolic acetyl-CoA [97]. The decrease of the

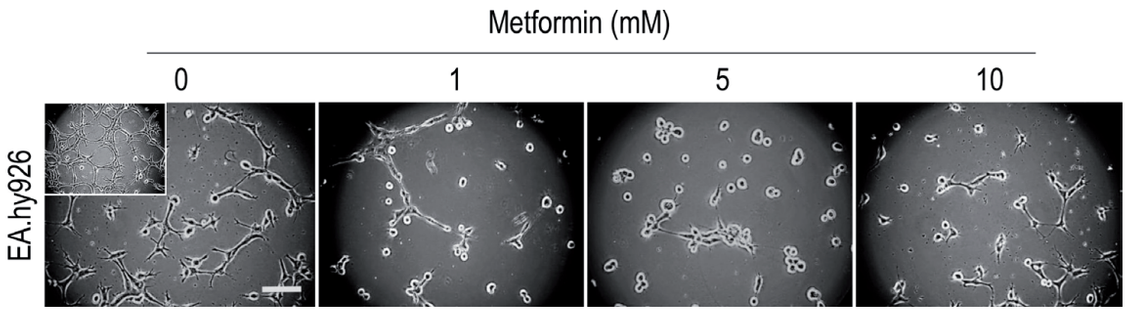

Figure 1.
Effect of metformin on the differentiation of endothelial cells. Metformin reduces the multicellular junctions and polygonal structures of endothelial cells EA.hy926 in a matrigel assay (4 h). Upper insert: positive control (NGF 100 ng/ml). Magnification bar: 50 μm.

conversion of acetyl-CoA to malonyl-CoA leads to an increase in the acetylation of histones in the chromatin and altered gene expression in ovarian cancer cells [67]. Because acetylation of nucleosomal histones is linked to nuclear processes as transcription, replication, and repair among other functions [98], it is possible that several antitumoral effects of metformin could be regulated by protein acetylation and transcriptional regulation of several oncosuppressor proteins.

The summary of the main studied antitumoral effects of metformin is shown in **Figure 2**.

3.6 Studies of metformin in diabetic patients with ovarian cancer

A recent meta-analysis shows that among available studies of relationship between metformin intake with ovarian cancer incidence and prognosis in diabetic patients, the majority of the studies indicate a negative correlation between the use of metformin and the incidence of ovarian cancer, as well as, a positive correlation with better prognosis [99]. The same study shows that metformin treatment in diabetic patients has a reduction of 24% risk of ovarian cancer occurrence and also a 42% of reduction in mortality [99]. The main studies that showed metformin benefits in the context of ovarian cancer diabetic patients are summarized in **Table 1**.

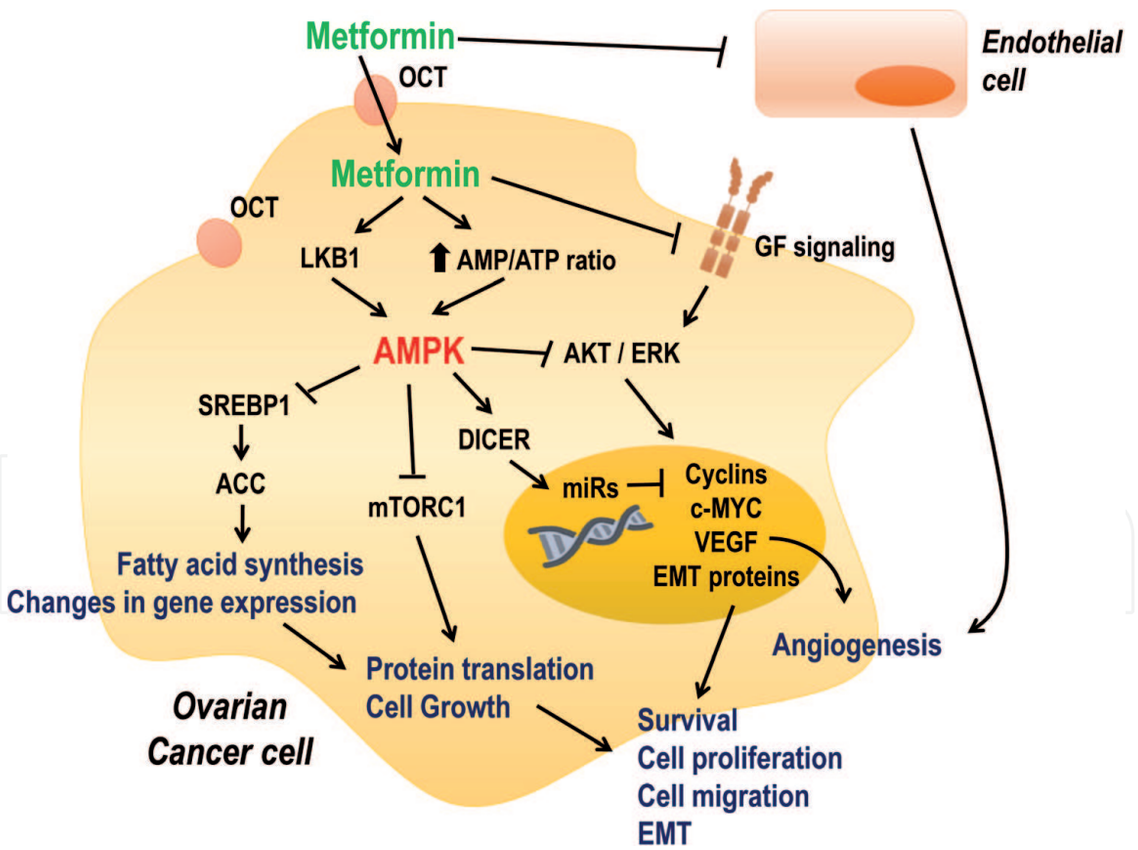

Figure 2.
Main antitumoral mechanism of metformin in ovarian cancer cells. Metformin enters the cell through organic cationic transporters (OCT) and produces the activation of liver kinase B1 (LKB1) and an increase of AMP/ATP ratio, which results in the activation of AMPK. This kinase has several targets as sterol regulatory element-binding protein 1 (SREBP) and acetyl-CoA carboxylase (ACC); the mechanistic target of rapamycin complex 1 (mTORC1) and AKT/ERK signaling; key proteins in the fatty acid synthesis and cell growth, survival, proliferation, and migration; and the processes of epithelial-mesenchymal transition (EMT). On the other hand, metformin can block the growth factor (GF) signaling dependent or independent of AMPK activation. Also metformin decreases the angiogenic potential of ovarian cancer cells, impairs the expression of vascular endothelial growth factor (VEGF), or acts directly on the endothelial cells.

Research	Study and population	Main finding
Wang et al. [12]	Retrospective cohort study N = 568, China	• Metformin group of OvCa patients had longer median PFS* than non-metformin, nondiabetic, and metformin-discontinued groups • Similar PFS* in dose (500 or 1000 mg of metformin) • Metformin treatment must be continuous to obtain beneficial effects
Bar et al. [114]	Retrospective cohort study N = 143, Israel	• Metformin was associated with a reduced risk of recurrence of OvCa (lower PFS*), and this association was stronger in diabetic patients
Tseng et al. [115]	Retrospective cohort study N = 479,475, China	• 601 metformin ever-users and 2600 never-users developed OvCa (incidence of 49.4 and 146.4 per 100,000 person-years) • Metformin use was associated with a decreased risk of OvCa
Kumar et al. [116]	Case-control study 72 cases (OvCa, metformin users), 142 controls (OvCa, non-metformin) USA	• Metformin was associated with a better survival in OvCa patients • 5-year DSS** was higher in metformin group • Metformin was an independent predictor of survival
Romero et al. [102]	Retrospective cohort study N = 341, USA	• Metformin group had a longer PFS* and overall survival of OvCa compared to nonusers or nondiabetic patients • Metformin group decreased hazard for disease recurrence
Bodmer et al. [117]	Case-control study 1611 cases (OvCa) and 9170 controls (non-OvCa), UK	Metformin use was associated with a decreased of risk of OvCa

**PFS: progression-free survival (length of time during and after the treatment of OvCa that a patient lives with the disease but it does not get worse).*
***DSS: disease-specific survival (percentage of people in a study or treatment group who have not died from OvCa in a defined period of time).*

Table 1.
Summary of studies that evaluated incidence and prognosis of ovarian cancer (OvCa) patients using and not using metformin.

Although several observational studies show positive effects of metformin in diabetic patients, it has not yet been elucidated if metformin could be beneficial in nondiabetic patients. In addition, ovarian cancer has a low incidence, and the number of participants in some of the available studies is low; therefore, the evidence should be interpreted with caution.

Because of the increased interest in the possible use of metformin in nondiabetic patients, there are currently six clinical trials inscribed in NIH ClinicalTrials.gov database to study metformin intake in association with carboplatin and paclitaxel (first-line chemotherapy) in nondiabetic woman with ovarian cancer (NCT02312661, NCT02437812, NCT03378297, NCT02122185, NCT01579812, and NCT02201381) from phase 0 to phase III of the study. The results of one of these trials show that metformin was well tolerated and the outcome results were favorable, because tumors from metformin-treated women have a threefold decrease in specific subpopulations of ovarian cancer stem cells with an increased sensitivity to cisplatin *in vitro* [100], supporting the use of metformin in the following phases of the study.

3.7 Role of metformin in metastasis and chemoresistance

Besides the abovementioned benefits, metformin treatment has a relevant role in the metastasis and chemoresistance prevention of several ovarian cancer models. For example, *in vitro* experiments have shown that metformin decreases the adhesion capacity, invasion, and migration of ovarian cancer cell lines [101]. In rodents, metformin treatment inhibits the growth of metastatic nodules in the lung product of ovarian cancer [33], and importantly, the use of metformin in diabetic women decreases the probability of disease recurrence [102].

The cancer stem cells, recently called "tumor-initiating cells," are a tumoral cell subpopulation with critical role in therapy resistance and metastasis [103–105]. There are several markers to identify them, as lactate dehydrogenase (LDH), aldehyde dehydrogenase (ALDH), or cell-surface antigens as CD44, CD133, or CD117 [106–108]. Metformin treatment decreases the abundance of ovarian cancer LDH+ and decreases its ability to form tumor spheres, an attachment-independent growth characteristic of these kinds of cells [109]. At the same time, a low dose of metformin (micromolar concentration) decreases the abundance of CD44+/CD117+ ovarian cancer cells selectively, whereas CD133+ or ALDH+ cell subpopulation were more sensitive to millimolar concentration of this drug [109, 110].

Another key point is that metformin decreases the expression of classical markers related with EMT. This process is necessary to confer an increased migratory capacity to tumor cells, participating in the intra-/extravasation and hence, in the tumor cell dissemination. In CD44+/CD117+ ovarian cancer cells, metformin treatment decreases snail2, twist, and vimentin protein levels (these are mesenchymal markers), increasing E-cadherin protein levels (a known epithelial marker) [110]. These observations are related with a study performed in diabetic patients with endometrial cancer, where in the biopsies of these patients using metformin were found increased levels of E-cadherin [111]. These findings suggest that metformin decreases the process of EMT in ovarian cancer cells, affecting preferentially tumor-initiating cells, which constitutes a relevant advantage, because this type of cells is not affected by traditional chemotherapy.

One important aspect in ovarian cancer treatment is the high percentage of chemoresistance developed by patients. In this context, metformin stands as a promising drug, since several studies showed that it could increase the susceptibility of ovarian cancer cells to chemotherapy and revert its acquired chemoresistance [34, 112, 113]. One recent study performed in ovarian cancer cell lines treated for 6 months with cisplatin and paclitaxel (for the acquirement of chemoresistance phenotype) shows that metformin treatment increases drug sensitivity and reduces migratory abilities of these ovarian cancer cells. In addition, the same study shows that metformin decrease the ovarian cancer stem cell population and the expression of specific biomarkers of pluripotent genes [112].

3.8 Main conclusions

Metformin is an antidiabetic drug that displays antitumoral effects in several *in vivo* and *in vitro* models of cancer, including ovarian cancer. The mechanism of its antitumoral effects could be either dependent or independent of AMPK, a key sensor of the cell energetic status. Metformin has several cell targets which include transcription factors and cell cycle regulators; wherewith it impairs cell proliferation by the arrest of the cell cycle. In addition, metformin modulates enzymes of metabolic pathways and lipid metabolism, as well as epigenetic and posttranscriptional regulation of the ovarian cancer cells, which can explain its pleiotropic actions. Another important point is that metformin regulates angiogenesis in

the ovarian cancer cells, mainly decreasing VEGF expression, which impairs the angiogenic potential of these cells. On the other hand, metformin acts directly in endothelial cells, decreasing its proliferation, migration and differentiation, which complement its anti-angiogenic effect.

An important niche for metformin treatment could be its selective effect in ovarian cancer cells with stem cell phenotype, which are responsible for ovarian cancer dissemination and chemotherapy resistance. Several studies show that metformin reduces ovarian cancer stem cells abundance and that it could have a chemosensitivity role when used in combination with first-line chemotherapy agents. This opens the possibility to the potential use of metformin as a coadjuvant agent in ovarian cancer treatment.

Finally, there are several observational studies in diabetic women with ovarian cancer which show that metformin is associated with less ovarian cancer incidence and better prognosis. However, it is important to consider that the number of participants using metformin in some of these studies is low and that several *in vitro* experiments have shown that metformin action depends on the metabolic context and nutrient and oxygen availability of ovarian cancer cells. For these reasons, the use of metformin in nondiabetic women with ovarian cancer should be considered with caution.

Currently, there are several clinical trials performed in women with ovarian cancer. These trials are studying the effect of metformin treatment together with standard chemotherapy in the ovarian cancer prognosis and clinic-pathological markers, which could be helpful to elucidate whether this drug could be considered as a coadjuvant alternative in the treatment of ovarian cancer.

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Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

ACC	acetyl-CoA carboxylase
AKT	activate protein kinase B
AMPK	adenosine monophosphate-activated protein kinase
ALDH	aldehyde dehydrogenase
EMT	epithelial-mesenchymal transition
ERK	extracellular signal-regulated kinase
HUVEC	human umbilical vein endothelial cells
IGF	insulin-like growth factor
LDH	lactate dehydrogenase
LKB1	liver kinase B1
mTORC1	mechanistic target of rapamycin complex 1
miRs	micro-RNAs
NGF	nerve growth factor
OCTs	organic cationic transporters
SREBP1	sterol regulatory element-binding protein 1
VEGF	vascular endothelial growth factor

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