Chapter

# Metformin in Cervical Cancer: Metabolic Reprogramming

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## Abstract

The reprogrammed metabolism plays a crucial role in intensively proliferating tumor cells to meet high energetic demands and adapt to metastasis and invasion. Metformin may counteract flexible metabolic phenotype of cervical cancer cells by restraining aerobic glycolysis (*Warburg effect*) and promoting mitochondrial-based metabolism. Metformin inhibits master oncogene c-Myc as well as hypoxia-inducible factor 1 (HIF-1α) and suppresses its downstream glycolytic regulatory enzymes and glucose transporters. Metformin targets bioenergetics of cervical cancer cells with aggressive phenotype and regulates the expression of enzymes controlling tricarboxylic acid cycle (TCA cycle) supplementation with substrates, glucose, and glutamine. The exposition of cervical tumor cells to Metformin alleviates their migratory capacity, restrains epithelial-to-mesenchymal transition (EMT) program implementation, and elucidates oxidative stress, which results in massive cell death due to apoptosis. The metabolic alterations caused by Metformin are specific to cancer cells. In summary, Metformin exerts antitumor effect in cervical cancer cells by regulating specific molecular targets in reprogrammed metabolism. Metformin selectively modulates metabolic pathways and thus may be potentially used in new precisely targeted therapeutic strategies for cervical cancer.

**Keywords:** Metformin, cancer, metabolism, metabolic reprogramming, *Warburg effect*, mitochondria, apoptosis, oncogenes, reactive oxygen species, epithelial-mesenchymal transition, targeted anticancer therapy



## 1. Introduction

The malignant transformation results in a specific rearrangement of metabolic processes called metabolic reprogramming of tumor cell. The altered metabolism causes a selective advantage to a transformed cell by facilitating its survival in a harsh environment and promoting the spread of tumor cells within the body.

Malignant cells very effectively adapt to high proliferation rate, metastasis, and invasion. Several molecular mechanisms were pointed out to drive such metabolic adaptation of cancer cells. The critical aspects of metabolic reprogramming in tumor cells substantially contribute to the *Warburg effect* [1], an increased catabolism of glucose to lactate in the presence of oxygen [2]. The altered metabolism of tumors results in elevated biosynthesis of macromolecules such as proteins, carbohydrates, and lipids and, in consequence, supports high proliferation rate of malignant cells [3].

In particular, the regulation of mitochondrial processes in cancer cells differs from normal counterparts, and it may be specific to the stage of tumor [4]. Therefore, cancer cells are sensitive to drugs that disrupt energy homeostasis, such as Metformin (1,1-dimethylbiguanide, Met) [5].

A generic drug, Metformin, has been widely used for treatment of *diabetes mellitus* in humans. However, it exerts pleiotropic effect in human organism. In particular, a great interest has been paid to Met, since retrospective analyses demonstrated that it significantly decreased the relative risk of cancer incidence in diabetic patients when compared with patients treated with other drugs. Clinical trials confirmed the epidemiological observations that Met exerted anticancer effects in humans [6]. It has been established that Met inhibits proliferation of various neoplastic cell lines in vitro, including breast, prostatic, colon, gastric, and cervical cancers [7, 8]. Currently, there is an intense ongoing research focused on molecular mechanisms behind these effects, since the implications of Met action in tumor cell are not completely understood [9].

To date, several molecular mechanisms were reported to play critical role in anticancer activity of Met. In particular, it was established that Met may affect energy metabolism of cancer cells by inhibition of complex I of mitochondrial electron transport chain (ETC) in mitochondria, which results in adenosine-5′-triphosphate (ATP) depletion and remodeling of the network of biosynthetic processes within the cell [9]. Met may act as an anticancer drug through the activation of the main energy regulator within the cell, adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) [7], and inhibition of mechanistic target of rapamycin complex-1 (mTORC1) [10] in tumor cells. Some of the pharmacological effects of Met seem to be independent of its action on glycemia homeostasis. Several reports demonstrated that treatment of tumor cells with Met results in cell cycle perturbations and apoptosis [11, 12]. The intracellular targets affected by Met were comprehensively reviewed by Ikhlas and Ahmad [9] and Pierotti et al. [13].

Along with the advent of human papillomavirus (HPV) vaccines, the primary prevention of cervical cancer has become more successful, but cervical malignancy still remains the significant cause of cancer mortality in women worldwide. Currently, chemotherapy using cytostatic drugs (mainly cisplatin, cis-dichlorodiammineplatinum (II)) is still the primal regimen, despite low specificity and substantial toxicity in patients [14].

Aerobic glycolysis has been recognized as the most common metabolic feature of malignant cells. The alterations in metabolism of cancer cells combined with the overexpression of oncogenes (c-Myc) and transcription factors (hypoxia-inducible factor 1a, HIF 1a) confer a great advantage to malignant cells to avoid apoptosis induced by reactive oxygen species (ROS). In this study we focused on the effects of Met on metabolism of metastatic cervical tumor cells. Based on recent data, we reported that Met inhibited glycolytic phenotype of aggressive cervical cancer cells by regulation of expression of oncogenes and their downstream proteins, which led to cellular death. Furthermore, Met regulated mitochondrial metabolism, especially via supplementation of tricarboxylic acid cycle (TCA cycle, Krebs cycle) with pyruvate and glutamine. Met, by targeting epithelial and mesenchymal markers of tumor cells, alleviated invasive properties of cervical cancer cells.

This review summarizes recent findings on Met and cervical cancer underscoring new implications of this drug in regulation of peculiar metabolism of tumor cells. We discuss new perspectives about targeting specific alterations in cervical tumor metabolic pathways using Met.

## 2. Metformin regulates metabolism of metastatic cervical cancer cells in vitro study

A growing evidence suggests that the screening for molecular targets for anti-cancer therapeutic treatments should take into account the existing differences in tumor cell phenotypes. Therefore, the metabolic effects exerted by Met were studied using SiHa cells (American Type Culture Collection, ATCC designation HTB-35) originating from aggressive cervical tumor, which acquired malignant characteristics [15]. The regulation of apoptosis pathways in HTB-35 (SiHa) cells highly reflects the specificity of cervical tumor in vivo [16]. HTB-35 cells, even unstimulated with cytokines, have mesenchymal-like characteristics, especially high vimentin expression, along with enhancement of cell scattering and ability to move [17]. Another cell line, C-4I cells (ATCC, designation CRL1594) with epithelial phenotype, was derived from primary in situ tumor [18]. HTB-34 cells (ATCC designation MS751) were isolated from metastatic site in lymph node [19]. HTB-35, C-4I and HTB-34 are human squamous cell cervical carcinoma lines and it is worth noting that squamous cell cancer is the most common cervical cancer and accounts for almost 80% of cervical carcinomas in patients [14]. HeLa human cervical cancer cells (ATCC designation CCL2), which have been extensively used in mechanistic studies, expressed epithelial traits and were derived from *adenocarcinoma* [8].

### 2.1 Metformin hampers the expression of oncogenes controlling glycolytic phenotype of cervical cancer cells under hypoxic and normoxic conditions and promotes apoptosis

The reliance on glucose supply is linked to the aggressiveness of malignant cells. Such reprogrammed metabolism makes migrating cancer cells more robust and independent of environmental conditions. The dysregulation of glucose metabolism is caused by alterations in functioning of several oncogenes. Malignant cells may gain metabolic plasticity by upregulation of only few oncogenes, such as c-Myc, p53, phosphoinositide 3-kinase (PI3K) and the mammalian target of rapamycin (mTOR) [20]. Additionally, the activation of transcription factors, such as HIF-1α, makes malignant cells more resistant to hypoxia (decreased oxygen level in microenvironment), which is one of the main factors affecting tumor growth [20]. The activation of HIF-1α is one of the crucial processes that promote glycolysis to generate ATP along with the decrease of mitochondrial pathways' activity in aggressive tumors. What is more, the migrating tumor cells may avoid oxidative stress by relying on glucose catabolism. As a result, tumor cells have higher chance to survive detachment from extracellular matrix (ECM), whereas normal cells undergo programmed death due to anoikis in the absence of attachment to ECM [21]. Following detachment from primary tumor bed and transportation to plasma and lymph, malignant cells may spread within the body and form secondary tumors. Therefore, the reprogrammed metabolism plays a crucial role in facilitating tumor metastasis.

We found that Met may regulate glycolysis in aggressive cervical cancer cells. The glycolytic phenotype of tumor cells is triggered mainly by a master regulator HIF-1α and its downstream proteins. Our study showed that Met alleviated the hypoxia-induced activation of HIF-1α, which was followed by decreased expression of HIF-1α downstream protein effectors in HTB-35 cells, as demonstrated in [22]. In particular, Met downregulated GLUT transporters (solute carrier family 2 member receptors, SLC2A), specifically GLUT1 and GLUT3. Additionally, Met inhibited the regulatory enzymes of the glycolytic pathway, hexokinase 2 (HK2), bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 (PFKFB4),

pyruvate kinase (PKM), and lactate dehydrogenase (LDH) (**Figure 1**). Met exerted greater effect on regulatory proteins in HTB-35 cells exposed to decreased oxygen level in the air than normal conditions.

Recent studies have reported that overexpression of c-Myc oncogene plays a significant role in the formation of cervical cancer. The enhanced expression of c-Myc is also of particular relevance to promoting invasive phenotype of cancer cells. What is more, the upregulated c-Myc may collaborate with HIF to effectively induce glucose and glutamine consumption in tumor cells. As a result, mitochondrial oxidative phosphorylation decreases. In particular, the upregulated c-Myc enhances glutamine catabolism in tumor cells, since the oncogene controls glutaminase (GLS) expression [23]. As measured using qPCR analysis, Met decreased *c-MYC* transcript level in HTB-35 cells [22], which was in compliance with inhibition of GLS protein expression [11]. The treatment of cervical tumor cells with Met decreased mRNA level for another c-Myc downstream protein, *CCND1* (cyclin D1), which regulates cell cycle progression [22]. Zhang et al. [24] reported that Met caused a substantial decrease of cyclin D1 expression in bladder cancer cells. The overexpression of oncogene cyclin D1 is positively correlated with chemotherapeutic resistance and apoptosis avoidance in squamous cell cancers [23]. The inhibition of *CCND1* expression in aggressive cervical tumor cells resulted in enhanced apoptosis [22].

Met triggered another pro-apoptotic mechanism in cervical carcinoma cells via regulation of Bcl-2 (B-cell lymphoma 2) protein family members' expression [22]. Bcl-2 proteins are key players in the regulation of mitochondrial-dependent programmed cell death. The activation of BAX protein leads to disruption of mitochondrial membrane potential and apoptosis, whereas Bcl-2 acts as an apoptotic suppressor. The counterbalancing pro- and anti-apoptotic effectors of Bcl-2 protein family play a crucial role in the regulation of the mitochondrial apoptotic cascade within the cell and constitute another important apoptotic checkpoint [25]. However, the disturbance of BAX/Bcl-2 pathway may result in the resistance to apoptosis by inducing compensatory mechanisms, thereby influencing the efficacy of some therapeutic regimens [26]. The exposition of cervical tumor cells to Met

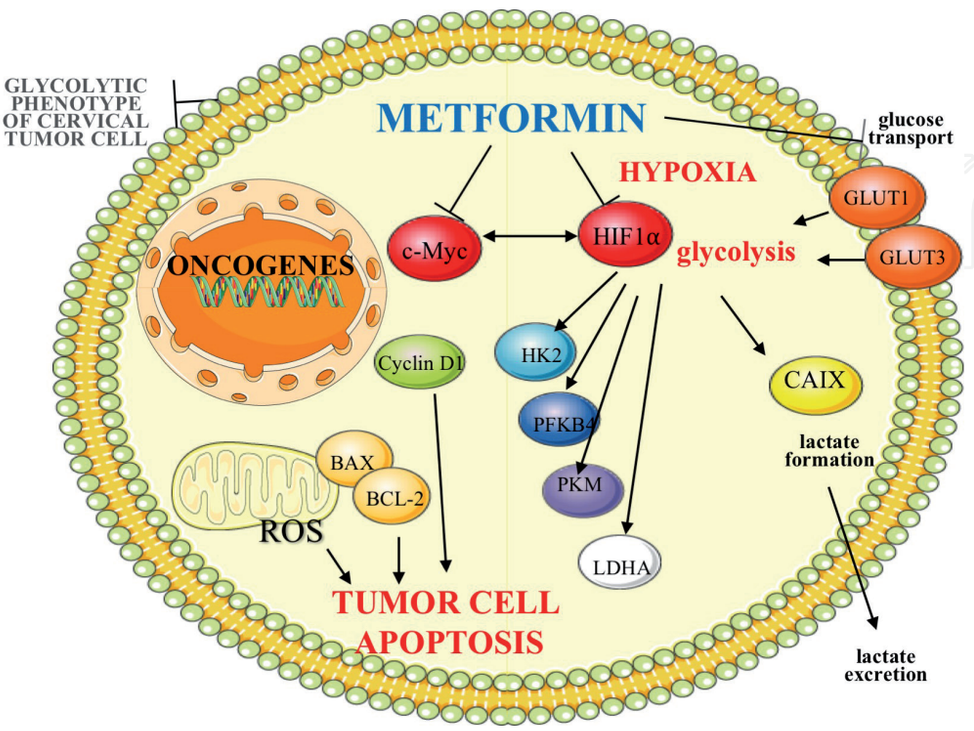


**Figure 1.**
*Metformin inhibits glycolytic phenotype of cervical carcinoma cells (↑—activation, ⊢—inhibition) [11, 12, 21, 22].*

significantly upregulated *BAX* transcript. It was found that the expression of *BAX* under hypoxic conditions was greater than in normoxia [22]. Additionally, Met downregulated transcript for *BCL-2* in HTB-35 cells in both, normoxic and hypoxic conditions.

The study using cervical cancer cells with metastatic phenotype cells showed that the downregulation of oncogenes/downstream regulatory proteins, together with the upregulation of pro-apoptotic BAX/Bcl-2, elucidated mitochondrial-dependent apoptosis in tumor cells. The obtained data suggest that Met was highly effective in facilitating cell death in cervical tumor cells [22], since it exerted its effect targeting independent events controlling mitochondrial apoptosis including the induction of ROS [11], the regulation of Bcl-2 protein family expression, and downregulation of cyclin D1. It should be emphasized that Met induced cell death solely in tumor cells, without causing detrimental effects to normal cells [11].

### 2.2 Metformin regulates TCA cycle supplementation in cervical cancer cells via pyruvate dehydrogenase (PDH) complex and generates oxidative stress in mitochondria

The reprogrammed metabolism of tumor cells not only meets high energetic demands but also provides intermediates for intensive proliferation. Therefore, glycolysis and mitochondrial oxidative phosphorylation may operate simultaneously in cancer cells. Many tumors may even switch between these pathways accordingly to the current requirements. Recent studies showed that most cancer cells have metabolically efficient mitochondria to provide intermediates for biosynthesis, generate reductive power (nicotinamide adenine dinucleotide phosphate, NADPH), and restore cofactor pool (e.g., nicotinamide adenine dinucleotide, NADH). In highly proliferating cancer cells, mitochondrial TCA cycle is active enough to sustain the biochemical reactions. Currently, the precise regulation of anabolic pathways and keeping their activities at adequate level is thought to play a key role in determination of "flexible" metabolic phenotype of cancer cells that enables their rapid division. Moreover, oxidative phosphorylation (OXPHOS) may represent a significant contribution to energy generation within malignant cell. On the other hand, inevitable products of OXPHOS are ROS and oxidative stress due to ROS overproduction may kill tumor cells [27].

It was demonstrated that the process of detachment of migrating squamous cancer cells from extracellular matrix (ECM) results in reprogramed metabolism toward glycolysis, particularly by PDH complex inhibition and following suppression of glucose respiration in mitochondria. Such metabolic phenotype of tumor cell enables efficient production of energy without excessive ROS generation. On the other hand, the stimulation of PDH activity may lead to increased anoikis sensitivity and attenuation of metastatic potential of cancer cells [28].

We found that Met may precisely regulate PDH metabolic checkpoint in cervical tumor cells (**Figure 2**). Met had great potency to activate oxidative decarboxylation of pyruvate to acetyl-CoA in HTB-35 cells expressing invasive phenotype, and it occurred via activation of PDH complex [11]. PDH complex plays a determinant role in the overall glucose disposal within the cell, since it funnels mitochondrial TCA cycle instead of lactate formation in cytosol. PDH activity is precisely regulated via covalent modification by the action of specific enzyme pyruvate dehydrogenase kinase (PDK). Several PDK activators were found to expand potent antitumor effect, also in cervical tumor HeLa cells [29]. We showed in aggressive cervical cancer HTB-35 cells that Met suppressed both PDK activity and the expression of gene encoding tumor-specific isoenzyme PDK1 [22]. This finding may have practical implications, since the screening strategy for PDK inhibitors should

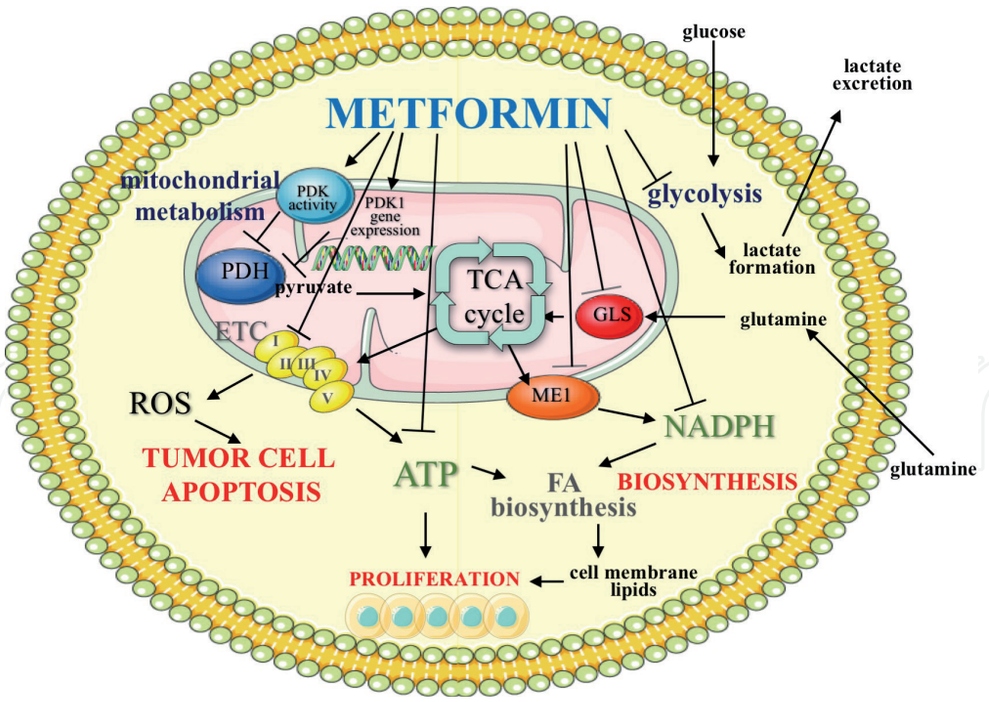


**Figure 2.**
*Metformin regulates mitochondrial metabolism of cervical carcinoma cells (↑—activation, ⊢—inhibition) [11, 13, 22, 27, 30].*

recognize the specificity among the PDK isoenzymes in order to avoid side effects in vivo [30]. Under hypoxic conditions inside tumors, the activation of HIF-1α decreases mitochondrial metabolism, which prevents the cell from oxidative stress and helps cancer cells avoid apoptosis [20, 23]. Our study showed that in aggressive cervical cancer cells Met counteracted these metabolic alterations by inhibiting PDK1, which is at the same time HIF-1α prime downstream effector. Furthermore, Met downregulated PDK1 gene expression also in normoxia [22].

In tumor cells that have functional mitochondria, the generation of oxidative stress may become an important therapeutic target [27, 30]. The imbalance of metabolic regulation and the resulting overproduction of ROS in mitochondrial ETC cause oxidative stress, which, at some point, becomes toxic to cancer cells, and that escalation of ROS elicits apoptosis-inducing factors and triggers death program through multiple mechanisms. In compliance, it has been newly reported that Met significantly increased ROS level, altered apoptosis-associated signaling, and induced cell death in human gastric adenocarcinoma cells [31] and human cervical cancer HeLa cells [32]. We found that in HTB-35 cervical cancer cells, Met caused excessive generation of mitochondrial ROS and elicited apoptosis [11, 22]. As shown in [22], the effect of Met was specific to tumor cells, and the formation of mitochondrial ROS was not affected in normal cells exposed to Met.

Met concomitantly targeted cytosolic glycolysis and mitochondrial pathways in HTB-35 cells, which increased apoptosis and suppressed survival of cervical tumor cells under normoxic and hypoxic conditions [22].

### 2.3 Met restrains glutamine entry into TCA cycle and inhibits cervical tumor cell proliferation

Glutamine may provide precursors to feed TCA cycle under limited flux of pyruvate from cytosolic glycolysis within tumor cells. The facilitated use of glutamine is a significant metabolic adaptation of cancer cell, besides enhanced glucose catabolism, and it provides intermediates sufficient for intensive biosynthesis and

energy production [20]. Glutaminase (GLS) is a key regulator of glutamine entry to TCA [33], and the inhibition of the enzyme may suppress tumor cell growth [25].

As shown in [11], the exposition of cervical cancer cells with invasive phenotype to Met downregulated the expression of GLS, thereby protecting mitochondrial anabolism from additional carbon supply for synthesis of macromolecules. Additionally, the effect of Met on GLS expression was specific toward cervical cancer cells, and in normal cells drug did not change the expression of the enzyme [11].

Glutamine entry to tumor cell not only improves carbon supply for macromolecules buildup, but it also replenishes the pool of cellular NADPH, since the conversion of malate to pyruvate catalyzed by malic enzyme 1 (ME1) is accompanied by the reduction of $NADP^+$ (**Figure 2**). NADPH is used for biosynthesis, but it also plays a significant role in the antioxidant protection of tumor cell by reducing glutathione molecule. Met downregulated expression of ME1 and alleviated generation of NADPH in cells, which, in conditions of limited supplementation of HTB-35 cells with glucose (suppressed expression of GLUTs), resulted in hampering of biosynthesis and alleviation of ROS detoxification [11, 22].

Furthermore, Met treatment caused acute drop in ATP concentration in HTB-35 cells. This is in compliance with data obtained by Parker et al. [34] who demonstrated that non-small cell lung cancer (NSCLC) cells may be uniquely sensitized to metabolic stresses by the action of other biguanide, phenformin (1-(diaminomethylidene)-2-(2-phenylethyl)guanidine). The inhibition of ATP generation may block biosynthesis in cervical tumor cells which results in restraining of cell proliferation.

### 2.4 Alterations of fatty acid (FA) de novo synthesis in cervical tumor cells upon exposition to Metformin affect cell proliferation

The facilitated fatty acid (FA) de novo synthesis together with upregulated glycolysis was recognized as one of the prime metabolic alterations in such tumor cells [35]. The enhanced FA biosynthesis meets high demands of rapidly proliferating malignant cells (generating components for cell membranes and signaling molecules). We found that Met decreased unsaturated lipid content in aggressive cervical cancer cells (**Figure 2**). The mechanism of Met action included downregulation of regulatory enzyme elongase 6 (ELOVL6), which catalyzes elongation of fatty acid molecule. Met also suppressed stearoyl-CoA desaturase (SCD1), which controls desaturation of FA. It was shown by Fritz et al. [36] that pharmacologic inhibition of SCD1 activity impaired unsaturated FA synthesis, which resulted in decreased proliferation of both androgen-sensitive and androgen-resistant prostate cancer cells. The treatment of cervical cancer cell lines [22, 37] with Met decreased cervical tumor cell proliferation, but Met did not affect the growth of normal cells [11].

### 2.5 Metformin inhibits epithelial-to-mesenchymal transition (EMT) process and migration properties of cervical cancer cells

Emerging data indicate that the enhanced activity of enzymes regulating lipid de novo synthesis may contribute to activation of EMT process in tumor cells [36]. The activation of EMT program in epithelial cancer cells facilitates tumor progression, invasion, and metastasis. It has been shown in independent studies that Met inhibits EMT in various cancer cell lines [8, 37]. Recently, it has been reported that Met reversed EMT phenotype induced with *transforming growth factor beta 1* (TGF-β1) in breast, lung, and cervical cancer cells by targeting the mechanisms regulating the

expression of E-cadherin. The exposition of tumor cells to Met resulted in suppression of their metastatic properties [8, 38].

In our study, EMT process was induced upon 48 h incubation of cervical cancer cells with 10 ng/mL of cytokine TGF-β1, as described in detail in [17]. HTB-35 cells, even unstimulated, expressed mesenchymal-like characteristics, and the incubation with TGF-β further enforced expression of mesenchymal marker, vimentin, along with enhancement of cell scattering and ability to move [17]. The study showed that Met was an effective suppressor of mesenchymal phenotype and, in particular, downregulated vimentin in HTB-35 cells (**Figure 3**). Recently, it was reported by Laskov et al. [39] that Met downregulated the expression of vimentin in endometrial cancers in vitro and in vivo in diabetic patients. The incubation of cervical cancer cell lines with Met reduced cells' ability to move, as shown using functional scratch test in C4-I and HTB-35 cells stimulated with TGF-β1 [17]. Mechanistic study revealed that Met inhibited the expression of transcription factors Snail-1, ZEB-1, and Twist-1. These mesenchymal markers facilitate EMT progress in cervical cancer cells.

Cheng and Hao [8] proposed another mechanism of Met action in cervical carcinoma cells via inhibition of mTOR/p70s6k signaling pathway and downregulation of glycolytic regulatory protein pyruvate kinase, isozyme M2 (PKM2), in HeLa cell line.

In order to clarify the molecular action of Met in cervical tumor cells with aggressive characteristics, the effect of the drug was tested in the hypoxic conditions. In cervical cancers, hypoxia and concomitant enhanced lactate formation result in acidification of microenvironment, which may promote the ability of metastatic cells to rapidly spread in tissue [41]. In such conditions, the activation of HIF1α induces its downstream protein carbonic anhydrase IX (CAIX). By regulation of tumor milieu pH, CAIX acts as a survival factor protecting malignant cells

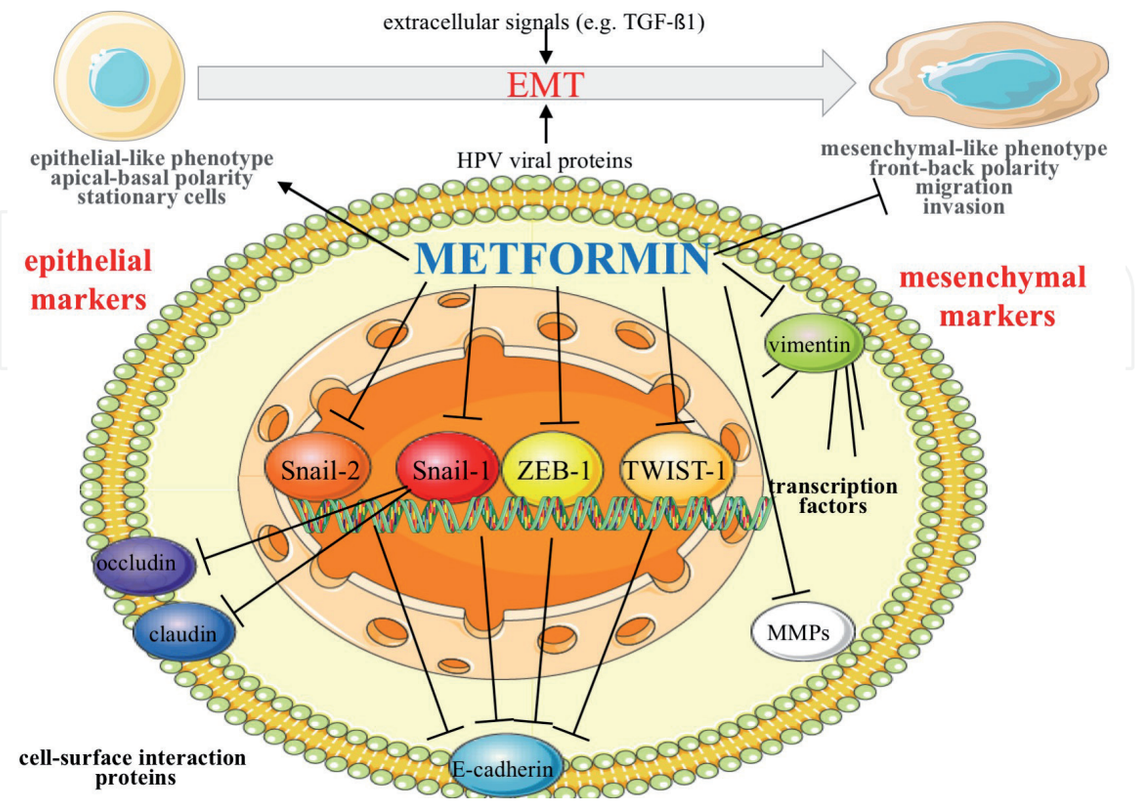


**Figure 3.**
*Metformin inhibits TGF-β1-induced EMT phenotype of cervical carcinoma cells (↑—activation, ⊢—inhibition) [8, 17, 40].*

against enhanced acidification of microenvironment. As a result, lactate damages adjacent normal cells and does not harm tumor cells [42]. Due to its relevant role in cell invasion, CAIX was proposed as a potential therapeutic target, also in cervical cancers [41, 42]. We showed that the exposition of HTB-35 cells to Met under hypoxia suppressed HIF-1α, which resulted in decreased transcription of *CAIX* gene, thereby alleviating invasive properties of cervical malignant cells [17].

## 3. In vivo findings related to the effect of Metformin

Recently, numerous beneficial activities of Met were reported. Met was shown to improve cardiovascular outcomes in humans [43], and the ability of Met to extend life-span in mammals has attracted great attention [44]. Emerging data indicate that Met may be applied as adjuvant in therapies aiming at combating diseases with high mortality rate, also in cervical cancer [45]. The clinical benefits of the use of Met in gynecologic oncology in humans were reviewed by Irie et al. [46] and Imai et al. [47]. Met also reduced the incidence of endometrial tumors and improved survival of patients with diagnosed local or advanced endometrial cancer [48]. Several clinical trials showed the potential of Met to elicit apoptosis in the uterus and prostate cancers in humans [49].

The potential pathological effects of Met have been well studied in long term in human population. One of the most undesirable effects in the context of peculiar metabolic alterations of cancer cell is the enhanced generation of lactic acid caused by biguanides. In fact, the application of phenformin (1-(diaminomethylidene)-2-(2-phenylethyl)guanidine) was associated with a much higher risk of lactic acidosis in patients, than Metformin. Therefore, the former drug was withdrawn from clinical use. Currently, the contraindication for the use of Met in patients is renal failure, since this group has greater risk of lactic acidosis. However, the concerns over lactic acidosis were shown to be largely unfounded, unless kidney disease was advanced. Yet, based on the recent data, Met can be safely used in patients with mild renal dysfunction, provided that patients are monitored appropriately [43, 50].

## 4. Conclusions

The exposition of aggressive cervical cancer cells to Met restrained the function of HIF-1α master regulator and downregulated HIF-1α downstream glycolytic genes. Met also downregulated glycolytic phenotype of HTB-35 cells through inhibition of oncogene *c-MYC* expression, which resulted in impairment of metabolic plasticity of cervical tumor cells, especially via downregulation of GLS.

Met precisely regulated PDH and GLS metabolic checkpoints in cervical tumor cells. In particular, in tumor cells Met targeted supplementation of mitochondrial pathways in pyruvate by downregulation of PDK1 gene expression and decreasing PDK activity. As a result, Met effectively enhanced TCA cycle flux in normoxic and hypoxic conditions. The downregulation of GLS and ME1 resulted in decreased regeneration of NADPH, the factor essential both for biosynthesis and cell protection against oxidative stress. The metabolic alterations of mitochondrial pathways caused by Met caused excessive generation of ROS which led to apoptosis. In cervical cancer cells, Met additionally induced apoptosis via upregulation of pro-apoptotic BAX protein expression and by downregulation of cyclin D1, oncogene *c-MYC* downstream protein. Met exerted its pro-apoptotic effect both in normal and decreased oxygen availability. This aspect of Met action may be important

when designing anticancer therapies targeting cells in hypoxic milieu inside solid tumors.

It is also important to highlight another cellular mechanism of Met action, namely, the suppression of EMT process in cervical tumor cells. EMT seems implicated into invasiveness and metastasis of cancer, and Met was able to inhibit EMT pathways. In cervical tumor cells stimulated with TGF-β1 as well as in unstimulated ones, Met decreased the expression of the main mesenchymal marker vimentin and reduced motility of cells. In addition, Met downregulated adaptive enzyme *CAIX* in tumor cells under hypoxia. CAIX promoted migration of malignant cells and acted as an important survival factor, and thus it has recently been proposed as therapeutic target in cervical cancers. Met might be considered as a potential factor targeting CAIX to hamper cervical tumor invasiveness.

These findings provide a new insight into regulation of glycolysis and mitochondrial pathways in cervical tumor cells using nontoxic and well-studied drug, Metformin, indicating the future prospect about utilization of this molecule in clinical oncological routine. The identification and targeting of specific alterations in tumor metabolic pathways may constitute a sole basis to design new precise therapeutic strategies in cervical malignancy. To date, very few innovative therapies against cervical malignancy are being tested in clinical trials; thus more specific and effective intervention is highly required.

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