Metformin: A Rising Star to Fight the Epithelial Mesenchymal Transition in Oncology

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Abstract: Metformin is a biguanide derivative which is widely prescribed as an oral drug for diabetes mellitus type 2. This old molecule has recently received a new attention because of its therapeutic properties in oncology, that seem to be independent of its action on glycemia homeostasis. The reappraisal of its pharmacological effects was supported by delineation of signaling pathways and more recently clinical trials. Numerous epidemiological studies showed that diabetics have an increased risk of several types of cancer and cancer mortality. Complex relationship between cancer and type 2 diabetes is going to be unraveled and recent observations revealed a significant action of metformin, but not other anti-diabetic agents, on cancer cells. As metformin may act as an anticancer drug through inhibition of mTOR, it might have greater benefit than suggested by insulin lowering alone. This review summarizes major publications on the link between cancer and metformin underscoring new implications of this chemical drug in oncology field. New perspectives about utilization of this molecule in clinical oncological routine, are described, particularly for patients without disturbance of glucose homeostasis. As the epithelial mesenchymal transition (EMT) seems implicated into invasive process and metastasis in cancer, and as metformin is able to inhibit EMT pathways, it is important to highlight cellular mechanisms of metformin.

Keywords: AKT, AMPK, cancer, clinical trials, dedifferentiated circulating tumor cells, diabetes, EMT, epidemiology, insulin, LKB1, metformin, mTOR, neoadjuvant therapy, S6KB, Stemness.

INTRODUCTION

Metformin (N,N-dimethylimidodcarbinomimidic diamide - IUPAC) is a N,N’-dimethylbiguanide, which belongs to the class of nitroenones. It was extracted from Galega officinalis (goat’s-rue or French lilac). As infusion, this plant was used in Middle Age to fight polyuria. Metformin is widely prescribed for type 2 diabetic patients and was approved in 1970’s in Europe and in 1995 in the United States. It has a wide variety of indications like: polycystic ovarian syndrome, metabolic syndrome management and diabetes prevention in high risk populations. The molecule was described as a product of N’, N’-dimethylyguanidine synthesis by Werner and Bell [1]. Metformin is a safety drug. The most common described side effects are diarrhea and dyspepsia. Renal function must be controlled before starting metformin as it can lead to lactic acidosis for patient with kidney deficiency. Phenformin and buformin were withdrawn from clinical use as they are less suitable for routine treatment due to the higher rates of lactic acidosis. Two galenic metformin formulations are available, immediate release, slow or extended release, the latter avoiding the most common gastrointestinal side effects. Metformin primary actions are neoglucogenesis inhibition, reduction of insulin resistance in peripheral tissues and then increased glucose uptake in skeletal muscle. It may exert its effects by activation of AMP-activated protein kinase (AMPK) able to inhibit neoglucogenesis during cellular stress. This old molecule received particular attention for its potential beneficial effects on fighting cancer cells independently from its role in diabetes [2-7]. This review depicts reasons for which metformin emerges today in oncology, the signaling pathways by which it acts, its potential mechanisms on cancer cells and its impact on epithelial mesenchymal transition (EMT).

METFORMIN AND CANCER

For more than 10 years medical literature has indicated 1) a relation between diabetes and cancer 2) a worse prognosis for diabetic patients with cancer than for non diabetic patients. Type 2 diabetes and development of cancers, particularly epithelial tumors localized to breast, pancreatic, colorectal, urinary and genital tractus, are linked [8-13]. To the opposite, studies reported a reduced risk for cancer prostate [14]. In breast cancer, epidemiological landmark studies noted prevalence of type 2 diabetes in newly diagnosed cancer patients (estimated from 8 to 18%) [15]. Moreover, compared with their non diabetic counterparts, patients with breast cancer and pre-existing diabetes have a greater risk of death [16,17]. Clinical and epidemiological incidences have underlined hyperinsulinemia, and insulin resistance as factors connected to poor cancer outcomes [18]. Hyperinsulinemia (exogenous insulin or secondary blood increased insulin) can directly promote tumorigenesis through the insulin receptor in epithelial tissues or indirectly acting on other modulators: insulin like growth factors, sex hormones [19, 20].

All these studies led to examine effect of treatment with oral drugs and or insulin on the development of cancer. Then, metformin seemed to be a new anticancer agent. Evans et al showed a lower risk for several cancers in type 2 diabetes patients treated with metformin rather than other diabetes medications [21]. Apart from previous epidemiological studies about cancer incidence and diabetes, new results have established that metformin may reduce cancer risks in metformin treated patients and enhance efficacy of chemotherapy. This new paradigm is based on numerous publications. In such a way, Bodmer et al demonstrated that long term metformin use is associated with decreased risk of breast cancer [22]. They identified case patients with a recorded incident diagnosis of breast cancer and they found decreased risk of breast cancer in women under metformin for several years. No such effect was seen for short-term metformin or sulfonylureas or other antidiabetes drugs utilization. These results were confirmed by the work of Libby et al who described that users of metformin are at low risk of incident cancer [23]. Many authors implemented this new aspect of metformin prescription [24-28]. When early-stage breast cancer patients received neoadjuvant chemotherapy and metformin, Jiralerspong et al showed a higher pathologic complete response rate. Upon 2529 patients with breast cancer who received neoadjuvant chemotherapy, the rate of pathologic complete responses was 24% in the metformin group, 8% in non metformin group and 16% in the non diabetic group. Even if comparison of
pathologic complete response rates between metformin and non-diabetic groups did not meet significance, it trended toward [29]. All these data strongly support the clinical development of metformin as an anticancer agent. Currently there are numerous ongoing clinical trials. Results in neoadjuvant breast cancer therapy indicated that metformin may potentiate classic chemotherapy [30]. It remains to be proved the relevance of metformin therapy to non-diabetic patients [31]. Hadad et al proposed to test metformin in a preoperative window of opportunity [32]. Yurekli et al suggested to introduce metformin in adjuvant treatment of Her-2 positive breast cancer [33].

**MODULATION OF AMPK ENERGY CELL SENSOR BY METFORMIN**

AMP-activated protein kinase (AMPK) is an energy sensor involved in the regulation of cell metabolism. Its activation is depending on the cellular AMP/ATP ratio. AMPK signal acts on its downstream regulators mTOR/S6K1. This pathway is a central one for the regulation of cellular energy. Its activation leads to switch on catabolic pathways (fatty acid beta oxidation, glycolysis) and inhibits ATP consumption (gluconeogenesis, protein and fatty acid synthesis and cholesterol biosynthesis) [34].

When AMP increases, AMPK is allosterically activated by phosphorylation of its catalytic α subunit by the upstream kinase LKB1. Secondary, AMP prevents dephosphorylation of AMPK by phosphatases [35].

Metformin leads to increased cellular AMP/ATP ratio, by disrupting mitochondrial complex I [36]. Thus, nitrogen species are generated, activating PKC which phosphorylates LKB1 leading to its nucleocytoplasmic translocation and activation of AMPK [37]. Metformin activates AMPK by at least two LKB1 dependent mechanisms [38]. Uptake of glucose and its utilization by skeletal muscle inhibits hepatic gluconeogenesis and decreases insulin resistance. Type 2 diabetes is characterized by relative insulin deficiency due to reduced tissue responsiveness to insulin, this is known as insulin resistance. Metformin lowers insulin level in the blood. These metabolic actions are mediated by activated AMPK on the mammalian target of rapamycin (mTOR). Activated AMPK induces phosphorylation of TSC2 (part of the heterodimeric tuberous sclerosis complex TSC including TSC1 and TSC2) [39]. Then TSC2 stimulates its GTPase activity toward Rheb [40]. Then the signaling pathway Rheb/mTOR is blocked, acting on mTORC1 (rapamycin-sensitive mTOR complex formed by the associated proteins : raptor, mLST8, PRAS40, and deoptor) [41]. Thus, S6K1 as well as 4E-BP1 (transcription factors), the down-stream effectors of mTORC1, are inefficient. By lowering insulin levels (as an indirect effect) metformin reduces the PI3K/AKT signaling pathway involved in cell survival [42, 43]. Metformin can also directly target mTOR independently of AMPK and TSC2 [44]. Mechanisms and pathways are summarized in Fig. (1a).

**POTENTIAL MECHANISMS OF METFORMIN ON CANCER CELLS**

Reported mechanisms include effects of the drug on cell growth, cell cycle, mTOR pathway, blood insulin level and finally on epithelial mesenchymal transition (EMT), a major key for cancer cell survival.

**Effect on Cell Cycle**

Metformin was able to inhibit breast cancer cells in vitro, in an AMPK-dependent manner, this action being associated with mTOR activation decrease. Many studies provided informations on the mechanisms by which metformin acts along with the arrest of cell cycle (G0/G1 or S phase arrest) and particularly leads to decrease cyclin D1. [45-48]. Cell division can be inhibited by tumor suppressor p53 when phosphorylated by AMPK leading cells, under bad nutrition conditions, to apoptosis [49-51].

Buzzai et al showed that p53 deficient cells and not p53 wild type are sensitive to metformin [52]. Others have shown that cell lines with or without functional p53 displayed similar sensitivity to metformin [45]. Moreover Ben Sahra et al indicated that the combined use of metformin and 2 desoxy glucose leads to p53 and AMPK mediated apoptosis [53] and that metformin acts independently of the p53 status on cell proliferation [54].

**Effect on Tyrosine Kinases**

Simultaneously to the suppression of mTOR activity, several protein kinases at once are inhibited. This is particularly true for the oncoprotein HER2. Vazquez-Martín et al demonstrated that HER2 oncoprotein itself may represent a key cellular target involved in the anti breast cancer action of metformin. They demonstrated that suppression of HER2 overexpression, under metformin, appears to occur via direct inhibition (AMPK independent) of S6K1 activity [55]. In the same manner, EGFR is inhibited in pancreatic cancer cells [56]. It becomes obvious that PI3K/mTOR/S6K1 axis is central to the occurrence of lapatinib (tyrosine kinase inhibitor) resistance in HER2 overexpressing breast cancer cells. Induced activation of AMPK has been described to suppress mTOR enzymatic activity and survivin protein synthesis involved in apoptosis [57-59]. By these effects metformin can reverse resistance to lapatinib.

**Effect via Insulin**

Beside the direct action (insulin independent) of metformin on AMPK/mTOR/S6K1 axis, other effects are indirect (insulin dependent) [60]. Effectively the reversal of hyperglycemia, the decrease of both insulin resistance and hyperinsulinemia may play a major role in anticancer activity of metformin. Insulin, a growth promoting hormone, has mitogenic, antiapoptotic and proinvasive activities on tumor cells. Insulin may bind and activate IGF1 receptors ([IGF-1R], which have much more potent mitogenic and transforming activity than the insulin receptors (IR). Furthermore, cancer cells often express high level IGF-1R and IR, underlining potential sensitivity to the growth promoting effects of insulin [61, 62]. Thus metformin by this indirect way may decrease the negative impact of the hormone on tumor development, as the flux through IRS1-AKT/mTOR/S6K1 axis is slowed down.

**Aging, Cancer and Metformin**

Metformin appears to have potent cancer chemopreventive properties by triggering DNA damage response signaling [63,64]. Metformin would be able to induce specific senescence like growth inhibition of premalignant cells or malignant cells. Cellular senescence would be a restricting cancer progression mechanism. Avoiding these safeguards, tumors can become immortal and proliferate. Early studies by Warburg demonstrated that cancer cells preferentially metabolize glucose by glycolysis although this pathway generates less ATP than the process of mitochondrial respiration [65]. Metformin able to inhibit the glucose flux, while stimulating lactate/pyruvate flux and mitochondrial biogenesis, causes ATP depletion and drastic increase of cellular AMP [66]. This modification of AMP/ATP ratio might induce an inhibition of mTOR via AMPK activation. Experimental data supported the fact that metformin slows aging rate, increases life span, inhibits tumor development of HER-2/neu transgenic mice [67-70]. Many studies are warranted to elucidate the relationship between senescence and cancer. The role of metformin in these pathological status must be highlighted.

**Metformin and Triple Negative Breast Cancer**

A distinct group of breast cancers called triple negative cancers (TN) fails to express HER2 estrogen and progesterone receptors. They have stem like and or mesenchymal features. They are frequently chemoresistant. Recent observational studies have shown that metformin had unique anticancer effects against TN. Thereby,
Liu et al reported that metformin inhibits cell proliferation, colony formation, and induces apoptosis. Two of the molecular effects in TN cells: reduction of cyclin D1 and activation of AMPK have been reported in other breast cancer subtypes. Specific effects in TN reported by these authors are induction of apoptosis, proteolytic cleavage of PARP, activation of caspase -3, -8 and 9, reduction of EGFR, P-Src, P-MAPK and cyclin E in dose and time dependent manner [46]. These results, arising from experiments on TN cell lines, seem not to be correlated to clinical trials. Effectively, Bayraktar et al in a retrospective study showed that metformin use during adjuvant therapy was not associated with improved survival outcomes. However they indicated a trend toward a decrease in the risk of developing distant metastases in diabetic patients receiving metformin [71]. TN cells are particularly sensitive to metformin. Deng et al demonstrated that the drug targets Stat3 to inhibit cell growth and induce apoptosis in TN. Prolonged upregulation of activation of Stat3 has been associated with TN characteristics. This activation has diverse procarcinogenic effects and particularly TGFβ-associated EMT. Thus, metformin down regulates Stat3 expression and or activity in TN [72]. These findings deserve to be tested with more studies.

**EMT and Metformin**

During the last ten years a new concept sparkled in cancer biology: involvement of an epithelial mesenchymal transition (EMT) in the metastatic dissemination of epithelial cancer cells. EMT endows cancer cells with stem cell properties. However there is still a too large gap between the concept and its clinical development. The EMT is a process in which epithelial cells lose their polarity and acquire migratory properties [73]. EMT was first noted during embryogenesis to drive the transformation of epithelial cells into mobile mesenchymal cells that travel to distant anatomical sites. This process normally tightly regulated is abnormally disrupted and activated during cancer metastasis and recurrence. It is considered to promote cancer cells progression and invasion into the surrounding environment [74]. As EMT may be sufficient to trigger ontogeny of cancer stem cells, Vazquez-Martin et al studied how metformin can act on the expression of key EMT drivers of the EMT machinery [75]. Based on the work of Liu et al, who reported that metformin has unique effect against TN breast cancer cells [46] they investigated whether the response of basal like breast cancer cells to metformin might relate to its ability to suppress the expression of key EMT drivers including ZEB1, TWIST1, SLC7A11, and pleiotropic cytokines TGFβ;87,88] thus eliminating the breast cancer stem cell phenotypes in cell populations bearing either
mesenchymal or epithelial markers. Their findings demonstrated that metformin down regulates the expression of several EMT controllers. Among them, they underlined TWIST1 and ZEB1 that regulate invasion, motility and senescence [89-91]. Metformin prevented generation of cancer stem cell phenotype by down regulating some EMT regulators (“EMT status”) independently of changes in EMT functioning (“EMT phenotype”). Moreover, Brown et al showed that a switch in CD44 alternative splicing is required for EMT. Additionally the CD44s isoform activated AKT signaling providing a mechanistic link to a key pathway that drive EMT [92]. All these data provide arguments to clinical development of metformin as a therapy able to forbid ontogenesis of cancer stem cells. The molecular action of metformin can also be explained by up regulation of tumor suppressive miRNA let-7a and miRNA-96 and down regulation of oncogenic miRNA181a. This can be the key preventing self-renewal of cancer-initiating cells arising from EMT [93]. EMT in human epithelial cancers was evidenced by detection of dedifferentiated circulating tumor cells showing mesenchymal and or stem characters [94-95]. Fig. (1b) indicated how metformin could act in breast cancer and target mesenchymal cells.

**METFORMIN DOSING**

Recommendations of prescription in type 2 diabetes are the following: the starting dosage of metformin is 500 mg twice daily. The maximum dosage for children age 10 to 16 is 2000 mg, and for adults age 17 and older is 2550 mg. The dose should be only increased when necessary and slowly in such a way to avoid side effects. The dose is increased by up 500 mg every week to reach the 2.50 g maximum. Extended-release metformin (long-acting) improves gastrointestinal tolerability and allows once-daily dosing. Total daily doses of metformin have been largely described for type 2 diabetes. Generally, dosing is choosed to decrease glycemia and blood HbA1c levels. However these prescriptions were established for diabetic patients but not for people safe of disrupted glycemia regulation. When looking at concentrations of metformin reported in oncology experiences, as well in vitro on cancer cell lines as in vivo in animal experiments, a wide concentration range was used. Based on these data, it seems us necessary to determine metformin dosing in cancer patients. Clinical trials could highlight the problem of metformin dosing in oncology.

**METFORMIN SIDE EFFECTS**

**Toxicity**

Metformin has been used for over 50 years and seems to be a safety drug. The most frequent adverse events are gastrointestinal symptoms [96, 97]. Digestive disorders (diarrhea, vomiting) represent the most common metformin side effects (around 30%) which are usually minor and transient and can be avoided by utilization of sustained release galenic formulations. In healthy individual metformin affects glucose, vitamin B12 and the digestive uptake of bile salts [98]. Long term metformin prescription has been shown to lead vitamin B12 malabsorption [99]. It has been suggested that this side effect may increase cancer tissue toxicity of adjuvant chemotherapy [100]. The only toxicity which has been described is lactic acidosis. The presence of clinical conditions, such as renal failure, increases the risk of metformin-associated lactic acidosis. This metabolic event is really very rare (4.3/100000/year) [101]. Although very unusual, one must be aware that carboplatin can cause mitochondrial toxicity and trigger lactic acidosis. Its occurrence can be promoted by metformin associated therapy [102]. Some biguanide analogs like phenformin were withdrawn from clinical use in the 1970s, because of their association with lactic acidosis.

**Angiogenease**

Phoenix et al reported in xenograph model of breast cancer that metformin appears to increase the production of VEGF which is a neoangiogenic signal [103]. Stambolic et al noted two concerns about this result. The first one is related to the used dose and the other criticism is that of the model system [104]. The prospect that metformin administration could result in neoangiogenic cancer phenotype deserves further attention.

**Resistance to Metformin**

As many other anticancer drugs used on a daily chronic basis metformin might generate refractory cancer cells. This concept has
been particularly described in the work of Martin-Castillo et al [105]. They demonstrated that disruption of AMPK/mTOR/ S6K1 axis on chronic exposure to metformin efficiently relieves negative feedback suppression on the IGF-1/IRS-1 axis [106]. This complex phosphorylates and activates PI3K and then AKT. Abolition of the negative feedback loop leads to increase of cell survival signals due to PI3K/AKT activation axis and thus counteracts the antitumor activity of metformin.

**CLINICAL TRIALS**

All the data reported in the literature strongly support the clinical development of metformin as an anticancer agent. Thus several clinical trials are ongoing to test metformin drug as an adjuvant treatment as well as combined with other oncotherapies. The largest trial (NCT01101438) will recruit more than 3000 patients with breast cancer (T1-3, NO-3, M0) and without diabetes to compare metformin and placebo. The primary outcome is disease free survival (DFS) and secondary are overall survival (OS), and relevant medical endpoints. Two phase II trials involving pancreatic cancer are underway (NCT01167738 and NCT 01210911). A phase II trial involving prostate cancer patients (NCT01215032) will test metformin to androgen derivation therapy. Other numerous trials are on the way, list and description can be find at http://clinicaltrials.gov. The future role of metformin in neoadjuvant treatment, its side effects, its synergy with conventional chemotherapies need to be defined. Results will have great interest as in some studies metformin will be given for the first time in non diabetic patients and moreover practically all types of cancer are included. This is important as clinical results with metformin in patients with insulin resistance do not take into account the problem of this drug prescription to insulin sensitive patients. In vitro studies have indicated that metformin is a strong mTOR inhibitor as other drugs do. Combined therapies with rapalogs could be interesting since metformin can counteract increased glucose levels resulting from mTOR inhibition. The major advantage of metformin can be, in combination with HER2 inhibitors, to overcome traztuzumab resistance and protection of cardiac cells from HER2-inhibition-related cardiotoxicity. Metformin exerts cardioprotective actions via AMPK and increases the expression of adiponectin and its receptors adipoR1 and adipo R2 in cardiomyocytes [107]. Key studies are summarized in Table 1.

**CONCLUSION**

The link between cancer and type 2 diabetes has been suggested for a long time by epidemiological studies. These latter indicated a decrease of cancer incidence in patients with metformin therapy compared to those with other antidiabetic drugs. The role of metformin was revealed when it was demonstrated that this molecule acts on AMPK/mTOR signaling pathway. Basic research experiments have been conducted to elucidate how this axis could be triggered. Metformin, an old molecule seems to have a new role among anticancer drugs. Metformin inhibits transcription of key glucoseogenesis genes, increases glucose uptake in skeletal muscle and decreases circulating insulin level. On cel

**CONFLICT OF INTEREST**

The author(s) confirm that this article content has no conflicts of interest.

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Metformin and Cancer


Barrière et al.


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