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Invited review

Metformin and cancer: Between the bioenergetic disturbances and the antifolate activity

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ABSTRACT

For decades, metformin has been the first-line drug for the treatment of type II diabetes mellitus, and it thus is the most widely prescribed antihyperglycemic drug. Retrospective studies associate the use of metformin with a reduction in cancer incidence and cancer-related death. However, despite extensive research about the molecular effects of metformin in cancer cells, its mode of action remains controversial. In this review, we summarize the current molecular evidence in an effort to elucidate metformin's mode of action against cancer cells. Some authors describe that metformin acts directly on mitochondria, inhibiting complex I and restricting the cell's ability to cope with energetic stress. Furthermore, as the drug interrupts the tricarboxylic acid cycle, metformin-induced alteration of mitochondrial function leads to a compensatory increase in lactate and glycolytic ATP. It has also been reported that cell cycle arrest, autophagy, apoptosis and cell death induction is mediated by the activation of AMPK and Redd1 proteins, thus inhibiting the mTOR pathway. Additionally, unbiased metabolomics studies have provided strong evidence to support that metformin alters the methionine and folate cycles, with a concomitant decrease in nucleotide synthesis. Indeed, purines such as thymidine or hypoxanthine restore the proliferation of tumor cells treated with metformin *in vitro*. Consequently, some authors prefer to refer to metformin as an "antimetabolite drug" rather than a "mitochondrial toxin". Finally, we also review the current controversy concerning the relationship between the experimental conditions of *in vitro*-reported effects and the plasma concentrations achieved by chronic treatment with metformin.

Contents

1. Why metformin and cancer?

Metformin, a biguanide, is an antihyperglycemic agent that has for decades been the first-line treatment for type II diabetes mellitus; indeed, it is the most widely prescribed antidiabetic drug. Despite the extensive use of metformin as an antidiabetic for 40 years, the first report indicating an anticancer effect in mammals

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was in 2001 [1], and the first report of a reduced risk of cancer in patients with type II diabetes treated with metformin was published only 10 years ago [2]. Since then, more than 150 articles reporting different effects of metformin in human cancers have been published. More importantly, more than 200 ongoing clinical trials, in almost all type of cancers, assessing the potential of metformin as an adjuvant or neoadjuvant chemotherapy agent or as an enhancer of classic chemotherapy are registered on www.clinicaltrials.gov. However, the mode of action explaining the antitumor and chemopreventive effects of metformin remains a matter of controversy. In this review, we present the current evidence suggesting some of the hypothesized mechanisms of action for this drug.

2. Mode of action of metformin as an antihyperglycemic drug

The first hypothesis to explain the mode of action of metformin as an antihyperglycemic drug involved the stimulation of glucose uptake by muscle [3]. There is a growing body of evidence suggesting that the primary effect of metformin as an antidiabetic is to decrease hepatic glucose production as a consequence of gluconeogenesis inhibition [4]. Furthermore, several reports indicate that metformin administration leads to a reduction in ATP levels in the liver and a decrease in the ATP/ADP ratio, resulting in a reduction in cellular bioenergetics [5,6].

At the molecular level, the most described mode of action of metformin is the inhibition of mitochondrial complex I (NADH ubiquinone oxidoreductase), the first component of the electron transport chain [7]. Complex I inhibition by metformin could interrupt mitochondrial respiration, inducing a decrease in proton-driven ATP production and causing an energetic stress and reduction in the AMP/ATP ratio. Consequently, it was shown in the last decade that AMP/ATP ratio decreases stimulates AMP-activated protein kinase (AMPK); this enzyme was therefore considered to be a major sensor and mediator of the glucose-lowering effect of metformin [8,9]. AMPK activation in muscle can also increase glucose consumption, and hepatic AMPK activation can inhibit gluconeogenesis and activate glycolysis [10]. Both of these effects of metformin may decrease blood glucose and contribute to its antihyperglycemic effect in type II diabetes.

Although AMPK was once considered the pivotal executor of metformin's glycemic action, loss of function of AMPK and the upstream kinase LKB1 in mice revealed that AMPK may not be required for the observed gluconeogenesis suppression induced by metformin [5]. In addition, an AMPK-independent mechanism has been proposed, wherein metformin may be antagonized by glucagon-dependent cyclic AMP (cAMP) signaling [11]. In this way, metformin disturbs the glucagon activation of adenylyl cyclase and consequent cAMP production, thereby inhibiting the activation of cAMP-dependent protein kinase (PKA). The activation of PKA decreases fructose-2,6,-bisphosphate levels, thus enhancing gluconeogenesis in the liver and increasing blood glucose levels [12]. Additionally, recent data show that metformin treatment alters the hepatocellular redox state by inhibiting mitochondrial glycerophosphate dehydrogenase (mGPD), an enzyme that transports cytosolic reducing equivalents from NADH to the mitochondria through the glycerol-phosphate shuttle. It is noteworthy that a decrease in reducing equivalents, as well as complex I inhibition, compromises the ability of mitochondria to provide reducing equivalents to the electron transport chain to promote the production of ATP [13].

Thus, considering the increasing evidence that mitochondrial metabolism plays a pivotal role in supporting tumor growth by delivering both ATP and metabolic intermediates that can be used in anabolic reactions [14,15], an understanding of the actions of metformin in energy metabolism, particularly mitochondrial function, is relevant within the context of the potential applications of metformin in oncology.

3. Metformin mode of action in cancer cell bioenergetics

The recognition that type II diabetes mellitus is associated with increased cancer risk has led to increased interest in the therapeutic potential of various antidiabetic drugs such as metformin [16,17]. Of the abovementioned modes of action, the first one explored was metformin's mitochondrial activity.

Efficient mitochondrial activity and complex I function have been shown to be essential for the promotion of aerobic glycolysis and the Warburg effect [18], and the induction of mitochondria-mediated apoptosis by metformin was described in glioma cells in 2007 [19]. In 2010, it was reported that metformin increases the fraction of uncoupled respiration [20], which is important because the induction of mitochondrial uncoupling itself *via* the overexpression of UPCs proteins is able to inhibit tumor growth in breast cancer cells (the MDA-MB-231 cell line) [21].

Although it has been shown that metformin inhibits mitochondrial complex I in cancer cells (Fig. 1), there is no molecular description to date of the interaction between metformin and this NADPH reductase. However, some recent evidence lends support to this hypothesis. Andrzejewski et al. [22] reported that metformin acts by directly inhibiting complex I-mediated mitochondrial respiration and citric acid cycle functions in breast cancer cells and their isolated mitochondria. These effects induce a shift in favor of uncoupling reactions, causing mitochondrial metabolism to become energetically inefficient. Thus, metformin inhibits oxygen consumption in isolated mitochondria only in the presence of complex I substrates (malate and pyruvate) but not in the presence of complex II substrates, such as succinate [22]. These data agree with classical evidence showing an inhibitory effect of complex I together with a membrane potential-driven accumulation of positively charged drug within the mitochondrial matrix in hepatocellular carcinoma [23]. Further evidence about the role of complex I in the effects of metformin was provided by Wheaton et al. [24]. These authors showed that metformin exerted an antiproliferative effect in colon cancer cells but that this effect is abolished when a metformin-resistant NADH reductase from *Saccharomyces cerevisiae* (NDI1) was overexpressed. These results were confirmed in mice overexpressing the NDI1 protein [24].

In addition to contributing to an energetic imbalance, the inhibition of mitochondrial complex I has been associated with a decrease in insulin/insulin-like growth factor-1 (IGF-1) signaling, inhibition of mammalian target of rapamycin (mTOR), activation of AMPK (Fig. 1), and reduction in reactive oxygen species (ROS) production and its associated DNA damage [25–28]. AMPK and mTOR signaling comprise a central pathway in tumor proliferation [29] and has been related to metformin activity in such cancers as breast carcinoma [30,31], esophageal cancer [32], pancreatic cancer [33] and gastric cancer [34]. Although the regulatory function of AMPK over mTOR is well established, there is also evidence that metformin can inhibit the activity of mTOR in an AMPK-independent manner, inducing cell cycle arrest through an increase in the expression of the Redd1 protein (Fig. 1) [35]. Nevertheless, AMPK-dependent expression of Redd1 induced by metformin has also been described [36]. Finally, other less well-known effects derived from mTOR inhibition have been suggested to be effects of metformin. Interestingly, recent studies show that metformin has the potential to antagonize the epithelial-mesenchymal transition (EMT) and stemness in cancer cells, an effect that is not an easily predictable consequence of mTOR inhibition [37–39].