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REVIEW

Metformin and Cancer

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■ Abstract

Metformin is well-known as an anti-diabetic drug, but it seems to possess anti-cancerous properties as well. Adenosine monophosphate-activated protein kinase (AMPK) is a highly conserved regulator of the cellular response to the presence of low energy in all eukaryotic cells. It is considered a key sensor of the balance of cellular ATP and AMP concentrations. LKB1 serine/threonine kinase is a divergent yet evolutionarily well-conserved kinase, biochemically sufficient to activate AMPK *in vitro* and genetically required for AMPK activation. Because of this potent connection to AMPK, LKB1 may act as a central regulator of metabolism *in vivo*. Once activated, AMP kinase phosphorylates the transcriptional activator TorC2, thereby blocking its nuclear translocation and inhibiting the expression of genes involved in gluconeogenesis. Data suggest that LKB1/AMPK signaling plays a role in protection from apoptosis, specifically in response to agents that increase the cellular AMP/ATP ratio. Active AMPK signaling offers a protective effect by providing the cell with time to reverse the aberrantly high ratio of AMP/ATP. If unable to reverse this ratio, the cell will eventually undergo cell death. These observations offer the provocative suggestion of a potential therapeutic window in which LKB1-deficient tumor cells may be acutely sensitive to AMP analogues or sensitized to cell death by other stimuli when treated in combination with agents that increase the AMP/ATP ratio. LKB1 therefore is a classical tumor suppressor. AMPK is a direct LKB1 substrate. A consequence of AMPK activation by LKB1 is the inhibition of the mammalian target of rapamycin (mTOR) C1 pathway. Metformin's anti-cancerous properties have been demonstrated in various cancer cells *in vitro*, such as lung, pancreatic, colon, ovarian, breast, prostate, renal cancer cells, melanoma, and even in acute lymphoblastic leukemia cells. To test metformin's action *in vivo*, mice were implanted with transformed mammary epithelial cells and treated with three cycles of metformin and with the anthracycline doxorubicin. When combined with doxorubicin, metformin wiped out tumors and prevented recurrence. Metformin alone had no effect, and doxorubicin as a single agent initially shrank tumors, but they regrew later. Virtually no cancer stem cells were recovered immediately after treatment and the complete response was sustained for nearly two months. Further studies are needed to assess the anti-cancerous potentials of metformin *in vivo*. This article reviews the current knowledge on the actions of LKB1/AMPK and the effectiveness of metformin in cancer, specifically in diabetes patients.

Keywords: AMPK · LKB1 · metformin · doxorubicin

Introduction

It is well-established that colorectal cancer incidence is increased among patients with type 2 diabetes mellitus [1-4]. Certain types of cancers are more common in people with diabetes than in those without. Diabetes is also associated with reduced survival after cancer [5-8]. Therefore, it is important to address the topic of cancer and in diabetes in future research.

Adenosine-monophosphate-activated protein kinase (AMPK)

Adenosine monophosphate-activated protein kinase (AMPK) is a highly conserved regulator of the cellular response to low energy expressed in all eukaryotic cells. AMPK is activated when intracellular adenosine triphosphate (ATP) concentrations decrease and AMP concentrations increase [9]. It is considered a key sensor for the balance of cellu-

lar ATP and AMP concentrations. AMPK is activated by:

1. Stimuli that induce stress including oxidative damage, osmotic shock, hypoxia, and deprivation of glucose or other nutrients.
2. Mitochondrial poisons.
3. Physiological stimuli including exercise, muscle contraction, and hormones such as leptin and adiponectin [10].

In mammals, AMPK has a critical role in metabolic processes, including glucose uptake and fatty acid oxidation in muscle, fatty acid synthesis and gluconeogenesis in the liver, and the regulation of food intake centrally at the hypothalamus level [11-13].

AMPK exists as a heterotrimer complex, composed of the catalytic kinase α subunit and two associated regulatory subunits, β and γ [11]. Upon energy stress, AMP directly binds to tandem repeats of crystathionine-β synthase (CBS) domains in the AMPK γ subunit, causing a conformation change that exposes the activation loop in the α subunit, allowing it to be phosphorylated by an upstream kinase [10]. The sequence flanking the activation loop threonine (Thr172 in human AMPKα1) is conserved across species and its phosphorylation is absolutely required for AMPK activation. Phosphorylation of a single invariant threonine residue in the activation loop of the catalytic subunit (Thr172 in human AMPKα1) has been shown to be required to activate all known AMPK homologues [12]. A number of laboratories have reported biochemical purification of a kinase activity, AMPK kinase (AMPKK), which is capable of phosphorylating Thr172 [14-17]. Calcium calmodulin-dependent protein kinase kinase (CAMKK) has been demonstrated to serve as a surrogate AMPKK *in vitro*, but not *in vivo* [18].

The LKB1 serine/threonine kinase is a divergent yet evolutionarily well conserved kinase that most closely resembles CAMKK in its catalytic domain. Threonine kinase LKB1 is biochemically sufficient to activate AMPK *in vitro* and is genetically required for AMPK activation by energy stress in a number of mammalian cell lines [19-20]. Because of this potent connection to AMPK, LKB1 may act as a central regulator of metabolism *in vivo* [21-26]. Once activated, AMP kinase phosphorylates the transcriptional activator TorC2, thereby blocking its nuclear translocation and inhibiting the expression of genes involved in gluconeogenesis (**Figure 1**) [23].

Abbreviations:
AMP - adenosine monophosphate
AMPK - adenosine-monophosphate-activated protein kinase
ATP - adenosine triphosphate
CAMKK - calmodulin-dependent protein kinase kinase
CBS - crystathionine-β synthase
IGF-IR - insulin-like growth factor insulin receptor
IR - insulin receptor
LKB1 - liver kinase B1 (a threonine/serine kinase)
MAPK - mitogen-activated protein kinase
mTOR - mammalian target of rapamycin
Thr172 - threonine 172
TORC2 - target of rapamycin complex 2

LKB1/AMPK and diabetes

The ability of metformin to lower glucose and insulin levels by inhibiting the expression of genes involved in gluconeogenesis is a satisfactory explanation of its therapeutic effect in diabetes [9]. Mice deficient in hepatic LKB1 develop hyperglycemia and are resistant to the glucose-lowering effects of metformin [21].

There is genetic and biochemical evidence that LKB1 is a critical regulator of AMPK *in vivo*. As such, LKB1 may play an unexpected role in multiple organ systems that mediate the diverse effects of AMPK on mammalian physiology. Importantly, AMPK has been shown to be a critical mediator of glucose uptake in skeletal muscle in mice. The kinase activity of AMPK is stimulated by two major anti-diabetic drugs, metformin and rosiglitazone [27-28]. Therefore, the identification of LKB1 as a major activator of AMPK *in vivo* may offer potential avenues to boost AMPK activity for the treatment of diabetes.

LKB1/AMPK and apoptosis

Data suggest that LKB1/AMPK signaling plays a role in protection from apoptosis, specifically in response to agents that increase the cellular AMP/ATP ratio. Active AMPK signaling induces a protective effect by providing the cell with time to reverse the aberrantly high ratio of AMP/ATP. If unable to reverse this ratio, the cell will eventually undergo cell death. These results offer the provocative suggestion of a potential therapeutic window in which LKB1-deficient tumor cells may be acutely sensitive to AMP analogues or sensitized to cell death by other stimuli when treated in combination with agents that increase the AMP/ATP ratio.

AMP kinase is activated by the product of the Peutz-Jegher tumor suppressor gene LKB1 [29].

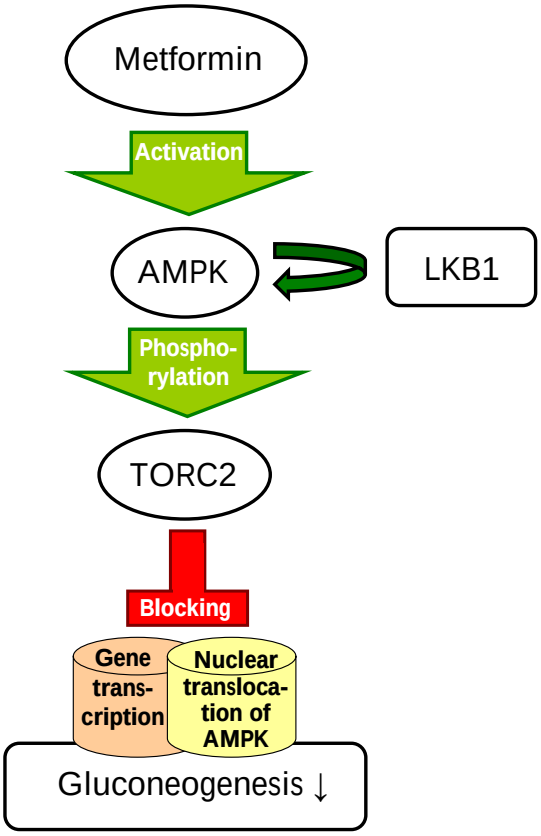

Figure 1. Action of metformin in diabetes. Metformin activates adenosine-monophosphate-activated protein kinase (AMPK), which phosphorylates the target of rapamycin complex 2 (TORC2), thereby blocking its nuclear translocation and transcription of the genes involved in gluconeogenesis. Liver kinase B1 (LKB1) is essential for the activation of AMPK. Positive AMPK signals prevent the mitogenic activity of mammalian target of rapamycin (mTOR) C1 pathway.

Peutz-Jeghers syndrome patients develop numerous benign tumors in the gastrointestinal tract and have a 20-fold increased risk of developing malignant tumors at other sites. Mutations in the LKB1 gene are also seen in some sporadic cancers, especially lung adenocarcinoma [30-31]. Therefore, LKB1 is a classical tumor suppressor [32]. AMPK is a direct LKB1 substrate. A consequence of AMPK activation by LKB1 is the inhibition of the mammalian target of rapamycin (mTOR) C1 pathway through phosphorylation of tuberous sclerosis 2 or hamartin and raptor [33-34].

As mentioned above, loss of LKB1 function is a frequent finding in lung adenocarcinoma and squamous cell carcinomas [20]. Interestingly, the anti-diabetic drug rosiglitazone is known to stimulate AMPK signaling through alterations in the intracellular AMP/ATP ratio, suggesting that rosiglitazone may be useful in the treatment of LKB1-deficient tumors as well [28]. The observation that altered AMP/ATP ratios result in cell death in the absence of AMPK signaling indicates that other cellular proteins that are regulated by AMP may contribute to the cell death observed.

LKB1 tumor suppression seems to be the major activating kinase for AMPK in the liver. As Shaw *et al*. suggested, minor roles for other kinases cannot be ruled out, but under all the conditions they have examined, the loss of LKB1 was mirrored by the loss of AMPK phosphorylation [26].

mTOR, insulin, and LKB1 pathways represent a fundamental eukaryotic network governing cell growth in response to environmental nutrients; dysregulation of one of these pathways contributes to both diabetes and cancer [29-32].

Metformin and various cancer cells

The involvement of a tumor suppressor pathway as a target for metformin's action in glucose homeostasis prompted studies of possible effects in tumor cells and animal cancer models. *In vitro* studies have shown that metformin inhibits the proliferation of colorectal cancer cells [33]. *In vivo* studies have demonstrated that metformin delays tumor onset in mouse models for p53 mutant colon cancer [34]. Another animal model of colon cancer has indicated that metformin inhibits colon carcinoma growth stimulated by a high-energy diet [35]. Two animal models of colorectal aberrant crypt foci showed that metformin significantly suppresses colonic epithelial proliferation by inhibiting the mTOR pathway [36-39]. This pathway has recently been found to be involved in T cell acute lymphoblastic leukemia [40]. Indeed, metformin has been shown to have therapeutic effects in T cells of acute lymphoblastic leukemia *in vitro* [41].

Metformin exerts *in vitro* inhibition of the proliferation of prostate, ovarian, and breast cancer cells [39]. This inhibitory effect is seen, however, at concentrations that are at least 10-fold higher than the peak plasma concentration attained with typical dosing in diabetics [42]. Even though most laboratory studies have been using doses that are much lower than the typical anti-diabetic dose of metformin used *in vivo*, there are emerging studies which show that even lower doses of metformin could have substantial anti-cancerous effects. For example, the proliferation of $CD133^+$, but not $CD24^+CD44^+ESA^+$ cells, which are considered pan-

creatic cancer stem cells, was inhibited by low doses of metformin [43]. Recently, it has been shown that the conventional anti-diabetic concentrations of metformin caused death in cancer cells and were preferentially cytotoxic to cancer stem cells related to non-cancer stem cells [44]. Also, to demonstrate the action of potential anti-cancerous properties of metformin *in vivo*, mice with transformed mammary epithelial cells were given three cycles of metformin and one cycle of doxorubicin, resulting in shrinking tumor cells and the prevention of recurrence. Mouse xenograft models demonstrate *in vivo* anti-tumor effects of metformin against pancreatic, prostate, and *p53* mutant colon cancers [33, 45-47].

AMPK is critically linked to the phosphatidylinositol-3 kinase/AKT/mTOR signaling pathway, a vital cellular signaling cascade that is essential for cell growth in response to mitogenic stimuli or pathways activated by growth factor receptors [48]. AMPK activation directly inhibits phosphorylation and subsequent activation of the mTORC1 complex and is controlled partly by the upstream kinase AKT, whose activation decreases the AMP:ATP ratio [35, 49-51]. AKT also directly inhibits the activation of AMPK by phosphorylation of AMPK at Ser 485/491 [52-53]. Renal cell carcinoma is a highly aggressive genitourinary cancer for which the treatment options are limited [54]. This malignancy is characterized by overactivation of this AKT/mTOR signaling pathway [55]. Extensive work over the last few years has demonstrated the effectiveness of targeting the mTOR pathway for the treatment of renal cell carcinoma [56]. Temsirolimus, a known mTOR pathway inhibitor, has clinically significant activity in the treatment of renal cell carcinoma and is now an FDA-approved agent in the treatment of patients with renal cell carcinoma [57]. Studies have demonstrated that addition of metformin exerted suppressive effects on the tumorogenicity of renal cancer cells *in vitro*.

It has been suggested that inhibitory effects on the LKB1/AMPK axis in melanoma may constitute an important mechanism of tumorigenesis [58]. Studies using both melanoma cell lines harboring this BRAF mutation and melanoma cell lines without this mutation suggest that AMPL plays a role in the control of malignant melanoma cell growth. Taken together, the above-mentioned studies provide evidence for potent inhibitory effects of AMPK on malignant melanoma cell growth and survival and raise the potential of AMPK manipulation as a novel future approach for the treatment of malignant melanoma [59].

Metformin and breast cancer

The discovery that metformin selectively kills cancer stem cells adds further interest and may explain its antineoplastic properties. Hirsch *et al.* genetically manipulated human breast epithelial cells to enrich for stem cells and tested these together with three distinct breast tumor cell lines [60]. Using flow cytometry to track the effects of metformin, researchers found that the drug is selectively toxic to cancer stem cells. To test metformin's action *in vivo*, mice were implanted with transformed mammary epithelial cells and treated with three cycles of metformin and with the anthracycline doxorubicin. When combined with doxorubicin, metformin wiped out tumors and prevented recurrence. Metformin alone had no effect and doxorubicin as a single agent initially shrank tumors but they regrew later. Virtually no cancer stem cells were recovered immediately after treatment and the complete response was sustained for nearly two months [61]. Among patients with breast cancer, the metformin-treated subgroup has been related with better outcomes than patients not treated with metformin [62].

Further studies will delineate whether the AMP kinase pathway is important in cancer stem cells, and whether the synergistic effect of metformin and anthracyclines is generalized to other types of cancer and to its combination with other drugs [63].

Metformin and colorectal cancer

Type 2 diabetes has been associated with increased incidence of colorectal cancer [64]. Recently, a study has revealed a significant association between highly intensive use of metformin and lower mortality from colorectal cancer in diabetics with stage I-III colorectal cancer compared with non-diabetics with stage I-III colorectal cancer [65]. Also, metformin use has been related with decreased incidence of colorectal adenomas in diabetic patients with previous colorectal cancer [66]. Overall survival has been found to be better among patients with colorectal cancer and type 2 diabetes taking metformin as part of their anti-diabetic medication compared with diabetic patients with colorectal cancer not taking metformin as part of their anti-diabetic regimen [67]. In Korean patients with colorectal cancer, the use of metformin has been associated with reduced risk of overall mortality, especially in patients with stage III colorectal cancer [68].

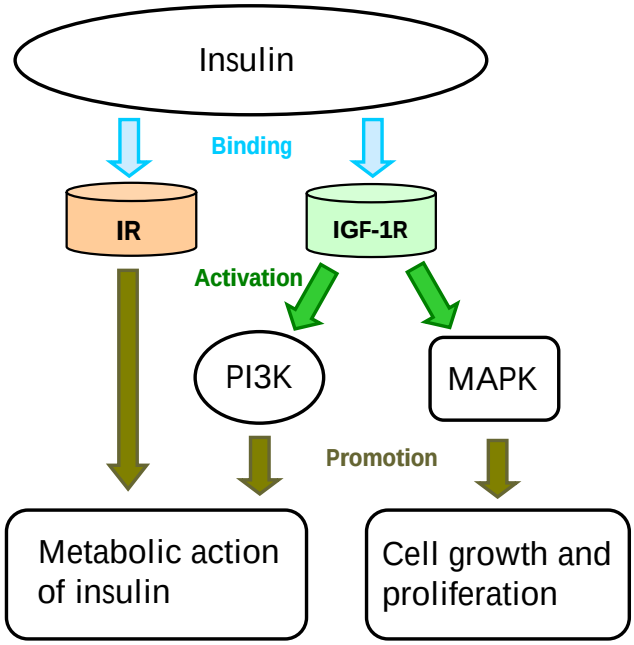

Figure 2. Insulin-binding and metabolic action of insulin. Insulin binds both to the insulin receptor (IR) and to insulin-like growth factor receptor 1 (IGF-1R). The binding of insulin to IGF-1R activates both phosphoinositol-3-kinase pathway (PI3K), which activates the metabolic pathway, and mitogen-activated-protein-kinase (MAPK), which promotes cell growth and cell proliferation. The binding of insulin to IR is responsible for the metabolic actions of insulin.

Metformin, cancer prevention, and mortality

As shown by Curie *et al.*, diabetic patients with type 2 diabetes taking metformin had a decreased risk of cancer compared with patients taking metformin plus sulfonylurea and patients on insulin treatment [69]. Metformin has been related with lower incidence of cancer among type 2 diabetes patients [70]. Many studies support the notion that the use of metformin results in lower incidence of cancer [71-75]. Lung, pancreatic, prostate, breast, ovarian, and hepatocellular cancer incidence has been found to be lower among patients receiving metformin [76-82]. Moreover, the mortality from cancer was lower in diabetes patients taking metformin as part of their anti-diabetic medication compared with those not taking metformin [83-86]. However, studies showing favorable effects of metformin on cancer are not always corroborated by large clinical trials. Larger studies are expected to investigate the possible antineoplastic effects of metformin more thoroughly [87-89].

Conclusions

Metformin exerts its anti-tumor effects mainly through the AMPK/LKB1/TORC1 signaling pathway, thereby causing apoptosis in cancerous cells [14, 21-23]. Another possible mechanism is amelioration of endogenous hyperinsulinemia by use of metformin therapy [90]. Insulin stimulates cellular proliferation, and multiple signaling pathways are activated after insulin receptors or insulin-like growth factor (IGF-I) receptors interact with their ligands [91-92].

Most cancer cells express insulin and IGF-I receptors. The A isoform of the insulin receptor is also commonly expressed, which may stimulate mitogenesis, even in cells deficient in IGF-I [93-94]. Metformin therapy decreases the levels of circulating insulin-like growth factors and insulin which, in turn, may reduce the risk of cancer (**Figure 2**).

Other possible mechanisms underlying the potential anti-tumor effect of metformin could be the antagonization of obesity and anti-inflammatory effects. Interleukin-6, plasminogen activator inhibitor-1, tumor necrosis factor-α, and monocyte chemoattractant are produced by adipose tissue and can enhance cancer cell proliferation, p-53 activation, downregulation of cyclin D1, and killing of cancer stem cells [95-98]. Further studies investigating potential mechanisms of the anti-cancerous properties of metformin are needed.

If metformin effectively helps cancer patients, it will finally join drugs such as thalidomide, retinoic acid, and arsenic, which have unique mechanisms of action and were first used elsewhere in medicine, but have also found their way into the field of anticancer drugs.

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