MINI REVIEW

Metformin: Antineoplastic Activity through the Mammalian Target of Rapamycin Pathway

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ABSTRACT

Metformin is used as a first-line therapy in individuals suffering from type 2 diabetes mellitus (T2DM) and has multiple mechanisms of action through which it impacts metabolism at the physiologic and molecular levels. In addition, metformin has been shown to positively impact outcomes in patients with T2DM suffering from many different forms of cancer. One pathway that seems to recur in many different cancers is the mammalian target of rapamycin (mTOR) pathway. The TOR protein, divided into the mTOR complex 1 (mTORC1) and complex 2 (mTORC2) subunits, upregulates mRNA translation and promotes anabolic processes through the phosphorylation of several downstream proteins. The activity of the mTOR pathway inhibits autophagy, the process of protein degradation in times of nutrient stress, thereby increasing processes that upregulate cell growth. Metformin modulates this pathway through the indirect activation of AMP-activated kinase (AMPK), a protein that inhibits mTOR and increases autophagy. The activity of metformin through AMPK has been shown to sensitize locally advanced non-small-cell lung carcinoma (LA-NSCLC) to radiotherapy (RT) and progression-free survival in patients with T2DM. Moreover, mTORC2 activity was found to be necessary for prostate cancer tumorigenesis, suggesting the viability of a therapy that directly targets the mTOR pathway. Understanding the antineoplastic activity of metformin, through both mTOR and other key pathways, may help in the development of adjunct cancer treatments in T2DM and beyond.

Key words: AMP kinase, antineoplastic, autophagy, cancer, mechanism, metformin, mTOR, type 2 diabetes

Although lifestyle changes are considered key to the treatment of patients with T2DM, the biguanide drug metformin remains an important agent in the control and possible prevention of this disease.[1] Metformin is believed to target the core defect of insulin resistance in T2DM, increasing insulin receptor sensitivity, and reducing hepatic glucose overproduction.[2] At the molecular level, metformin is believed to inhibit mitochondrial respiration at complex I of the electron transport chain, resulting in increased levels of AMP, and leading to the upregulation of AMPK.[3] AMPK acts to restore energy balance by turning on catabolic processes that generate ATP and turning off processes that consume ATP.[4] Through this and other mechanisms, metformin's impact on metabolism has been repeatedly shown to have benefits in cardiovascular diseases, inflammatory states, and of special interest to us, cancer treatment and prevention.[5-7]

Metformin has been shown to increase overall survival in T2DM patients suffering from colon, lung, gastroesophageal, thyroid, and prostate cancers.[8-10] Although many mechanisms are currently being investigated, a key pathway that has been shown to recur in multiple cancers is the mTOR pathway. This pathway regulates cell growth and proliferation and is an important stimulator of anabolic processes including protein synthesis.[11] Metformin targets this pathway through its actions on AMPK and inhibits mTOR signaling, leading to robust antineoplastic activity.[12,13] This review will detail the functions of mTOR and examine metformin's effects through it on cancers in T2DM patients.

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The TOR protein, which acts as a downstream component of the PI3K/AKT pathway, is a Ser/Thr kinase belonging to the phosphoinositide 3-kinase (PIKK) family.[14] This family of kinases, which includes ataxia-telangiectasia mutated (ATM), ATM and rad3-related (ATR), DNA-dependent protein kinase catalytic subunit (DNA), suppressor with morphological effect on genitalia family member (SMG1), and mTOR regulates an organism's response to metabolic, environmental, and genetic stress.[15] Abnormalities in these proteins are associated with immunodeficiency, chromosomal aberrations, and increased incidence of cancer. In one study, silencing of ATR and ATM resulted in a statistically significant decrease in senescence-associated beta-galactosidase, further suggesting the role of PIKK proteins as a site of tumor suppression.[16] As a member of this family of proteins, mTOR influences cell proliferation by upregulating protein synthesis and modulating ribosome biogenesis.[17,18] Furthermore, mTOR has been shown to inhibit protein degradation in nutrient-rich conditions; a process called autophagy.[19] Mutations leading to the chronic activation of mTOR have been documented in many malignancies, and it is believed to play an important role in the tumorigenesis and progression of some cancers.

mTOR is composed of two distinct dimeric subunits known as mTORC1 and complex 2 (mTORC2).[20] mTORC1 is the rapamycin-sensitive component of mTOR and forms a functional unit with its accessory protein raptor.[21] Their combined activity phosphorylates eIF-4E binding protein 1 (4E-BP1), undoing its repression of mRNA translation by allowing for the binding of initiation factor eIF4G.[22] mTORC1 and raptor also work to phosphorylate s6 kinase 1 (S6K1), which further promotes translation through its kinase activity on downstream substrates.[23] The activity of mTORC1 is guided by nutrient availability, growth factor signaling, energy, and stress, allowing it to play an important role as a regulator of autophagy.[24] In starving conditions, low levels of ATP activate AMPK, which goes on to phosphorylate raptor.[25] The phosphorylated raptor binds protein 14-3-3, leading to the inhibition of mTORC1, and the induction of autophagy. In nutrient-rich conditions, mTOR activity is promoted by feeding and increasing levels of insulin, leading to its activation, and the cessation of autophagy.[26] mTORC1 regulation of anabolic metabolism is fundamental for cell growth, and its abnormal activation has been implicated in 80% of human cancers.[27] mTORC2, the rapamycin-insensitive portion of mTOR, acts as a mediator of cytoskeletal organization and polarity alongside its accessory protein rictor. A study utilizing *Saccharomyces cerevisiae* found that TOR2 knockout samples arrested in the G2/M phase of the cell cycle,[28] revealing that the mTOR pathway's effect on growth is intrinsically tied to both metabolism and cell cycle progression. The regulation of mTORC2 remains a topic of debate, but it is believed to be influenced by growth factor activity, including insulin stimulation. Together, mTORC1 and mTORC2 integrate environmental cues that signal local conditions and prevent the cell from depleting its valuable resources during food scarcity.

Although there are multiple prospects in shaping this pathway in the case of malignancy, metformin is of particular interest due to its multivalent effects. Metformin indirectly upregulates AMPK, which is normally active during starvation and fasting conditions, leading to the inhibition of mTOR through multiple mechanisms.[4] In addition to the direct phosphorylation of the raptor accessory protein, AMPK phosphorylates tuberous sclerosis complex 2. In conjunction with tuberous sclerosis complex 1, this set of proteins inactivates S6K1 and promotes the function of 4E-BP1, thereby inhibiting the upregulation of mRNA translation by mTOR.[29] Metformin has also been found to inhibit mTORC1 independently of AMPK and TSCs. In response to amino acid abundance, Rag GTPases interact with raptor, leading to the clustering of mTORC1 near the nucleus and its activation by Ras homolog enriched in brain.[30] This nutrient-centric response was shown to be inhibited by the presence of biguanides, which are believed to inhibit GTPase activity and prevent perinuclear aggregation.[31] In addition to metformin's effects on cell growth, it has recently been observed that this inhibition of the mTOR pathway may also contribute to cancer cell apoptosis. A study found that breast cancer cells plated in low glucose medium (5.5 mM) and treated with metformin (10 mM) over a period of 48 h showed significant levels of cytotoxicity and mTOR activation.[32] Interestingly, this model revealed that the apoptotic effect was heavily reliant on glucose availability, with high glucose mediums (12.5 mM and higher) showing significantly less cytotoxicity. Moreover, the high glucose medium did not alter the activation of the mTOR pathway, as shown by similar levels of phospho-mTOR and phosphor-RPS6 across experimental models, suggesting that other mechanisms involving metformin's metabolic activity were also at work.[32]

The pronounced antineoplastic effects of metformin through the mTOR pathway can be traced through multiple cancers and have even been found in particularly malignant species including non-small-cell lung cancer. In patients with LA-NSCLC, metformin use in conjunction with chemo-RT was associated with significantly improved progression-free survival.[33] This synergistic effect was followed in an *in vitro* study, which found that metformin improved the sensitivity of lung cancer cells to RT, even at low micromolar doses (5–100 μM).[34] This response was found to be mediated through enhanced activation of ATM and AMPK, and overall suppression of the Akt-mTOR-4EBP1 pathway. The sensitization effect is particularly impressive in light of the traditionally poor outcomes associated with RT in NSCLC. Furthermore, it was found that samples treated with RT and metformin expressed twice as much sustained activation of AMPK versus RT or metformin alone, suggesting that

combination therapies in T2DM patients may optimize the longevity of antitumor activity.

Reducing the activity of mTOR has been theorized to be particularly effective in cancers that manifest through the PI3K/Akt pathway, of which mTOR is a downstream component.[35] This signaling pathway influences cell growth and is susceptible to activating mutations or loss of tumor suppression. The loss of PTEN, a key tumor suppressor that functions as a phosphatase, has been closely associated with the dysfunction of this pathway and has been found in nearly 70% of prostate cancers.[36] In knockout mutations of PTEN, large accumulations of phosphatidylinositol (3,4,5)-triphosphate (PtdIns(3,4,5) P3) result in the concentration of protein kinase B (Akt), a substrate of mTOR activity. The phosphorylation of abundant Akt by mTOR is believed to greatly contribute to the malignant potential of cells with this mutation. A study done on PTEN knockout mice in 2009 found that mTORC2 activity was necessary for Akt phosphorylation and the eventual tumorigenesis of cancer in prostate epithelium with this mutation.[37] It was also shown that mice with only partial loss of the rictor gene were protected from tumor formation, with only 1 in 10 heterozygous models showing visible tumors. Moreover, an age-matched comparison of prostate tissues between wild type and rictor knockout samples revealed no differences in histology, suggesting that therapies that target mTORC2 and rictor function may have limited damaging effects.[37]

Metformin's ability to reduce the function of the mTOR pathway through activation of AMPK serves an important role in understanding its antineoplastic effects. Although mTOR inhibition through rapamycin has been found to have toxic side effects, the potential for a tolerable adjunct therapy that works through this pathway may exist in the form of the biguanides. Moreover, it has also been shown that metformin's effects on cancer are not limited to mTOR: Its impact on cellular respiration has been found to reduce the Warburg effect and negatively impact the growth of cancer stem cells.[38] With malignant conditions being more common in T2DM patients, metformin's application may serve a protective effect, whether through the PI3K/Akt/mTOR pathway or through a different channel.[39] The use of metformin or a unique derivative to combat malignancy may eventually be possible regardless of diabetic status.

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