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Perspective
Perspective on Memmott et al., p. 1066

Cancer Prevention Research

Chemoprevention Meets Glucose Control

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Abstract

The report by Memmott et al. (beginning on page 1066 in this issue of the journal) assessing the efficacy of the antidiabetes drug metformin in a mouse model of lung carcinogenesis suggests protective effects via two possible avenues: Decreased circulating insulin and insulin-like growth factor levels and energy stress leading to inhibition of mammalian target of rapamycin signaling. These potential mechanisms are discussed in this perspective, as are their implications for cancer prevention and therapy. *Cancer Prev Res; 3(9); 1049–52. ©2010 AACR.*

Targeted therapies have affected the course of cancer treatments, but they have yet to be widely used as agents for chemoprevention. Over the past few years, preclinical studies have repeatedly shown that inhibition of mammalian target of rapamycin (mTOR) signaling may prevent cancer progression. mTOR is a serine/threonine kinase that serves as a central regulator of cellular growth, a function that is highly conserved from yeast to mammals. In mammalian cells, it integrates several different inputs—ranging from growth factor signaling to nutrient, energy, and oxygen availability—to ultimately determine if a cell should grow and divide (Fig. 1; refs. 1–5). mTOR exists in two distinct intracellular protein complexes: TOR complex 1 (TORC1) and TORC2. TORC2 is a complex of several proteins, including rapamycin-insensitive companion of TOR (Rictor), and is necessary for phosphorylation of AKT on residue Ser473 in response to phosphatidylinositol 3-kinase (PI3K) activation (6). TORC1 is a distinct complex consisting of mTOR and three other proteins: rapamycin-sensitive regulatory associated protein of TOR (Raptor), mLST8, and PRAS40. Unlike TORC2, TORC1 is effectively inhibited by rapamycin and its analogues. TORC1 phosphorylates p70 S6 kinase and 4E-binding protein-1, -2, and -3. These phosphorylation events lead to enhanced translation of mRNAs that encode many cell cycle regulators (e.g., c-Myc and cyclin D1) as well as certain ribosomal proteins and elongation factors (reviewed in ref. 7). In addition, activation of TORC1 results in increased expression of hypoxia inducible factor 1α, a transcription factor involved in the control of glucose uptake and metabolism (8, 9).

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The central role of TORC1 in cancer cell growth spurred the development of rapamycin analogues as potential therapeutics for cancer. With the exception of their use in renal cell carcinoma, mantle cell lymphoma, and pancreatic neuroendocrine tumors, these compounds have failed to show substantial activity as single-agent therapies in advanced cancers (10–12). There are several likely reasons for this lack of efficacy that include: reflex activation of other signaling pathways, such as the PI3K-AKT pathway, resulting from TORC1 inhibition (i.e., loss of a negative feedback); the inability of rapamycin analogues to completely inhibit TORC1; and the fact that rapamycin analogues often induce cytostasis rather than cell death. However, preclinical data over the last few years have suggested that TORC1 inhibitors may be effective chemoprevention agents. In a recent study, there was a substantial increase in the life span of normal mice that were randomized to receive rapamycin (13). These mice were randomized to rapamycin at the relatively advanced age of 600 days. At the time of death, the rapamycin-receiving mice had the same incidence of tumors and lymphomas as the control mice, and it seemed that rapamycin retarded tumor progression rather than suppressed tumorigenesis. In a related set of studies, rapamycin was administered to mice that were induced to develop lung cancers with the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; ref. 14). Rapamycin hindered tumor progression, resulting in an ~90% reduction in tumor burden. If rapamycin treatment was initiated contemporaneously with NNK, it showed evidence of blocking tumor formation. Rapamcyin similarly inhibited the lung tumor burden, but not the tumor incidence, induced by benzo(*a*)pyrene (15). Last, the rapamycin analogue CCI-779 halted the progression of mutant-*KRAS*–induced lung cancers in mouse models (16). Taken together, these findings suggest that early administration of rapamycin or other agents that inhibit TORC1 may retard cancer progression and, if initiated early in the course of tumorigenesis, may have utility as chemoprevention agents.

Although these results are promising, immunosuppression and other side effects of direct TORC1 inhibition may

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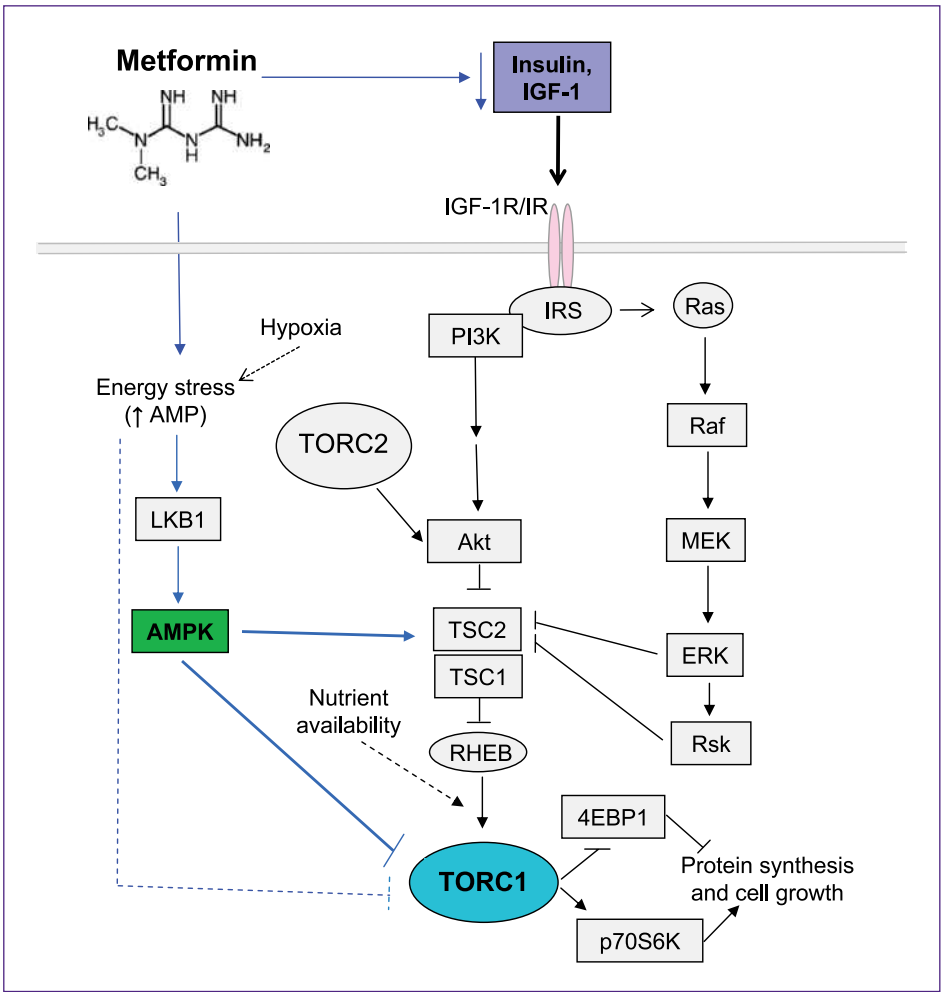

Fig. 1. mTOR exists in the intracellular complexes TORC1 and TORC2. TORC1 phosphorylates p70 S6 kinase (p70S6K) and 4E-binding protein 1 (4EBP1) to control cell growth. Various inputs into TORC1 regulation directly affect the TSC1-TSC2 complex (often through direct phosphorylation), which in turn controls the activation of the Ras homologue enriched in brain (RHEB) small G protein that directly activates TORC1. Growth factor signaling [through PI3K/AKT and extracellular signal–regulated kinase (ERK)/-ribosomal S6 kinase (Rsk) signaling] and energy homeostasis [through AMP-activated protein kinase (AMPK)] directly lead to TSC2 phosphorylation. *In vivo*, metformin downregulates TORC1 via several potential mechanisms including AMPK-dependent and -independent mechanisms. IRS, insulin receptor substrate; LKB1, liver kinase B1; MEK, mitogen-activated protein kinase/extracellular signal–regulated kinase kinase.

limit the utility of these inhibitors as chemoprevention agents. Thus, other strategies that indirectly downregulate TORC1 may be more acceptable as cancer preventives. One pathway that regulates TORC1 is the liver kinase B1 (LKB1)/AMP-activated protein kinase (AMPK) signaling axis. Under conditions of energy deprivation and elevated AMP, AMPK is activated through phosphorylation by LKB1. AMPK, in turn, phosphorylates tuberous sclerosis 2 (TSC2), leading to activation of its guanosine triphosphatase activating protein (GAP) activity toward Rheb, leading to inactivation of TORC1 (Fig. 1; refs. 1, 5). AMPK also directly phosphorylates Raptor in the TORC1 complex to downregulate signaling (17). Thus, activation of AMPK has the potential to downregulate TORC1 in cancer cells.

Metformin, an oral antidiabetes drug, activates AMPK by elevating cellular AMP levels, and studies have shown that it suppresses TORC1 signaling in cancer cell lines (18). In these studies, metformin notably failed to downregulate TORC1 in *LKB1*-deficient cancers and *TSC2*-deficient mouse embryo fibroblasts. However, recent studies have shown that metformin and phenformin (another antidiabetes drug) can downregulate TORC1 signaling in TSC2-deficient mouse embryo fibroblasts by a mechanism independent of AMPK (17, 19). Indeed, Thomas and colleagues found that phenformin phenocopies amino acid withdraw by inhibiting Rag function and disrupting colocalization between mTOR and Rheb. Taken together, these studies show that metformin and phenformin suppress TORC1 signaling, and this suppression may occur through both AMPK-dependent and AMPK-independent mechanisms. Considering the potency of rapamycin in delaying the progression of tumors, these studies suggest that metformin may have anticancer or chemoprevention activity. Indeed, retrospective studies have revealed that diabetic patients treated with metformin had substantially lower incidences of cancer than did patients treated only with sulfonylureas or insulin (20, 21). [The history, epidemiology, and clinical context of metformin and other biguanides are reviewed by Pollak elsewhere in this issue of the journal (22).] Of course, it is unknown whether metformin would provide similarly impressive results in people without diabetes. It is also unknown if the protective effects are through direct TORC1 suppression in malignant (subclinical) or premalignant

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cells or by decreasing the levels of circulating insulin (which may promote the growth of malignant cells; Fig. 1).

In this issue of the journal, Memmott and colleagues extend these retrospective findings with work to specifically determine if metformin prevents lung cancer progression in preclinical models (23). They used the mouse model of lung tumorigenesis induced by NNK. Two doses of metformin were administered via drinking water and initiated 1 week after administration of NNK. Metformin plasma concentrations were similar to those observed in human diabetes patients. The investigators found that tumor burden was reduced by ~50% in the cohort of mice receiving the higher dose of metformin. However, similar to several of the rapamycin studies, tumor incidence was unaffected. Indeed, the effect on TORC1 signaling was modest, as determined by immunohistochemical analysis of phospho-S6. Higher blood metformin levels were achieved by i.p. injection, and this correlated with greater inhibition of phospho-S6 and a more substantial decrease in tumor burden (~70%). Of note, there was no evidence of activation of AMPK in lung tumors, suggesting that the antitumor activity and TORC1 inhibition were independent of AMPK activation. Furthermore, metformin led to decreased levels of circulating insulin and insulin-like growth factor (IGF), as well as decreased phosphorylation of IGF-I receptor, AKT, and extracellular signal–regulated kinase (ERK), in tumor tissue. Thus, the profound effects on tumor growth may have been a consequence of perturbations of glucose homeostasis and hormone levels rather than of direct autonomous TORC1 suppression in cancer cells (Fig. 1).

The findings in this report add greater enthusiasm for using agents such as metformin and/or phenformin as chemopreventive agents. It is possible that the lower serum IGF and insulin levels may have provided a large proportion of therapeutic efficacy. These findings are particularly relevant in light of recent studies showing that dietary restriction retards tumor growth, and this effect likely resulted from lower insulin and IGF levels (24). Indeed, cancers with "constitutive" PI3K signaling through phosphatase and tensin homologue (*PTEN*) loss or *PIK3CA* mutation were resistant to the effects of dietary restriction. Unlike the cancers sensitive to dietary restriction, the growth of these resistant cancers was also unaffected by IGF or insulin *in vitro*. Thus, if metformin and/or phenformin abrogated tumor progression by lowering IGF or insulin levels, lung cancers with constitutive PI3K signaling, such as those with *PTEN* loss, *PIK3CA* mutation, and even perhaps epidermal growth factor receptor (*EGFR*) mutation or anaplastic lymphoma kinase (*ALK*) translocation, may be resistant to the protective effects of these agents. Experiments such as those performed by Memmott et al. in cancers with these genetic backgrounds may directly address this concept.

The findings of these experiments point to potential clinical trials and to pragmatic clinical decisions. From a practical standpoint, the Memmott and retrospective studies suggest that diabetics might have a lower risk for cancer if treated with metformin rather than sulfonylureas or insulin. Thus, primary care physicians and endocrinologists may consider this information when choosing an antidiabetes regimen for patients, especially those at a higher risk for developing cancer. Additionally, these studies and those showing that rapamycin delays tumor growth and prolongs life suggest that agents that inhibit mTOR, activate AMPK, lower cellular ATP levels, and/or lower insulin or IGF-I levels may have roles in chemoprevention.

Chemoprevention studies with these agents would ideally target patients with a high risk of developing cancers, such as those with premalignant conditions (e.g., atypical adenomatous hyperplasia of the lung or dysplastic oral lesions; refs. 25, 26) or those with a high risk of disease recurrence (such as in the adjuvant setting). Because these patients will not have cancer at the time of treatment, these interventions will have to be relatively tolerable. Of note, recent studies suggest that direct mTOR catalytic site inhibitors do not induce as much immunosuppressive activity as do rapamycin analogues, and thus may be better suited for chemoprevention studies (27). For agents that directly inhibit mTOR or activate AMPK, there is the possibility that the release of feedback inhibition of PI3K and ERK signaling may ultimately have untoward consequences (28–30); therefore, these approaches may benefit from careful preclinical modeling before transitioning to the clinic. Ultimately, these therapies hold promise for delaying or preventing cancer progression and having a substantial, beneficial effect on cancer mortality.

Disclosure of Potential Conflicts of Interest

J.A. Engelman is a consultant for Agios; L.C. Cantley is founder and a consultant for Agios.

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