Rapamycin and mTORC1 Inhibition in the Mouse: Skin Cancer Prevention

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Abstract

Therapeutic and preventive effects of rapamycin include reduced risk of nonmelanoma skin cancer (NMSC). In this issue of the journal (beginning on page 1011), Checkley and colleagues report that rapamycin inhibits mTOR complex 1 in murine epidermis, thereby inhibiting tumor promotion mediated by tetradecanoxyl phorbol-13 acetate in association with a strong anti-inflammatory effect. Rapamycin is an immunosuppressive drug for preventing graft rejection in organ transplant recipients and reduces the risk of NMSC and Kaposi’s sarcoma in this population, albeit by mechanisms distinct from immunosuppression. Important future directions include identifying molecular predictors of rapamycin/rapalog sensitivity or resistance (potentially, for example, PI3K pathway alterations and KRAS mutations) and combined non-rapalog, mTOR-targeting approaches, all of which should increase efficacy and minimize toxicity. Cancer Prev Res; 4(7); 957–61. ©2011 AACR.
polyposis coli (APC) loss-of-function mutations (10). Tuberous sclerosis complexes 1 and 2 (TSC1 and TSC2) function as tumor suppressors and are implicated in kidney cancer (9), presumably through their effect on the mTOR signaling pathway. The TSC1–TSC2 complex acts as a GTPase-activating protein. Following phosphorylation by 5'-AMP–activated protein kinase (AMPK), TSC2 stimulates Ras homologue enriched in brain (RHEB), thereby inhibiting mTORC1 signaling. TSC2 also can be phosphorylated by AKT or GSK3β, which negatively regulates mTORC1, providing additional evidence for the existence of multiple regulatory mechanisms of the mTOR signaling pathway and cell growth.

mTORC2 is associated with the regulation of cytoskeletal organization and cell polarization. mTORC2 phosphorylates and activates AKT through a feedback loop [at serine 473 (Ser473)], including serum- and glucocorticoid-regulated kinase and protein kinase C (PKC), thus playing an important role in regulating cell survival, cell-cycle progression, and anabolism. After phosphorylation at Ser478, AKT is primed for further phosphorylation at threonine 308 (Thr308), which leads to its full activation. TPA is a potent inducer of PKC in this context, enhancing phosphorylation of AKT at both Ser473 and Thr308. Rapamycin inhibits mTORC1, but chronic use of this drug may also inhibit mTORC2 (16). Rapamycin binds to a 12-kDa FK506-binding protein, also known as prolyl isomerase (PPIase), thereby inhibiting the ability of mTORC1 to phosphorylate its downstream effectors. mTORC1 inhibition by rapamycin or other rapalogs has antineoplastic effects, suggesting that mTORC1 is a relevant common effector protein complex involved in the pathogenesis of various cancers (Table 1). Specifically, epidermal transgenic expression of RHEB, a direct activator of mTORC1, sensitized murine

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**Table 1. Summary of animal studies supporting the role of rapamycin-mediated inhibition of mTOR in cancer therapy/prevention**

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Description of effects</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Skin</td>
<td>Reduction in tumor numbers, tumor size, and progression of benign lesions to SCCs in UVB-induced SKH-1 hairless, DMBA–TPA 2-stage and cyclosporine A–mediated carcinogenesis in various murine models</td>
<td>22, 26, 38–41</td>
</tr>
<tr>
<td>Head and neck</td>
<td>Reduction in the oral carcinogen-specific mouse model, human xenograft, and oral-specific Kras/p53 two-hit carcinogenesis murine models</td>
<td>22, 42–44</td>
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<td>Gastrointestinal (liver, intestine, stomach, and pancreas)</td>
<td>Inhibition of hepatocellular carcinoma in xenograft and orthotopic murine models; reduction in intestinal polyp formation and mortality in ApcΔ716 engineered mice; inhibition of gastric cancer in xenograft immune deficient mouse models; mimicked the anticancer effects of calorie restriction in a murine model of pancreatic cancer</td>
<td>28, 36, 45–47</td>
</tr>
<tr>
<td>Kidney</td>
<td>Inhibition of tumor growth in A/J Tsc2+/− mice and xenograft models</td>
<td>48, 49</td>
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<td>Breast</td>
<td>Inhibition of tumor growth of premalignant and malignant lesions in ductal carcinoma in situ and Erb2-dependent transgenic breast cancer mouse models</td>
<td>50, 51</td>
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<tr>
<td>Prostate</td>
<td>Diminution of prostate adenocarcinoma in Pten+/− and Pten−/−/Tsc2+/− mice.</td>
<td>52</td>
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<tr>
<td>Bladder</td>
<td>Prevention of invasive bladder cancer in a murine model carrying bladder epithelium-specific deletion of p53 and Pten.</td>
<td>53</td>
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<tr>
<td>Rhabdomyosarcoma</td>
<td>Lack of published studies in murine models, however, a clear benefit of combinatorial chemotherapy in humans</td>
<td>54</td>
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<td>Lung</td>
<td>Inhibition of benzo(a)pyrene-, tobacco carcinogen (NNK)-induced lung tumorigenesis in A/J as well as in Tsc1/Kras double-mutant mice</td>
<td>23, 55, 56</td>
</tr>
<tr>
<td>Anal</td>
<td>Rapamycin slowed the growth of anal cancer both in a murine model and in humans.</td>
<td>57</td>
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<td>Uterine</td>
<td>Eker rat model of uterine leiomyomatata faithfully recapitulates the human transcriptional profile of this tumor. In this model, mTOR activation (followed by IRS-1 phosphorylation) occurs early, or in endometrial hyperplasia, and the rapamycin analogue WAY-129327 significantly decreased hyperplasia, proliferation, and tumor incidence, multiplicity, and size</td>
<td>58</td>
</tr>
</tbody>
</table>

Abbreviations: DMBA, 7,12-dimethylbenz[a]anthracene; NNK, nicotine-derived nitrosamine ketone; IRS-1, insulin-receptor substrate 1.
skin to SCC induction (17). The phosphoinositide 3-kinase (PI3K)/AKT/mTOR signaling pathway is also activated by mutations in the p110α subunit of PI3K called PI3CA, and preclinical investigations suggest that PI3CA mutations may predict the clinical outcome of PI3K/AKT/mTOR-axis inhibitors (18).

In this issue of the journal, a report by Checkley and colleagues further expands our understanding of the mechanisms by which rapamycin inhibits phorbol ester-mediated skin tumor promotion (19). These investigators showed that the inhibition by rapamycin of TPA-induced mTORC1 [characterized by enhanced phosphorylation of mTOR (Ser2448), p70S6K (Thr389), p4E-BP1 (Ser65 and Thr37/46), pS6-ribosomal protein (Ser240/244), and AKT (Thr308, Ser473)] resulted in a significant reduction in the epidermal labeling index as well as TPA-induced hyperplasia. Of importance, these events were followed by a significant decrease in dermal inflammation that was associated with decreased infiltration of inflammatory cells (T cells, macrophages, neutrophils, and mast cells). Chronic inflammation is a known risk factor for various human cancers, including skin cancer (20). These data suggest that mTORC1 is a key regulator of proliferation and inflammation at the site of TPA treatment of mouse skin. Presumably, therefore, rapamycin inhibits expression of multiple cytokines/chemokines and their receptors, known to promote proliferation and inflammation responses (21). However, the anti-inflammatory effects of rapamycin on macrophage and T-cell infiltration may depend on the type of treatment protocols, including routes of administration (22, 23).

An important finding in this article was that, contrary to its inhibitory effects at lower doses on AKT phosphorylation, rapamycin increased AKT phosphorylation at both Ser473 and Thr308 when given at 200 nmol in multiple administrations. Although the mechanisms for these observations are unclear, the authors suggest that an mTOR-dependent negative feedback loop may be responsible. It is conceivable, however, that the effects of rapamycin on cell survival signaling are associated with a biphasic dose response which involves distinct mechanisms. A clarification of this as yet unresolved issue would require the demonstration that rapamycin-mediated inhibition of AKT phosphorylation at both Ser473 and Thr308 also occurs through its inhibition of mTORC2 and that rapamycin is able to inhibit TPA-induced PKC (vide supra). Loss of RICTOR results in a complete loss of AKT phosphorylation at Ser473, leading to suppression of phosphorylation of the transcription factors forehead box O 1 (FOXO1) and FOXO3 but not of its other substrate TSC2 (tuberin). Apparently, mTORC2 favors cell survival through AKT-mediated inhibition of FOXO1 and FOXO3, which are known to activate genes that promote apoptosis (10, 20). This year, Zinzalla and colleagues provided evidence that mTORC2 is activated by its direct physical association with the ribosomal machinery which ensures
that mTORC2 activity is calibrated to complement the intrinsic growth capacity of the cell (24, 25).

Of importance, Checkley and colleagues also observed that rapamycin is a potent inhibitor of TPA-mediated tumor promotion in murine skin (Fig. 1). It abrogates papilloma formation and reduces the progression of papillomas to SCC (19). Similar anticarcinogenic effects of rapamycin (Table 1) were previously reported in UVB-induced photocarcinogenesis and 2-stage chemical carcinogenesis murine models (22, 26). Consistent with the observations of Checkley and colleagues, rapamycin has preventive and therapeutic effects on cancer in humans (27–31). A significant clinical effect of rapamycin treatment is its ability to delay the development of premalignancies and to reduce the incidence of new NMSCs in organ transplant recipients (3).

In conclusion, by using the TPA-induced skin cancer model in mice, Checkley and colleagues have provided new insights into the potential utility of rapalogs in preventing and treating environmentally induced skin cancer. Their data indicate that signaling through mTORC1 contributes significantly to inflammation and ultimately to skin tumor promotion (Fig. 1). On the basis of the understanding of compensatory feedback loops between mTORC1 and mTORC2, second-generation inhibitors of this pathway have been developed (32). These agents simultaneously inhibit both mTOR complexes, and some of them also inhibit PI3K, which is a key player in regulating this feedback loop (18). mTOR inhibitors require a word of caution, however: despite their strong effect in preventing cancer in both animals and humans, their chronic use in humans is associated with a number of side effects which often require discontinuation of therapy and are a major concern in the cancer prevention setting (3, 18, 33). Metformin and other biguanides may have a role in addressing this concern. These agents are widely used in treating diabetes, where they have a tolerable risk profile, and recent data suggest that they may be effective cancer chemoprevention agents (34, 35). In vivo, metformin downregulates mTORC1 via several potential pathways, including AMPK-dependent and -independent mechanisms (34, 35). Important data show that caloric restriction partially mimics the anticancer effects of rapamycin in a murine model of pancreatic cancer (36). Substantial data on potential predictive markers of mTOR-inhibitor efficacy for cancer therapy and prevention have highlighted the predictive potential of KRAS mutations and markers of PI3K/AKT pathway activation (37). Predictive markers and combinations of non-rapalog approaches for targeting mTOR should increase efficacy and minimize toxicity. A specific cancer prevention approach combining metformin and caloric restriction modalities as part of an mTOR-targeted regimen should prove useful, particularly because safety is such a critical factor for chemoprevention trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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