Review

The Mammalian Target of Rapamycin Pathway as a Potential Target for Cancer Chemoprevention

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Abstract

The mammalian target of rapamycin (mTOR) is a key signaling node coordinating cell cycle progression and cell growth in response to genetic, epigenetic, and environmental conditions. Pathways involved in mTOR signaling are dysregulated in precancerous human tissues. These findings, together with the intriguing possibility that mTOR suppression may be associated with antitumor actions of caloric restriction, suggest that mTOR signaling may be an important target for chemopreventive drugs. (Cancer Epidemiol Biomarkers Prev 2007;16(7):1330–40)

The National Cancer Institute, Division of Cancer Prevention has recently published a series of reviews on mechanism-based targets for cancer-preventive intervention including peroxisome proliferator–activated receptors (1), inducible nitric oxide synthase (2), and epigenetic modulators, primarily histone deacetylases and DNA methyl transferases (3, 4). Here, the potential of mammalian target of rapamycin (mTOR) signaling in chemoprevention strategies is reviewed.

mTOR Signaling

mTOR is the target of rapamycin, a macrolide antibiotic and immunosuppressant of the phosphoinositide kinase family. It is a component of two protein complexes, mTORC1 and mTORC2. mTORC1 consists of mTOR, mLST8, and raptor (regulatory-associated protein of mTOR), and mTORC2 consists of mTOR, mLST8, mSIN1 (mitogen-activated protein kinase–associated protein 1), and rictor (rapamycin insensitive companion of mTOR; refs. 5, 6). Rapamycin bound to FKBP12 inhibits mTORC1, but, with important exceptions described below, not mTORC2 (6). mTOR is a serine-threonine kinase with lipid kinase activity. Its signaling is activated when the genetic and environmental milieu is optimal for cellular growth, and diminishes under stressful conditions including insufficient nutrients, energy, or growth factors, as well as DNA damage (refs. 7-13; Fig. 1). Thus, TOR protein is essential for cell growth and development and is involved in regulating cell cycle progression, cell size, cell migration, and survival; it also negatively governs autophagy, wherein proteins and organelles are degraded during nutrient deprivation (8-13). Disruption of the gene encoding TOR is lethal in all species.

mTOR regulates both transcription of genes relevant to carcinogenesis, including, for example, hypoxia inducible factor (HIF)-1α (14-17), and activity of the procarcinogenic phosphotidylinositol 3-kinase (PI3K)/AKT pathway (18, 19). As described below, mTOR activity mediates AKT-activated cell proliferation and survival; however, one of mTOR effectors, ribosomal protein S6 kinase 1 (S6K1), is a feedback inhibitor of insulin- and insulin-like growth factor (IGF)–induced PI3K activation. S6K1, stimulated by activated mTOR, phosphorylates insulin receptor substrate proteins, inhibiting their function, which in turn diminishes signaling through the PI3K/AKT pathway (reviewed in refs. 19, 20). Thus, cells regulated by this mechanism can become resistant to mTOR inhibition as S6K1 production decreases and AKT rebounds (5, 19, 21). An additional complexity is the finding that mTORC2 may also participate directly in activation of AKT (refs. 5, 6; Fig. 2), and so mTOR inhibitors may reduce AKT activity regardless of losing feedback inhibition by S6K1. Based on studies in cancer cell lines and in leukemia patients treated with rapamycin, Sabatini et al. (5, 6) have suggested that where rapamycin is effective against PI3K/AKT signaling, it interferes with mTORC2 assembly and function.

Many inputs that signal to mTOR converge on the tuberous sclerosis complex (TSC) of tumor suppressor protein hamartin, encoded by the *TSC1* gene, and tuberin, encoded by the *TSC2* genes. The small GTP-binding protein Ras homologue enriched in brain (Rheb), which can bind directly to and up-regulate mTOR (22), is inactivated by the GTPase activating protein activity of TSC (9-12, 23). Tumor suppressors phosphatase and tensin homologue (PTEN; associated with Cowden disease) and LKB1 (associated with Peutz-Jeghers syndrome; ref. 24) also down-regulate mTOR via mTORC2 and mTORC1, respectively, and their loss is similarly associated with autosomal dominant hamartoma syndromes.

The best characterized targets of mTOR phosphorylation are two families of proteins that control translation, ribosomal protein S6 kinases (S6K1 and S6K2 in mammals) and eukaryotic initiation factor 4E (eIF-4E)–binding protein 1 (4E-BP1). In mTORC1, raptor may act as a scaffolding protein, linking mTOR to S6K1 and 4E-BP1, and has generally been reported as a positive regulator of mTOR (reviewed in ref. 11). S6K1 is activated by phosphorylation and regulates ribosomal protein translation and ribosome biogenesis (9-11). The role of S6K1 in feedback inhibition of AKT was described above, and other studies in S6K-deficient organisms have established its importance in the control of cell and organism growth (25, 26).

Phosphorylation by mTOR inactivates 4E-BP1. In quiescent cells, unphosphorylated 4E-BP1 binds and inhibits eIF-4E, which is present in rate-limiting quantities relative to other components of the translational apparatus and is a key regulatory factor for protein synthesis. eIF-4E binds to the 7-methylguanosine–containing cap of mRNA and participates

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in the transfer of mRNA to the 40S ribosomal subunit. mTOR phosphorylation decreases the binding affinity of 4E-BP1 for eIF-4E, which leads to increased translation of cap-dependent mRNAs (9-11).

mTOR as a Hormone and Growth Factor Sensor. Although the mechanisms are not yet completely understood, effects of hormones and growth factors seem to be mediated initially by mTORC2 (5). Besides insulin and IGF, epidermal growth factor and platelet-derived growth factor interact with mTORC2 primarily by activating PI3K. PI3K activation is blocked by PTEN as well as by S6K1 (9, 10, 23, 24); PTEN dephosphorylates lipid products generated by PI3K, down-regulating PI3K signaling (Fig. 2). The lipid product of PI3K localizes AKT to the plasma membrane, where it is phosphorylated and activated by 3-phosphoinositide–dependent protein kinase 1 and mTORC2 (5). AKT may then activate mTORC1 signaling, largely by directly phosphorylating and inactivating TSC2. In addition to signaling via PI3K/AKT, growth factors activate mTOR by a pathway involving phosphatidic acid and phospholipase D1 (27-29).

mTOR as a Nutrient and Energy Sensor. mTORC1-mediated activity is positively regulated by the level of intracellular amino acids (8, 10-12, 23). The branched-chain amino acid leucine, principal indicator of amino acid supply in mammals (30), is an effective regulator of mTOR activity in most cell types (8, 31-34); TSC proteins (35, 36) and Rheb (37-39) have also been implicated in the nutrient-sensing branch of the mTOR pathway. However, molecular events involved in nutrient sensing remain largely unknown (10-12, 23).

Molecular mechanisms involved in mTORC1-mediated energy sensing have been associated with both TSC and LKB1. Although initial studies suggested mTOR as the direct cellular sensor of ATP levels (40), increasing evidence implicates AMP-activated protein kinase A (AMPK) in regulation of mTOR activity. A more sensitive indicator of cellular energy status than ATP, AMP increases when the ATP/ADP ratio decreases, and a high ratio of AMP/ATP signals that the energy status of the cell is compromised (41). AMP-stimulated AMPK activity is sensitive to very small changes in intracellular AMP levels (41). In energy-deprived cells, AMPK directly phosphorylates and enhances the ability of TSC2 to inhibit mTORC1 signaling (42). LKB1 can directly phosphorylate and activate AMPK (43-45), and under conditions of energy stress, LKB1 is required for mTORC1 suppression, which is dependent on AMPK and TSC (46). AMPK can also directly phosphorylate mTORC1 under conditions of energy stress (47). Additional stress-induced mechanisms of mTOR regulation have been described (12, 17, 24, 48-51).

mTOR Signaling and Cancer

Knowledge about consequences of dysregulated mTOR signaling for tumorigenesis comes mostly from studies in which mTOR has been pharmacologically disrupted by rapamycin and its analogues CCI-779, RAD-001, and AP23573 (5, 13, 48).

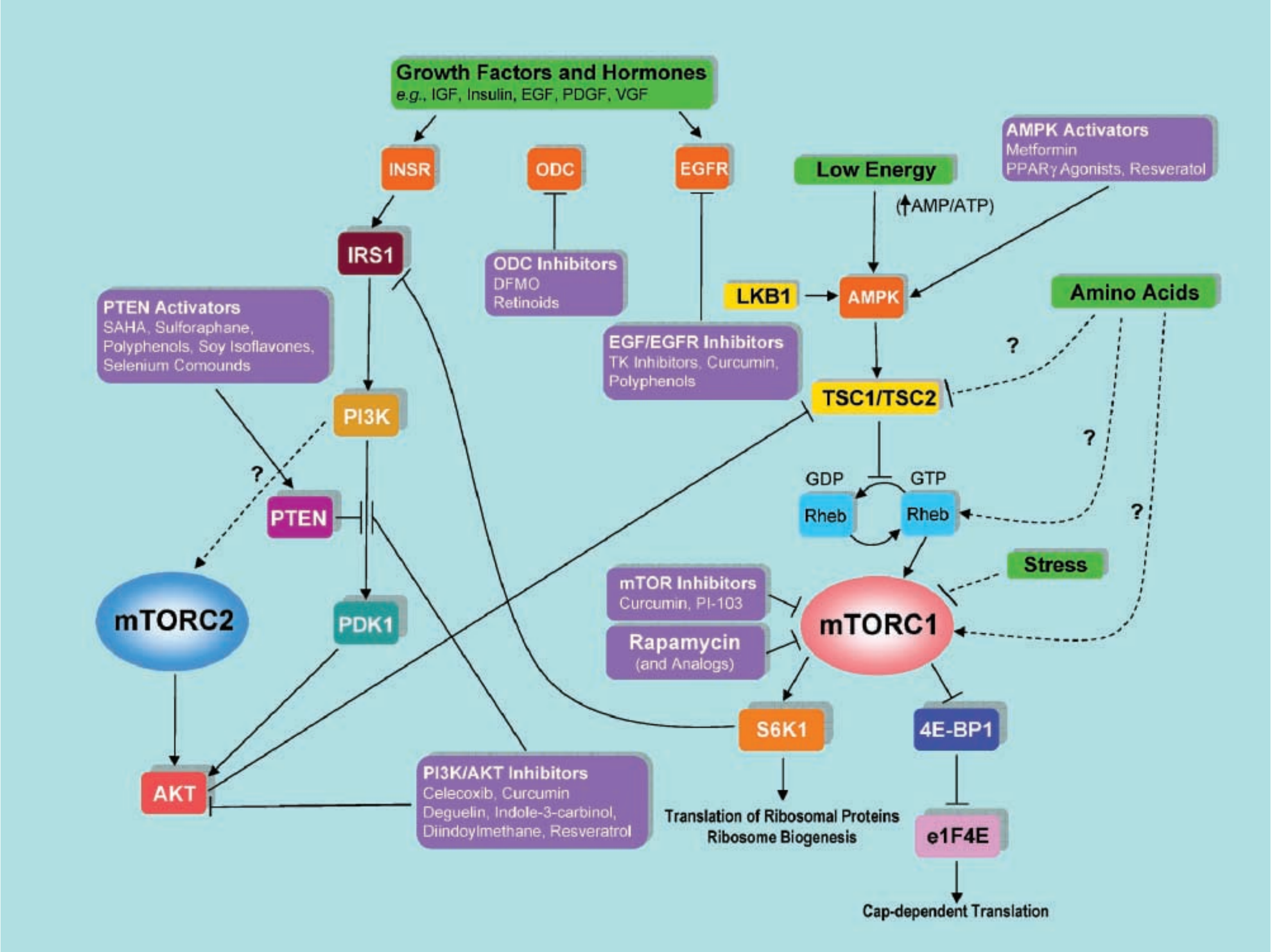

Figure 1. mTOR functions as a central control protein integrating signals from a host of environmental factors, including amino acids, energy, hormones, growth factors, cytokines, and other stress factors.

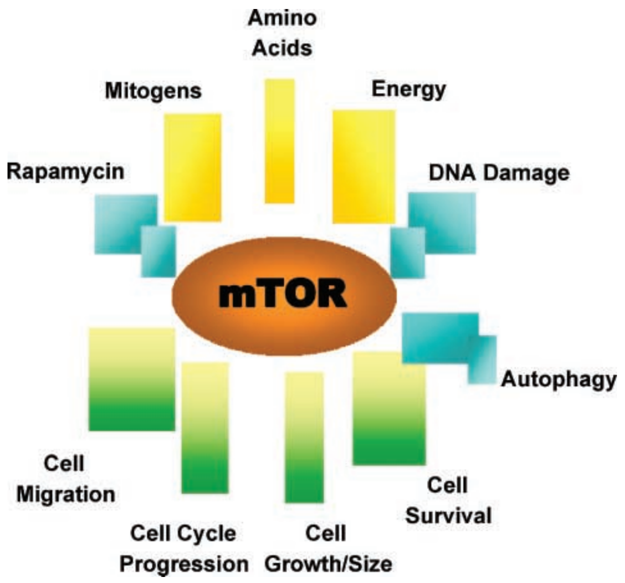

Figure 2. Cancer preventive modulation of mTOR signaling pathways and targets for cancer preventive intervention. Upstream of mTOR: Growth factors and hormones signal to mTOR by activating the PI3K/AKT/PTEN axis. Lipid products of PI3K activate AKT via mTORC2 complex. Activated AKT phosphorylates and inactivates TSC2, thereby allowing signaling (which occurs via mTORC1 complex). PTEN dephosphorylates lipid products generated by PI3K, down-regulating PI3K signaling. Rheb functions as a positive regulator of mTORC1; it is inactivated by TSC GTPase activating protein function. Amino acids and stress activate mTORC1 independently of PI3K/AKT. TSC proteins and Rheb are involved, but the mechanisms are largely unknown. In energy-deprived cells, AMPK phosphorylates and increases TSC2 inhibition of mTORC1. LKB1 phosphorylates and activates AMPK. In energy-deprived cells, LKB1 is required for mTORC1 suppression, which is dependent on AMPK and TSC2. Downstream of mTOR: mTORC1 directly or indirectly activates S6K1, which regulates ribosomal protein translation and ribosome biogenesis. S6K1 also is a feedback inhibitor of PI3K/AKT signaling by blocking insulin receptor substrate. mTORC1 also directly phosphorylates and inactivates 4E-BP1, decreasing its binding affinity for eIF-4E, which leads to increased translation of cap-dependent mRNAs. Known tumor suppressors that negatively regulate mTOR pathways appear in yellow boxes. *Dashed lines,* unknown/uncertain pathways. Targets for cancer preventive intervention (see text and Table 2): In addition to direct inhibition of mTOR by rapamycins and a few other compounds, other targets upstream on mTOR signaling pathways are affected by known chemopreventive agents. Examples shown on this figure in lavender boxes include inhibitors of protein kinase–mediated cell proliferation signaling [ornithine decarboxylase (*ODC*) and epidermal growth factor (*EGF*)/epidermal growth factor receptor inhibitors (*EGFR*)], activators of tumor suppressor (PTEN) gene transcription (DNA methyl transferase, histone deacetylase, and histone acetyltransferase inhibitors), direct inhibitors of PI3K/AKT signaling, and activators of AMPK.

In therapeutic models, these selective mTOR inhibitors suppress growth of a diverse range of cancer types, with their effectiveness apparently depending on concomitant blockage of PI3K/AKT signaling (48, 52, 53). Although their immediate utility for long-term cancer prevention is not yet established (see Strategies for the Development of mTOR Inhibitors in Clinical Prevention Studies), rapamycins also seem to be efficacious in initial prevention proof-of-principle experiments (discussed in mTOR Inhibition to Prevent Cancer).

eIF-4E seems to be the most crucial downstream effector of mTOR-associated carcinogenesis (54). Overexpression of S6K in rat cells induces morphologic changes but does not lead to oncogenic transformation (55), and S6K1 alterations are rare in human cancers (9). On the other hand, overexpression of eIF-4E alone or in combination with other oncogenes transforms cells *in vitro* (reviewed in ref. 54). In transgenic mice, overexpression of eIF-4E significantly enhances transformation in cooperation with c-Myc, albeit with a long time lag (56, 57).

mTOR inhibition affects tumorigenesis by slowing or arresting cells in the G_1 phase of the cell cycle, promoting apoptosis, and affecting angiogenesis pathways (9, 10, 23, 58). The effects on cell cycle progression are mediated, at least in part, by blocking the ability of eIF-4E to enhance translation of mRNAs encoding positive cell cycle progression regulators, such as cyclin D1 and ornithine decarboxylase, and to inhibit translation of negative regulators, such as cyclin-dependent kinase inhibitors (9, 54). Although the effects of rapamycins are often cytostatic, they can also induce apoptosis in cell lines (59, 60) and *in vivo* in AKT-dependent precancerous prostatic mouse tissue (17). In AKT-dependent mouse lymphomas, rapamycin restored apoptotic response to cytotoxic agents, which was then reversed by eIF-4E expression; rapamycin alone was minimally effective in this system (56).

Rapamycins also exert antiangiogenic effects *in vivo*, in conjunction with decreased production of proangiogenic vascular endothelial growth factor (VEGF; refs. 58, 61, 62), which, along with its receptor, is controlled by HIF-1α (63). These findings are consistent with studies showing that PI3K/AKT/mTOR signaling can up-regulate HIF-1α–dependent responses in hypoxic cells (16) and precancerous lesions *in vivo* (17).

Varying levels of AKT activity may also explain the differences in malignancy potential of tumors arising in subjects with hamartoma syndromes. Unlike cells lacking PTEN, in which AKT is constitutively active, TSC1/2- and LKB1-deficient cells show diminution of AKT activation, which is attributed to higher mTOR activity and S6K feedback inhibition of the PI3K/AKT pathway (reviewed in ref. 64). Malignant tumors are common in Cowden disease (associated with mutated PTEN) but less frequent in TSC and Peutz-Jeghers syndrome (associated with mutations in TSC1/2 and LKB1, respectively). It is unknown whether losing S6K1 feedback inhibition of PI3K/AKT will eventually promote malignant progression in those with benign hamartoma syndromes. However, as rapamycin prevents development of macroscopic renal lesions in a TSC rat model (65), and activation of eIF-4E rather than S6K may be responsible for oncogenic actions of aberrant mTOR signaling, perhaps mTOR inhibition will obstruct signaling pathways promoting tumor growth in TSC despite AKT (64).

Thus far, there is very little published information on clinical studies relevant to inhibition of mTOR signaling in patients with hamartoma syndromes. One very interesting, albeit preliminary, approach is the use of PI3K inhibitors in patients with Cowden syndrome. A phase I study of a new PI3K inhibitor, BEZ235, opened in January 2007 and will include advanced-stage cancer patients with Cowden syndrome (66). In July 2003, the National Institute of Neurological Disorders and Stroke published a research plan for TSC stemming from discussions and results presented at an international symposium on TSC held in September 2002 (67). Recommendations for clinical studies were made in the research plan and at the symposium, including phase I/II studies of mTOR inhibitors in TSC patients. One of the recommended studies, evaluation of the effect of rapamycin on TSC-associated renal angiomyolipoma, is in progress (68). Another phase I/II study is evaluating the effects of RAD-001 on astrocytomas in patients with TSC (69).

Dysregulation of mTOR Signaling in Precancerous Human Tissues: Targets for Chemoprevention

Neither mutation nor amplification of mTOR has been found in human cancers (70). However, dysregulation in mTOR signaling pathways in premalignant (Table 1) as well as early malignant (9, 13, 70, 71) human tissues suggests mTOR as a promising target for cancer prevention strategies. For example, loss of PTEN and AKT up-regulation occur during human carcinogenesis generally as relatively late events more prevalent in advanced dysplasia or carcinoma *in situ* than in early dysplasia. In a study using tissue array analysis, it was found that 11% (13 of 113) of ductal carcinoma *in situ* human breast lesions had PTEN loss, increasing to 26% (35 of 134) of invasive cancers; AKT up-regulation occurred in 33% (38 of 114) of ductal carcinoma *in situ* cases (72). In testicular carcinogenesis, PTEN is lost during progression from intratubular germ cell neoplasias to cancers (73). Methylation (silencing) of the PTEN promoter has also been reported in cervical intraepithelial lesions (74). AKT is activated in intraepithelial prostate lesions (75, 76); one study found that transition from histologically normal epithelium to prostatic intraepithelial neoplasia was marked by a surge in AKT activation, concurrent with suppression of apoptotic pathways (76). Other studies observed early onset of high-grade prostatic intraepithelial neoplasia in PTEN mutant mice (77, 78). Endometrial carcinogenesis is one notable exception to late-occurring PTEN loss; PTEN mutations are prevalent in both precancerous and cancerous endometrial lesions (79, 80).

Amplification of the catalytic subunit of PI3K (81) and activation of AKT (81-83) are associated with development of severe dysplasia in the lung; tobacco-specific carcinogens activate AKT in primary human lung epithelial cells *in vitro* (84). eIF-4E expression also increases during progression from atypical adenomatous hyperplasia to invasive lung cancer (85). In the colon, both AKT (86) and eIF-4E are overexpressed in adenomas (87, 88); the latter is often associated with elevated cyclin D1 levels (87). AKT activation is also seen in precancerous stages of malignant melanoma (89, 90). In head and neck lesions, expression of eIF-4E increases with increasing dysplasia (91).

mTOR Inhibition to Prevent Cancer

Five recent studies with rapamycin and its analogues supply compelling data to assess chemopreventive potential of mTOR inhibition. In the first study, a 2-week treatment with the rapamycin analogue RAD-001 (10 mg/kg body weight/d, i.g., as a microemulsion) completely reversed the prostatic intraepithelial neoplasia phenotype in ventral prostates of mice expressing human AKT1 by inducing apoptosis (blocked by coexpression of proapoptotic Bcl-2) and inactivating HIF-1α target genes, including genes encoding most glycolytic enzymes (17). In the second study, another rapamycin analogue, CCI-779 (20 mg/kg body weight/d, i.p., for 8 days), diminished sizes and total number of murine lung lesions (atypical alveoli, hyperplasias, and adenomas) induced by somatic K-RAS activation. Most of this effect was due to decreased progression to adenomas (92).

The third study is relevant to prevention in adjuvant settings and to prevention of AKT-induced antiestrogen resistance (93). The mTOR pathway was up-regulated in aromatase expressing breast cancer cells with hormones (estradiol or androstenedione). Up-regulation was reversed by treatment with RAD-001 or the aromatase inhibitor letrozole (94). Both letrozole and RAD-001 inhibited androstenedione-induced proliferation. The agents showed synergistic activity in combination, which is attributed to increased inhibition of G_1 progression and increased apoptosis. The combination is now being evaluated in a phase II trial in breast cancer patients before surgery (neoadjuvant setting; ref. 93).

In the fourth study, rapamycin showed chemopreventive activity against mammary gland tumors in transgenic mice bearing activated ErbB2 (HER-2/*neu*) receptor either alone (NeuYD) or with VEGF expression (NeuYD × VEGF; ref. 58). Low-dose rapamycin (0.75 mg/kg, i.p., every other day) was given to groups of 20 females of both strains, starting ~2 weeks before the expected appearance of spontaneous mammary gland tumors (i.e., at 92 days of age in NeuYD mice and 38 days of age in NeuYD × VEGF mice) until sacrifice (at 138 days of age). Rapamycin dramatically inhibited tumor formation in NeuYD mice. One third of rapamycin-treated NeuYD mice had no clinically detectable tumors at sacrifice, whereas 100% of controls had multiple tumors; tumor weight and volume per mouse were significantly reduced compared with controls ($P < 0.0007$). A lesser, yet significant, chemopreventive effect was seen against more aggressive tumors in NeuYD × VEGF mice, and tumor weight and volume per mouse were significantly reduced ($P < 0.001$). In both strains, rapamycin suppressed growth of established tumors, more profoundly in NeuYD × VEGF mice. Of note, the effect of rapamycin was cytostatic rather than cytotoxic because tumor regrowth was apparent by 10 days after discontinuation of rapamycin. Additional experiments in monolayer and three-dimensional culture suggested that chemopreventive and growth-inhibiting effects of rapamycin in this setting could result from suppression of ErbB3 (thereby inhibiting heterodimerization of ErbB2 and ErbB3 receptors; ref. 95) and of HIF-1α. ErbB2 (HER-2/*neu*) is overexpressed in approximately one third of human breast tumors, suggesting mTOR inhibition as a possible chemopreventive strategy against metachronous tumors or recurrence in high-risk patients whose primary tumors overexpressed ErbB2 or in patients showing dysregulation of the PI3K/AKT/mTOR signaling pathway.

The fifth study also evaluated chemopreventive effects of rapamycin in a transgenic mouse model of human breast carcinogenesis (96). Mammary intraepithelial neoplasia outgrowths model human ductal carcinoma *in situ*, carry the oncogene PyV-mT (which activates ErbB2 signaling, including the PI3K/AKT pathway), are transplantable, and develop into invasive mammary gland tumors. Rapamycin (0.75 and

Table 1. Alterations upstream and downstream of mTOR in precancerous human tissues

Target	PI3K/AKT/mTOR pathway alteration	Reference
Endometrium	PTEN mutation	(70, 79)
Lung	PI3K catalytic subunit amplification, AKT activation, eIF-4E overexpression	(76-78, 81, 148)
Head and neck	AKT activation, eIF-4E overexpression	(91, 149, 150)
Esophagus (Barrett's)	AKT activation	(151)
Oral cavity	eIF-4E overexpression	(152)
Colon	AKT overexpression; eIF-4E overexpression	(86-88)
Prostate	AKT activation and overexpression; PTEN loss (late)	(70, 75-78)
Cervix	PTEN promoter Methylation	(74)
Skin (melanoma)	AKT activation, HIF-1α expression	(89, 90, 153)
Kidney	TSC loss, HIF-1α expression	(147)
Leukemia	PTEN loss in hematopoietic stem cells	(154)
Breast	AKT activation and overexpression, mTOR activation, S6 activation, PTEN loss (late)	(72)
Testicles	PTEN loss (late)	(73)

Table 2. Chemopreventive agents with potential to modulate mTOR signaling

Agent	Chemopreventive activity	References
DNA methylation inhibitors (activate *PTEN* gene transcription)		
5-Aza-2′-deoxycytidine (decitabine)	Intestine, lung, prostate	(4)
Tea polyphenols (epigallocatechin gallate, polyphenon E)	Colon, prostate, esophagus, bladder, forestomach, liver, lung, breast, small intestine, skin	(155-160)
Catechin	Intestines, skin	(161-163)
Soy isoflavones (genistein)	Breast, prostate, skin, stomach	(164-167)
Flavonoids (quercetin, myricetin)	Lung, skin	(161)
Caffeic acid	Forestomach	(168)
Chlorogenic acid	Intestines, lung, skin	(168)
Sodium selenite	Colon, esophagus, liver, breast, pancreas, stomach	(169)
Benzyl selenocyanate	Colon, forestomach, breast	(169)
1,4-Phenylenebis(methylene)selenocyanate	Colon, lung, breast	(169)
Histone deacetylase inhibitors (activate *PTEN* gene transcription)		
Sulforaphane	Colon, forestomach, breast	(170-173)
Sodium butyrate, phenylbutyrate	Colon (aberrant crypts)	(4, 174)
Diallyl sulfide	Colon, esophagus, forestomach, liver, lung, breast, skin, thyroid	(4, 175, 176)
Phenethyl isothiocyanate	Esophagus, forestomach, lung, breast, pancreas	(177, 178)
Suberoylanilide hydroxamic acid	Breast, lung	(4, 179, 180)
Valproic acid	Intestine	(4, 181)
Histone acetyltransferase inhibitors (activate *PTEN* gene transcription)		
Curcumin	Colon, duodenum, forestomach, breast, skin, tongue	(182, 183)
Ornithine decarboxylase inhibitors (inhibit protein kinase–mediated cell proliferation)		
2-Difluoromethylornithine	Colon, bladder, skin	(156, 157)
Retinoids (fenretinide)	Breast, skin, head and neck, bladder, ovary, colon	(156, 157)
PI3K/AKT inhibitors		
Celecoxib	Colon, bladder, skin, lung, esophagus, head and neck, breast, prostate	(120-122, 157)
Curcumin	Colon, duodenum, forestomach, breast, skin, tongue	(160, 182-184)
Deguelin	Lung, breast, colon	(119, 185, 186)
Indole-3-carbinol, diindolylmethane	Breast	(116, 117)
Resveratrol	Colon, prostate, breast	(187-192)
Rosiglitazone	Breast, prostate, colon	(1, 125)
Soy isoflavones (genistein)	Breast, prostate, colon, skin, stomach	(164-167)
Tea polyphenols (epigallocatechin gallate, polyphenon E)	Colon, prostate, esophagus, bladder, head and neck, forestomach, liver, lung, breast, small intestine, skin	(155-160)
Epidermal growth factor/epidermal growth factor receptor inhibitors (inhibit protein kinase–mediated cell proliferation)		
Tyrosine kinase specific inhibitors (erlotinib, gefitinib, EKB569)	Lung, colon, head and neck, breast	(193-195)
Curcumin	Colon, duodenum, forestomach, breast, skin, tongue	(111, 160, 182, 183)
Resveratrol	Colon, prostate, breast	(160, 187-192, 196)
mTOR inhibition		
Curcumin	Colon, duodenum, forestomach, breast, skin, tongue	(182, 183)
AMPK activators (inhibit AKT-driven blockade of TSC1/TSC2 tumor suppressors)		
Resveratrol	Colon, prostate, breast	(124, 160, 187-192, 196)
Metformin	Breast, pancreas	(45, 127, 128, 197)
Rosiglitazone	Breast, prostate, colon	(1, 125)

3.0 mg/kg body weight, i.p., every other day for 35 days starting 3 weeks posttransplantation) significantly inhibited growth of mammary intraepithelial neoplasia outgrowths, invasive tumor incidence, and tumor burden.

Other studies suggest the utility of inhibiting mTOR signaling in preventive settings. The area of complex atypical hyperplasia in uterine secretory epithelium of $PTEN^{+/-}$ mice diminished when CCI-779 was administered late in life, after most mice had developed these lesions (97). As noted above, rapamycin prevents development of macroscopic renal lesions in a rat model of TSC (65, 98), although without affecting formation of microscopic precursor lesions [ref. 65; as the role of TSC1/2 loss in sporadic human cancers is unknown (99), it is unclear how these findings affect more general prevention]. Lastly, studies in renal transplant patients treated with rapamycin suggest that mTOR inhibition may prevent development of skin cancer in this population (100-102). Rapamycin and CCI-779 have limited antitumor activity against gliomas (particularly medulloblastomas), often seen in patients with basal cell nevus syndrome, presumably because of an effect on the *gli* gene (13). These observations and the high frequency of basal cell skin cancers seen in basal cell nevus syndrome patients (103) led the National Cancer Institute, Division of Cancer Prevention to evaluate topical rapamycin in a phase I proof-of-principle and biomarkers study in basal cell nevus syndrome patients.

The mechanistic data and experimental results presented above suggest that mTOR inhibition will have clinical chemopreventive efficacy primarily in preventing progression of precancerous lesions. They also suggest that mTOR inhibition could be efficiently evaluated in clinical trials with subjects at high risk for developing metachronous lesions. Despite these promising activities of the rapamycins, potential up-regulation of PI3K/AKT (18, 21, 53) by mTOR inhibition may temper the use of mTOR-specific inhibitors as single agents. However, blocking effects of mTOR inhibition on procarcinogenic cell proliferation and angiogenesis and identification of other molecular targets associated with carcinogenic activities affected by mTOR signaling (particularly along PI3K/AKT–mediated pathways) suggest additional cancer prevention strategies that do not rely exclusively on directly inhibiting mTOR; these strategies are described below (Table 2; Fig. 2).

Strategies for the Development of mTOR Inhibitors in Clinical Prevention Studies

Direct Inhibition of mTOR. Ease of administration (i.e., availability of oral formulations) and chronic safety risk are critical considerations for clinical development of chemopreventive agents, although, as suggested above, ''personalized'' medicine may lead to cohort enrichment with high-risk subjects who are more likely to find net benefit in treatment despite inconvenience and some potential toxicity. As potential chemopreventive agents, immunosuppressive activity, cutaneous toxicity (e.g., rashes, nail effects), nephrotoxicity in diseased kidneys, and limited oral bioavailability are concerns with rapamycin and its analogues CCI-779 (rapamycin prodrug), AP23573, and RAD-001, despite their improved solubility and stability (13, 48, 104). RAD-001 may be the most useful of the analogues in chemopreventive settings because of its well-developed oral formulation.

Use of mTOR as a direct target in cancer prevention may also require consideration of other strategies to minimize risk/benefit ratio of using these agents. For example, in preclinical and clinical cancer therapy studies, antitumor effects of mTOR inhibitors are maintained using intermittent dosing schedules, which minimize immunosuppression (13, 52, 70). Using this strategy, partial responses and stable disease have been seen in phase I and II studies in patients with a variety of tumor types, and only mild to moderate toxicities have been observed (13, 48). Topical application to the drug target tissue where feasible (e.g., on skin and lung) may also enhance efficacy and avoid many safety issues (i.e., by decreasing the need for systemic bioavailability and the potential for systemic toxicity). As noted above, topical application of rapamycin is, in fact, being evaluated for prevention of basal cell carcinomas in basal cell nevus syndrome patients. Inhalational exposure to chemopreventive agents also seems to be a promising strategy for chemoprevention of lung cancer (105-107), as evidenced by proof-of-principle studies of budesonide in laboratory animals (105, 106) and in patients with bronchial dysplasia (108). Interestingly, a liposomal formulation of rapamycin for inhalational delivery has been proposed for local immunosuppressive treatment of cytokine-induced inflammation in the lung (109).

A few recent studies found that rapamycin analogues are not the only agents which include direct inhibition of mTOR among their activities. PI-103, a novel dual inhibitor of mTOR and the p110α isoform of PI3K, was recently reported to block phosphorylation of AKT and cause G_0-G_1 cell cycle arrest in glioma cells (110). Unlike the broad-spectrum PI3K inhibitor LY294002, which suppresses AKT phosphorylation and induces cytotoxicity in the same dose range, the effective dose of PI-103 is much lower than the cytotoxic dose. PI-103 also shows significant activity against established tumors in nude mice (110). The dual blockade of AKT and mTOR at relatively low, relatively nontoxic doses makes PI-103 an appealing candidate for chemoprevention. However, its potential toxicities include inhibition of insulin signaling in mice, suggesting possible diabetogenic activity. PI-103 also inhibits DNA protein kinase, a key enzyme for repairing double-stranded DNA, suggesting interference with DNA repair.

Indirect Inhibition of mTOR. Combinations of mTOR inhibitors with established chemopreventive agents, or use of other targets of these chemopreventive agents as surrogates for mTOR, may be effective cancer prevention strategies (Table 2; Fig. 2). The most attractive potential combinations are those that inhibit both mTORC1- and AKT-driven signaling, while taking advantage of the good safety profiles of established chemopreventive agents (e.g., food-derived products or drugs approved for other indications) to attain low effective doses and minimize the potential for toxicity. An example would be the combination of a rapamycin analogue (e.g., RAD-001) with a food-derived chemopreventive agent with AKT-inhibiting activity [e.g., curcumin (111, 112), resveratrol (113), epigallocatechin gallate (114), soy isoflavones (genistein; ref. 115), indole-3-carbinol (116), or diindoylmethane (116, 117)]. Recent preliminary studies have even suggested that some chemopreventive agents with AKT-inhibiting activity may dampen mTOR signaling partially through direct mTOR inhibition. For example, curcumin has shown chemopreventive activity in animal models of carcinogenesis (118) and is under evaluation in phase I clinical trials. It inhibits phosphorylation of mTOR, S6K1, and 4E-BP1 at physiologic concentrations (2.5 μmol/L) in a panel of cancer cells (111). Whereas this activity may not be specific for mTOR, it is noteworthy that much higher concentrations of curcumin (>40 μmol/L) were required to inhibit AKT (111, 112).

mTOR-specific inhibitors might also be combined with other chemopreventive agents that inhibit PI3K or AKT [e.g., deguelin (119) or celecoxib (120-122)] or affect ancillary pathways, such as 2-difluoromethylornithine, which inhibits ornithine decarboxylase and hence protein kinase C–mediated proliferative activity (123), or DNA methyltransferase inhibitors or histone deacetylase inhibitors, which reactivate the hypermethylated PTEN promoter (4).

Some agents may exert chemopreventive activity, at least in part, via the energy-sensing component of mTOR signaling, possibly by activating AMPK. These chemopreventive agents may prove to be effective as the mTORC1 inhibitor component in agent combinations targeting mTOR signaling. For example, resveratrol increased levels of AMPK in mice fed a high-calorie diet and also increased insulin sensitivity and reduced IGF-1 levels (124). Rosiglitazone, an antidiabetes peroxisome proliferator–activated receptor-γ agonist with chemopreventive activity (reviewed in ref. 1), increased PTEN expression, inhibited AKT phosphorylation, increased AMPK phosphorylation, and decreased S6K1 phosphorylation in non–small-cell lung carcinoma cells (125). Metformin, another type 2 diabetes drug, also activates AMPK (126) and has shown chemopreventive activity, suppressing development of carcinogen-induced pancreatic cancers in hamsters fed a high-fat diet (127) and decreasing size and increasing latency of mammary adenocarcinomas in HER-2/*neu* transgenic mice (128). Because type 2 diabetes has been associated with increased risk for pancreatic (129), liver (130), and, presumably, other cancers, treatment for type 2 diabetes could also be cancer chemopreventive. Because mTOR inhibition down-regulates expression of genes encoding enzymes of the glycolytic pathway, [^{18}F]fluorodeoxyglucose-positron emission tomography scanning may provide noninvasive readouts of mTOR activity in tissue to help identify likely responders to treatment with mTOR-inhibiting cancer prevention strategies (17).

Does mTOR Mediate the Effects of Caloric Restriction on Tumorigenesis? A Primary Prevention Model

Human epidemiologic studies of calorie restriction are difficult to conduct and interpret, particularly because of requirements for extensive estimation of food intake and lack of clarity about the appropriate time period or food sources for observation (131-133). Nonetheless, several carefully documented studies in subjects with access to high-quality diets (i.e., where effects would not as likely be confounded by malnutrition) have suggested that calorie restriction can increase longevity and reduce cancer and other disease incidences (reviewed in ref. 132). For example, Kagawa et al. showed that Okinawans ingesting ~20% fewer calories than the overall Japanese population also had lower incidences of cerebral vascular disease, cancer, and heart disease (132, 134). In addition, cancer incidences in prostate, breast, and colon have been associated with calorie (energy) intake. As one example, Platz and colleagues showed modest associations of energy intake and body size or physical activity with increased risk of metastatic or fatal prostate cancer in younger men (age ≤65 years; refs. 135, 136). As a second example, among women 55 to 74 years old in the screening arm of National Cancer Institute Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, those in the highest quartile of energy intake (≥2,084 kcal/d) had a significantly increased risk for breast cancer compared with those in the lowest quartile (<1,316 kcal/d) based on data reported in a food frequency questionnaire at baseline (137). Finally, in a study of 2,073 cases of primary colon cancer and 2,466 age- and sex-matched controls, high-energy intake was associated with increased risk of colon cancer in both men and women [OR, 1.74 (95% CI, 1.14-2.67) for men and 1.70 (95% CI, 1.07-2.70) for women; ref. 138].

In experimental studies, caloric restriction has been shown to prevent cancer and increase life span in a wide variety of mammalian and nonmammalian species. In rodent models, caloric restriction inhibits the development of chemically (e.g., with benzo[*a*]pyrene, *N,N*-diethylnitrosamine, 7,12-dimethybenz[*a*]anthracene, or *p*-cresidine), radiologically, or genetically (e.g., in p53-deficient, WNT-1 transgenic mice) induced tumors (139). Once thought to be a secondary response to decreased cell growth, the inhibitory effects of caloric restriction on tumor development are increasingly recognized as stemming from alterations in specific molecular response pathways (124, 133, 140, 141).

mTOR senses the overall growth milieu of the cell by integrating signals from hormones, growth factors, nutrients, energy, and other environmental factors. During caloric restriction, this checkpoint would not be traversed even in the context of aberrant growth factor stimulation—a hallmark of transformation (142). Cells starved for amino acids (34) or energy (40) do not activate mTOR in response to growth factors. Because mTOR also senses DNA damage (50), decreased signaling through mTOR in caloric restriction may check cell growth subsequent to genetic insult. IGF-1, which is also associated with the antitumor effects of caloric restriction (139, 141), signals through mTOR as well as other downstream effectors.

Rapamycin affects expression of many genes involved in nutrient and energy metabolism, protein synthesis and turnover, stress response, immune modulation, and chromatin remodeling. Genes affected by rapamycin in human B lymphoma cells and mouse T lymphocytes (14) significantly overlap with genes affected by calorie starvation in animals (143). Similar antiproliferative effects are observed in mouse T lymphocytes treated with rapamycin, low levels of glutamine, or glucose. Moreover, rapamycin does not increase the degree of growth inhibition when cells are deprived of nutrients, suggesting that the antiproliferative effects of rapamycin result from its ability to induce a starvation-like signal (14). Thus, studies of rapamycin-induced gene expression support the hypothesis that diminished signaling through mTOR contributes to antitumor effects of caloric restriction.

Finally, it is worth noting that increased longevity, which is also associated with caloric restriction (139, 141), may be at least partially dependent on TOR-mediated pathways. TOR deficiency doubles the natural life span of *Caenorhabditis elegans*; these effects seem to interface with the known negative regulatory effects of the IGF receptor homologue gene on longevity in this organism (144). Inhibition of TOR signaling in *Drosophila melanogaster* extends life span in a manner similar to dietary restriction (145). In mice fed a high-calorie diet, resveratrol both mimicked the effects of calorie restriction and increased survival of the animals; as noted above, the calorie restriction effects were mediated at least partially via AMPK and IGF-1/mTOR signaling (124).

Conclusions

Although discovered little more than a decade ago (146), mTOR is established as a critical central controller which permits cells to progress through G_1 only when all conditions are favorable for growth. Numerous elements of mTOR signaling pathways are dysregulated in precancerous lesions and early evidence shows promising effects of mTOR pathway inhibition in preventive settings. For example, precancerous lesions or early cancers with PTEN loss, or those dependent on PI3K/AKT signaling due to other molecular lesions, may be highly sensitive to mTOR inhibition. Observing clinically normal cells from persons at risk for cancer because of conditions such as TSC suggest that the initial underlying lesion (i.e., TSC2 mutation) seems to be related to cancer development via activation of mTOR pathways (147), potentially leading to identification of the earliest biomarkers and new agents for cancer prevention.

Promising mechanism-based prevention strategies combining rapamycin inhibitors with established chemopreventive agents are suggested by published results. These combination strategies could be directed both to specific cohorts who would likely benefit and to specific cancer settings. For example, dependence of AKT-activated cells on functioning mTOR signaling for growth and proliferation suggests a potential synergy of combinations of mTOR inhibitors with inhibitors of other targets on the hormone/IGF receptor/PI3K/AKT signaling pathways.

Besides IGF receptor/PI3K/AKT, other components of mTOR signaling may also prove to be useful targets for chemoprevention. For example, if eIF-4E is central to mTOR-dependent tumorigenesis, selective targeting of this branch of the mTOR signaling pathway may offer a more favorable therapeutic strategy. An especially promising approach targets the energy sensing component of mTOR signaling by activation of AMPK. Finally, the effect of mTOR inhibition on prevention therapy could be much greater if mTOR pathways are indeed integral to transmitting signals associated with beneficial effects of caloric restriction on tumor development and longevity. This intriguing possibility merits further exploration.

To conclude, despite successful proof-of principle studies with rapamycin and its analogues, use of these drugs alone or in combination with other agents in clinical prevention settings will necessitate development of acceptable chronic toxicity profiles and formulations with improved ease of administration and bioavailability. An alternative approach should involve use of established chemopreventive inhibitors of collateral targets on mTOR pathways (Table 2). Many of these agents are natural food-derived products or drugs developed for chronic oral administration with well-characterized safety profiles. Moreover, it seems that combinations of rapamycin or its analogues together with established chemopreventive agents would most likely be efficacious in clinical cancer prevention trials.

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