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Clinical development of mammalian target of rapamycin inhibitors

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Rapamycin, a natural product, has antimicrobial, immunosuppressant, and antitumor activities that result from modulating signal transduction pathways that link mitogenic stimuli to the synthesis of specific proteins needed for cell cycle progression from G_1 to S phase [1]. Today, rapamycin (sirolimus, Rapamune^{TM}) is approved as an immunosuppressive drug for renal transplant recipients. Two related compounds are in under development: SDZ RAD as an immunosuppressant and the ester CCI-779 as a cancer therapeutic. The immunosuppressant effects of rapamycin are due to its inhibition of the biochemical events required for IL-2 stimulated T cells to progress from G_1 to S phase of the cell cycle [2]. However, the growth-inhibitory actions of rapamycin and its related compounds are not restricted to lymphoid cells; these agents have cytostatic or cytotoxic activities against solid and lymphoid tumor cell lines. This article focuses on recent advances in the understanding of the mechanisms of cell growth inhibition by rapamycin and the issues surrounding the development of this class of agent as a potential cancer therapy.

Target of rapamycin

The phosphoprotein kinase, target of rapamycin (TOR), was first described in the yeast *Saccharomyces cerevisiae* as the functional target of rapamycin. Two distinct genes have been identified in yeast, but only a mammalian homolog (mTOR) of TOR2 has been described. In mammalian cells, mTOR is a large polypeptide kinase of 290 kDA [3] (also known as FRAP [4], RAFT1 [5], and RAPT1 [6]). The yeast TOR proteins exhibit a high degree of overall sequence identity (>40%) to mTOR, with even greater identity (>65%) observed in their carboxy-terminal catalytic domains [7].

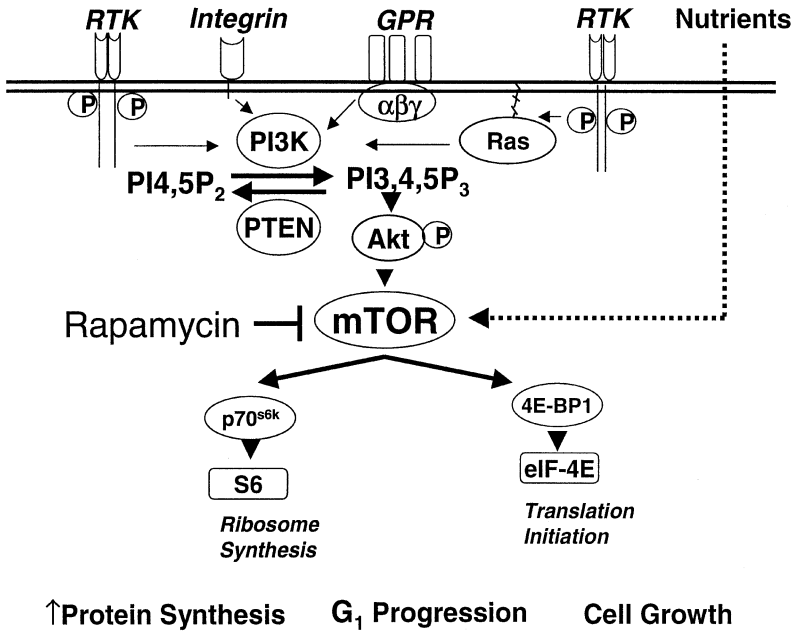

Fig. 1. Rapamycin-sensitive signaling pathways. Receptor-ligand binding activates the PI3K/Akt/mTOR pathway. The TOR regulates the activities of the translational regulators 4E-BP1 and $p70^{S6}$ kinase. Rapamycin binds to FKBP12 and the complex inhibits mTOR. Rapamycin may cause G_1 block by inhibiting translation of proteins important for G_1/S transition although other mechanisms may be involved in the drug's antiproliferative effect. RTK, receptor tyrosine kinase; GPR, G-protein receptor.

mTOR, a downstream component in the phosphoisinositol-3 kinase (PI3K)/Akt pathway (Fig. 1), acts as a nutrient sensor and regulator of translation [8,9]. In the presence of mitogen stimulation of the PI3K/Akt pathway and sufficient nutrients, mTOR participates in the activation of $p70^{S6}$ kinase ($p70^{s6k}$) and in the inactivation of 4E-binding protein-1 (4E-BP1). These events and possibly signals to other kinases result in the activation of the translation of specific mRNA subpopulations important for cell proliferation and survival. Although mutations of mTOR have not been reported in human cancers, mTOR is a component of the PI3K/Akt pathway, which is of considerable interest to cancer therapeutics development because of the high frequency of mutations in components of the pathway seen in human malignancies (Table 1).

Yeast and mammalian TOR proteins are members of phosphoinositide 3 kinase (PI3K)-related kinases (PIKK) family [10]. Among these PIKK family members are the cell cycle regulatory protein kinases ataxia-telangiectasia–mediated, ataxia-telangiectasia–related, and DNA-dependent protein kinase catalytic subunit. The PIKK family members share a carboxyl-terminal catalytic domain that bears significant sequence homology to the lipid kinase domains of PI3Ks,

Table 1
Abnormalities in the phosphatidyl-inositol 3 kinase/Akt-mTOR pathway in human cancers

Abnormality	Function	Tumors
Growth factor receptors (eg, EGFR, PDGFR, IGF-R, IL-2)	Oncogene	Lung, bladder, ovary, endometrium, cervix, prostate carcinomas, glioma, lymphoma
PI3 kinase	Oncogene	Ovary
PTEN	Tumor suppressor gene	Prostate, endometrium, breast carcinomas, melanoma
Akt	Oncogene	Breast, gastric, ovary, pancreas, prostate carcinomas
eIF-4E	Oncogene	Breast, bladder, and head, and neck carcinomas; lymphoma
Cyclin D	Oncogene	Mantle cell lymphoma; breast, head and neck carcinomas
P16	Tumor suppressor gene	Familial melanoma, pancreas carcinomas

Abbreviations: EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; IGF-R, insulin-like growth factor receptor; IL-2, interleukin-2 PI3, phosphoisinositol-3; eIF-4E, eukaryotic initiation factor-4E.

range of essential cellular functions, including cell cycle progression, cell cycle checkpoints, DNA repair, and DNA recombination [11,12].

The upstream signaling pathway that couples growth factor receptor occupancy to mTOR protein activation is only partially understood. mTOR is a phosphoprotein, and its phosphorylation state and catalytic activity have been reported to be modulated by the mitogen-activated PI3K/Akt [13,14]. PI3 kinase and Akt are considered to be proto-oncogenes, and the pathway is inhibited by the tumor suppressor gene PTEN [15]. Activation of the pathway through overexpression of PI3K, Akt, or loss of PTEN augments the activity of mTOR and may increase the importance of this pathway in tumor cell survival and cell sensitivity to rapamycin compounds [16–18].

The downstream actions of mTOR on translation are better understood than its upstream effectors. For the subset of mRNAs that contain regulatory elements located in the 5′-untranslated regions, the binding of the mRNA to the ribosomal subunit and the efficient initiation of translation is mediated by the multi-subunit eukaryotic initiation factor-4 (eIF-4) complex [19]. 4E-BP1 is a low-molecular-weight protein that inhibits the initiation of translation through its association with eIF-4E, the mRNA cap binding subunit of the eIF-4F complex [4]. Binding of 4E-BP to eIF-4E is dependent on the phosphorylation status of 4E-BP1. In quiescent cells, 4E-BP1 is relatively underphosphorylated and binds tightly to eIF-4E [19]. Stimulation of cells by hormones, mitogens, growth factors, cytokines, and G-protein–coupled agonists results in 4E-BP1phosphorylation through the action of mTOR and possibly other kinases, which promotes the dissociation of the 4E-BP1/eIF-4E complex. The eIF-4E can then bind to the eIF-4F complex, and this interaction leads to an increase in translation rates of a subset of mRNAs.

phosphorylates S6 on multiple sites, and these modifications favor the recruitment of the 40S subunit into actively translating polysomes [20] and enhance the translation of mRNAs bearing 5′ terminal oligopolypyrimidine tracts. Although these transcripts represent only 100 to 200 genes, they can encode up to 20% of the cell's mRNA [21].

In summary, under appropriate physiologic conditions, mTOR activation results in the transduction of signals that initiate the translation of specific subsets of mRNA transcripts and the activation of cyclin-dependent kinases, promoting progression through the cell cycle. This results in the activation and proliferation of T and B cells and such nonimmune cells as fibroblasts, endothelial cells, hepatocytes, and smooth muscle cells [22]. In tumor cells, activation of the pathway through inappropriate growth factor stimulation, overexpression of PI3K, Akt, or loss of PTEN augments the activity of mTOR and may increase the importance of this pathway in tumor cell survival and cell sensitivity to rapamycin compounds.

Agents that specifically inhibit mTOR are limited to rapamycin and the structurally related compounds CCI-779 and SDZ RAD. Wortmannin and LY294002 are structurally unrelated molecules that, at low concentrations, are relatively specific, cell-permeable PI3K inhibitors [10]. However, wortmannin also directly inhibits mTOR autokinase activity with an IC_{50} that is $\sim$100-fold higher than that required for PI3K inhibition ($\sim$200 nM in vitro and 300 nM in vivo) [10]. LY294002 inhibits mTOR autokinase activity in vitro, with an IC_{50} of 5 μM [10]. Rapamycin and SDZ RAD are being developed as immunosuppressants, and CCI-779 is being developed as a cancer therapy.

The discovery of rapamycin and its antiproliferative activity

Rapamycin, a macrolide, was first identified as product of the fungus *Streptomyces hygroscopicus*, an organism isolated from the soil samples from Easter Island [23,24]. Although it was originally identified as an antifungal agent, subsequent studies demonstrated impressive anti-tumor and immunosuppressant activities. The National Cancer Institute (NCI) originally evaluated rapamycin in the late 1970s. It was found to have antiproliferative activity in a variety of murine tumor systems, including B16 melanoma and P388 leukemia models [25,26]. Rapamycin has since been shown to inhibit the growth of B-cell lymphoma cell lines [27], small-cell lung cancer cell lines [28], rhabdomyosarcoma cell lines [29], and MiaPaCa-2 and Panc-1 human pancreatic cancer cell lines [30]. Rapamycin also augmented cisplatin-induced apoptosis in murine T-cell lines, the human promyelocytic cell line HL-60, and human ovarian cancer cell line SKOV3 [68]. These data suggest that rapamycin has intrinsic antiproliferative activity and may enhance the efficacy of selected cytotoxic agents.

Similar to other natural immunosuppressants, such as cyclosporin A and

activity as a prolyl isomerase [31,32]. Although this enzymatic function is important for altering protein conformation, it is not relevant to the action of rapamycin [33]. However, rapamycin must complex with FKBP-12 to inhibit mTOR. Thus, rapamycin may be considered a ''prodrug'' for the active agent at the cellular level, the FKBP12-rapamycin complex.

Because inhibiting mTOR-mediated $p70^{S6K}$ and 4E-BP1 phosphorylation by rapamycin are coupled to growth arrest in G1, rapamycin's anti-proliferative properties may be due to its effects on the regulation of protein translation [34–36]. Inhibiting these key signaling pathways results in inefficient translation of mRNAs of proteins, such as cyclin D1 [69] and ornithine decarboxylase [37], which are important for cell cycle progression through the G_1 phase. However, in addition to its actions on $p70^{s6k}$ and 4E-BP1, rapamycin prevents cyclin-dependent kinase (cdk) activation and retinoblastoma protein (pRb) phosphorylation [38–41]. Rapamycin also seems to accelerate the turnover of cyclin D1 at the mRNA and protein levels, resulting in a deficiency of active cdk4/cyclin D1 complexes required for pRB phosphorylation and release of E2F transcription factor, and to increase association of $p27^{kip1}$ with cyclin E/cdk2. These two events, decreased cdk4/cyclin D and increased $p27^{kip1}$ with cyclin E/cdk2, along with the inhibition of translation of other mRNAs, can explain the observed inhibition at the G_1/S-phase transition [34,42]. However, cells derived from mice in which the p27 gene has been disrupted by homologous recombination are only partially rapamycin resistant, which indicates that rapamycin can inhibit cell cycle progression by p27-independent mechanisms [43]. In addition, there are data showing that proliferation can proceed despite rapamycin-induced inhibition of 4E-BP1 and S6 kinase phosphorylation [43,44]. Thus, although the target of rapamycin has been identified, the downstream pathway from target to inhibition of cell cycle progression is uncertain.

Clinical development

Although rapamycin, RAD, and CCI-779 share many biochemical and physiologic properties [22,45,46], they are being developed for different indications, in part because of their different pharmacologic features. Rapamycin and RAD are available in oral formulations and are being developed as immunosuppressants, whereas an intravenous formulation of CCI-779 is being evaluated as an anti-cancer therapeutic. Preclinical studies of rapamycin and the 40-O-(2-hydroxyethyl)-sirolimus derivation, RAD, showed that both compounds are effective in preventing and treating acute allograft rejection in a variety of transplant models as single agents and function synergistically with standard immunosuppressants [22]. Both agents are orally administered, and the efficiency of absorption is modulated by p-glycoproteins. Rapamycin has a terminal half-life of 62 hours in stable renal transplant recipients treated with cyclosporine, and

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