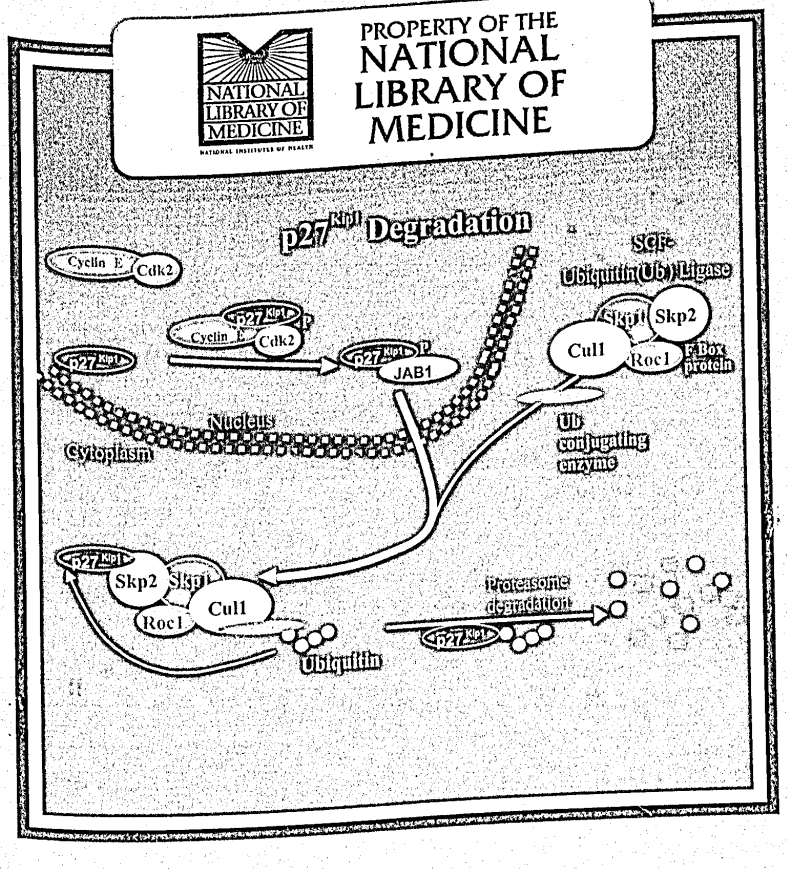
Current Cancer Drug Targets



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Aims and Scope

Current Cancer Drug Targets aims to cover all the latest and outstanding developments in the medicinal chemistry, pharmacology, molecular biology, genomics and biochemistry of contemporary molecular drug targets involved in cancer e.g. disease specific proteins, receptors, enzymes, genes.

Each issue of the journal contains a series of timely in-depth reviews written by leaders in the field covering a range of current topics in drug targets involved in cancer.

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Mammalian Target of Rapamycin (mTOR) Inhibitors as Anti-Cancer Agents

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Abstract: Highly specific signal transduction inhibitors are being developed as anti-cancer agents against an array of molecular targets, with the promise of increased selectivity and lower toxicity than classic cytotoxic chemotherapy agents. Rapamycin and its analogues are a promising class of novel therapeutics that specifically inhibit signaling from the serine-threonine kinase, mammalian target of rapamycin (mTOR). mTOR is a key intermediary in multiple mitogenic signaling pathways and plays a central role in modulating proliferation and angiogenesis in normal tissues and neoplastic processes. Rapamycin potently inhibits T-cell proliferation, and is approved for clinical use as an immuno-suppressant following kidney transplantation. Hyperactivation of mTOR signaling has been implicated in tumorigenesis, and promising pre-clinical studies in several tumor types suggest that the anti-proliferative and anti-angiogenic properties of rapamycin may be useful in cancer therapy. These studies have led to several clinical trials evaluating the safety and efficacy of rapamycin analogs in cancer therapy. The goal of this article is to review the mechanism of action of rapamycin as an anti-cancer agent, and to review the clinical experience with rapamycin and rapamycin analogs as immunosuppressive and anti-neoplastic therapeutic agents.

Key words: Rapamycin, CCI-779, temsirolimus, RAD-001, everolimus, AP23573, sirolimus, cancer, cytostatic anti-cancer drugs, mTOR, renal transplant, immunosuppressive agents.

INTRODUCTION

Rapamycin and its analogues are novel, molecularly targeted drugs that are being developed as anti-cancer agents. The parent compound, rapamycin (Sirolimus, Rapamune; Wyeth-Ayerst) is approved by the Food and Drug Administration (FDA) for the prevention of allograft rejection following renal transplantation, and for incorporation into drug-eluting stents to prevent re-stenosis following coronary angioplasty. Experience in the transplant setting suggests that long-term use of this agent is safe and well tolerated. Rapamycin analogues with more favorable pharmacokinetic properties are currently being developed as anti-cancer drugs. Rapamycin and its analogs inhibit the signaling activity of the serine-threonine protein kinase, mammalian target of rapamycin (mTOR). mTOR functions downstream from multiple growth factor receptor tyrosine kinases to promote cell growth and proliferation. Key downstream targets of mTOR include p70S6 kinase and eukaryotic initiation factor 4E-binding protein (4EBP1), which modulate the translation of select mRNA transcripts that ultimately impact on cell growth and cell cycle progression. More recent data have linked mTOR signaling with the cellular response to hypoxia and the expression of vascular endothelial growth factor (VEGF), which suggests that mTOR may be an important mediator of tumor angiogenesis. In tumors that are reliant on mTOR signaling, disruption of these key signaling pathways by rapamycin results in cell cycle arrest and inhibition of angiogenesis, and these effects may account for the anti-neoplastic activities of mTOR inhibitors seen in multiple tumor types. Based on promising pre-clinical studies, rapamycin and its analogs currently are being tested as anti-neoplastic agents, both given alone or in combination with conventional cancer therapies. In this review, the biology of mTOR signaling and the cellular pharmacology of mTOR inhibition will be discussed as will the clinical development of mTOR inhibitors.

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History

Rapamycin is a macrocyclic lactone antibiotic that was first isolated from the bacterium *Streptomyces hygroscopicus* found in soil samples taken from Easter Island (called 'Rapa Nui' by its native inhabitants; hence the name 'Rapamycin'). After its isolation and purification, studies revealed that rapamycin was a potent anti-fungal agent and an effective immunosuppressant[1]. Subsequent studies demonstrated that rapamycin inhibited proliferation in several tissues including IL-2 stimulated T-cells, vascular endothelium, smooth muscle, and tumor cells. These observations prompted the development of rapamycin and its analogs as immunosuppressive agents, inhibitors of vascular re-occlusion and as anti-cancer agents.

Rapamycin and three analogs, CCI-779, RAD-001 and AP23573, have been developed for human use (Fig. (**1**)). Among these, only rapamycin (Sirolimus, Wyeth Pharmaceuticals) is currently approved, for preventing kidney allograft rejection following renal transplantation and in drug-eluting stents to reduce the incidence of re-stenosis following coronary artery angioplasty. CCI-779 (Temsirolimus, Wyeth Pharmaceuticals) is an ester of rapamycin, with superior oral bioavailability compared to the parent compound rapamycin. This drug is available in oral and intravenous formulations, and clinical development of this drug is well underway with several phase II and III trials being conducted. RAD-001 (Everolimus, Novartis Pharmaceuticals) is an orally available hydroxyethyl derivative of rapamycin developed by Novartis for applications in the transplant, cardiovascular and oncological

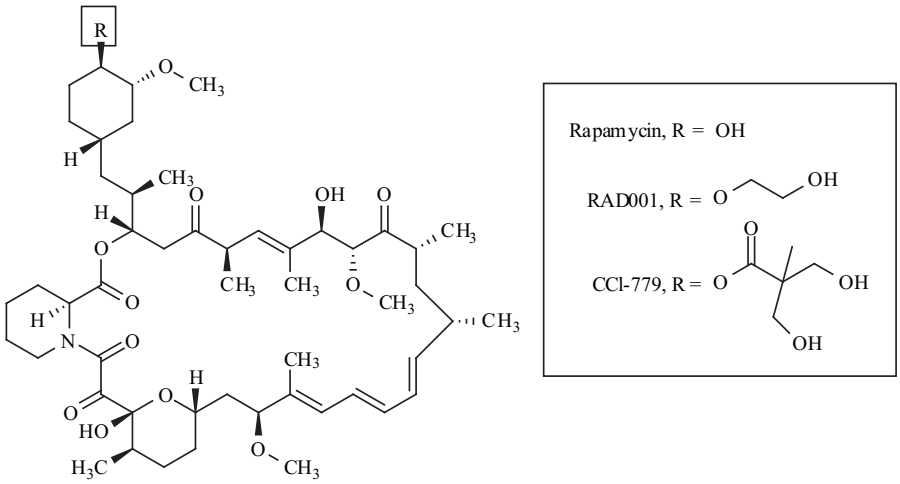

Fig. (1). Structure of rapamycin and rapamycin derivatives.

settings, and clinical testing for all these indications are ongoing. The newest mTOR inhibitory agent to be developed for clinical use is AP23573 (Ariad Pharmaceuticals). Early phase I clinical trials with this agent, which is also an analog of rapamycin, are now underway.

BIOLOGY OF mTOR

mTOR is a serine-threonine-directed kinase that belongs to the family of phosphatidyl-inositol 3-kinase-related kinases (PIKK). All members of this PIKK family contain a C-terminal kinase domain that shares significant homology with that of the phosphatidyl-inositol 3-kinase (PI3K); other members of this family include ataxia telangiectasia mutated (ATM), ATM and Rad3 related (ATR) and DNA-dependent protein kinase (DNA-PK) [2,3]. These latter 3 kinases play key roles in orchestrating DNA damage checkpoint responses and DNA repair [4]. In contrast, mTOR monitors intracellular nutrient and energy availability and promotes cell growth and proliferation following mitogenic stimuli, dependent upon the availability of requisite nutrients [5].

Rapamycin is a highly specific inhibitor of mTOR function. Rapamycin is unable to bind directly to mTOR, but forms a complex with the immunophilin, 12 kDa FK506-binding protein (FKBP12; FK-506 is an unrelated immunosuppressant); it is this drug-protein complex that binds to mTOR through an FKBP12-rapamycin binding (FRB) domain[6]. The FRB domain is adjacent to the kinase domain in mTOR and formation of this tri-molecular complex markedly attenuates downstream signaling from mTOR. Interestingly, rapamycin treatment does not inhibit mTOR catalytic kinase activity directly, since autophosphorylation of mTOR is unaffected by rapamycin treatment. Instead, binding of the FKBP12/rapamycin complex is thought to prevent interaction of mTOR with its kinase substrates and thus prevent downstream signaling [7]. The interaction of the rapamycin/FKBP12 complex with mTOR is highly specific and is so stable that inhibition of mTOR by rapamycin is essentially irreversible. The cellular and biochemical effects of rapamycin are generally believed to result exclusively from inhibition of mTOR signaling

The mTOR signaling network (Fig. (**2**)) is important for driving cell growth and proliferation in multiple tumor types. Several receptor tyrosine kinases (RTKs), including the epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR) and insulin-like growth factor (IGFR) can activate PI-3 kinase activity, which, in turn, phosphorylates phosphatidylinositol (PI) on the D-3 position [10]. The resulting accumulation of phosphatidylinositol-3, 4, 5-triphosphate on the cytoplasmic surface of the plasma membrane leads to activation of a number of kinase signaling pathways including that regulated by protein kinase B (PKB, Akt). Akt stimulates mTOR function both through direct phosphorylation of a negative regulatory domain within mTOR [11] as well as through its effects on the tuberous sclerosis complex-2 (TSC2) protein [12-15]. TSC2, in a complex with tuberous sclerosis complex-1 (TSC1) protein, functions as a GTPase-activating protein towards the Rheb1 GTPase. Akt-mediated phosphorylation of TSC2 disrupts the TSC1/TSC2 complex and relieves inhibition of Rheb1 activity; activated Rheb1 then can stimulate mTOR phosphorylation and signaling (see Fig. (**2**)) [16-19]. The inhibitory effects of TSC2 on mTOR activity are stimulated in nutrient deprived conditions by activation of LKB-1, which signals through AMP-activated protein kinase (AMPK) to enhance TSC2 activity [20,21]. Signaling from mTOR also is regulated by association with Raptor, which probably functions as a scaffolding protein to promote transient association and phosphorylation of downstream targets [22,23]. Collectively, these data highlight the idea that mTOR functions within a molecular complex of multiple proteins that regulate its activity [24].

PI3K-mediated activation of Akt is normally opposed by the lipid phosphatase PTEN (phosphatase and tensin analogue), which dephosphorylates phosphatidylinositol at the D-3 position. Deletion or mutation of the gene encoding this tumor suppressor protein commonly occurs in multiple tumor types and results in constitutive activation of PI3K-dependent signaling pathways that include Akt and activated Ras as signaling mediators. Consistent with the potential role of the Akt/mTOR signaling pathway in tumorigenesis, overexpression of activated Akt and activated Ras in glial

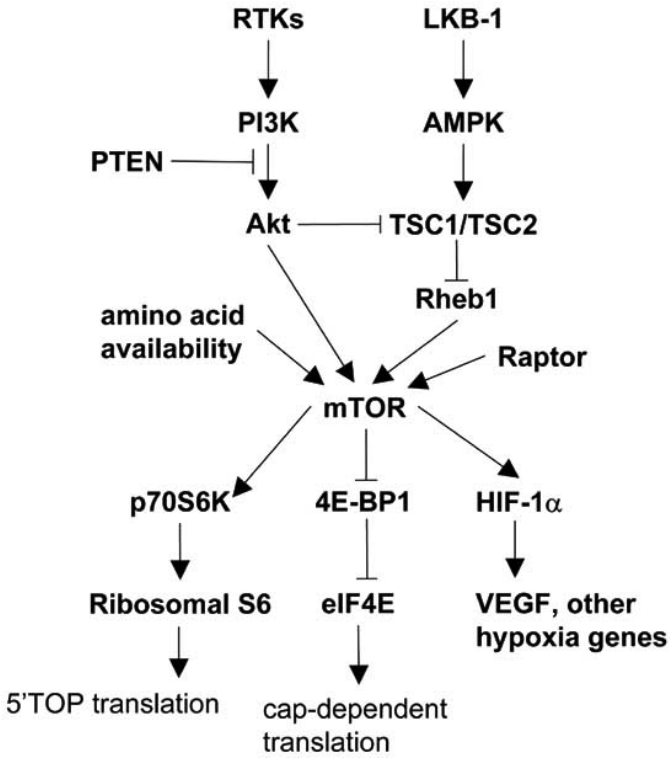

Fig. (2). The mTOR signaling network.

transgenic mouse model [25], and these tumors are exquisitely sensitive to treatment with rapamycin. Germ-line inherited deficiencies in PTEN, TSC or LKB-1 result in Cowden's disease, Tuberous Sclerosis and Peutz-Jeghers syndrome, respectively, which are all characterized by the development of multiple benign hamartomas. Loss-of-function in any one of these three proteins results in hyperactivation of mTOR signaling, and this presumably accounts for the development of the characteristic hamartomatous lesions. Thus, constitutive activation of mTOR signaling can be an important contributor to tumorigenesis in both benign and malignant tumors.

mTOR-Dependent Signaling Pathways

mTOR signals downstream to multiple protein targets: p70 S6 kinase (p70S6K), eukaryotic translation initiation factor 4E binding protein (4E-BP1), and the hypoxia-inducible transcription factor, HIF-1α. These 3 well-characterized downstream signaling targets have been implicated in control of hypoxia- and mitogen-induced tumor proliferation and disruption of these pathways may play an important role in the anti-tumor effects of rapamycin. In the sections below, we will describe the potential links between the anti-tumor effects of mTOR inhibitors and disruption of downstream signaling to p70S6K, 4EBP1 and HIF-1α in more detail.

p70S6K

mTOR regulates translation of select mRNA transcripts through phosphorylation of p70S6K. Following PI3K-dependent phosphorylation of residues within an auto-inhibitory domain, mTOR regulates the phosphorylation of Thr-389 [26]. Modification of this residue is essential for subsequent phosphorylation of other residues within the activation loop of the kinase domain, which allows for full catalytic activity. After mitogen stimulation, activated p70S6K phosphorylates the S6 component of the 40S ribosomal subunit, and this promotes translation of mRNA containing 5'TOP [27]. Because transcripts for many ribosomal proteins and translation elongation factors contain this 5'TOP motif, rapamycin-mediated suppression of p70S6K activity may inhibit cell growth and proliferation by limiting ribosomal biogenesis and restricting protein synthesis capacity.

4EBP1

mTOR modulates protein translation initiation by regulating the assembly of the eukaryotic initiation factor 4F (eIF4F) complex on the 5'-methyl-GTP cap of mRNA transcripts. The eIF4F complex is a heterotrimer composed of the mRNA cap-binding protein eIF4E, a scaffolding protein eIF4G, and a helicase eIF4A (reviewed in [5]). This tripartite complex regulates the rate of cap-dependent protein translation by mediating the rate-limiting step of mRNA loading onto the small 40S ribosomal subunit. Formation of a functional eIF4F complex is controlled by the phosphorylation status of an eIF4E binding protein (4E-BP1). In nutrient- or growth factor-deprived cells, the association of hypophosphorylated 4E-BP1 with eIF4E blocks binding of eIF4G to the cap structure and inhibits

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