9 Combinations of Cytotoxic Drugs, Ionizing Radiation, and Mammalian Target of Rapamycin (mTOR) Inhibitors

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9.1 Introduction

Rapamycin and its analogs are novel, molecularly targeted drugs that are being developed as anti-cancer agents. The parent compound, rapamycin (Sirolimus, Rapamune, Wyeth, Madison NJ, USA) 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 analogs with more favorable pharmacokinetic properties are currently being developed as anti-cancer drugs (Fig. 9.1). CCI-779 (Temsirolimus, Wyeth Pharmaceuticals) is an ester of rapamycin, with superior oral bioavailability compared with 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 phase-III trials being conducted. RAD001 (Everolimus, Novartis International AG, Basel, Switzerland) is an orally available hydroxyethyl derivative of rapamycin developed for applications in the transplant, cardiovascular, and oncological settings, and clinical testing for all these indications is ongoing. The newest mammalian target of rapamycin (mTOR) inhibitor to be developed for clinical use is AP23573 (Ariad Pharmaceuticals, Inc., Cambridge, Massachusetts, USA). Early phase-I clinical trials with this agent, which is also an analog of rapamycin, are now underway.

Rapamycin and its analogs inhibit the signaling activity of the serine-threonine protein kinase 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 preclinical 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 chapter, the biology of mTOR signaling and the cellular pharmacology of mTOR inhibitor monotherapy and combination therapy are reviewed.

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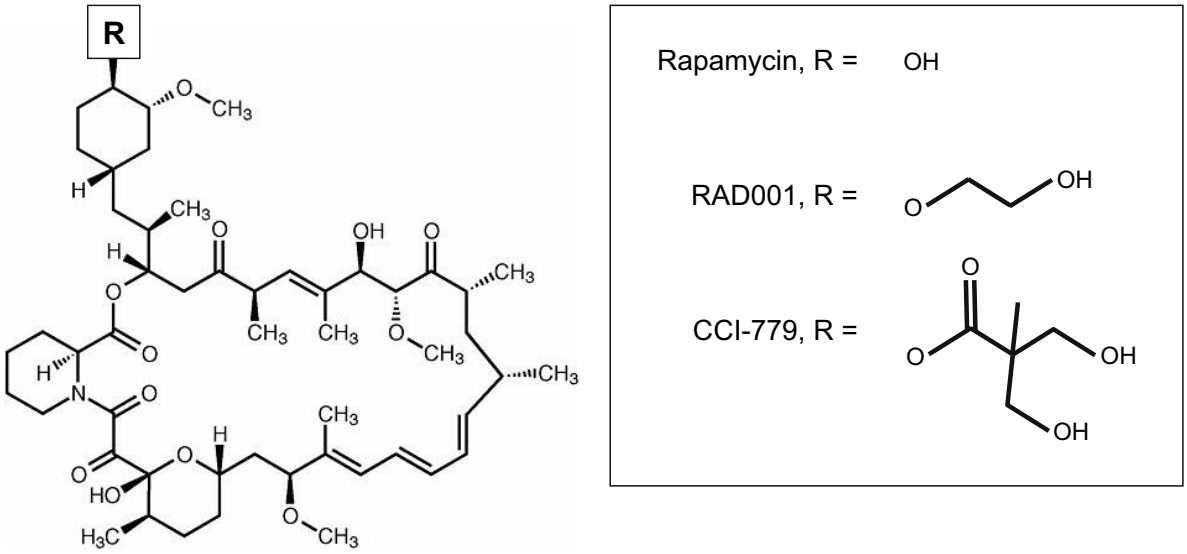

Fig. 9.1. Structure of rapamycin and rapamycin derivatives

9.2 Biology of mTOR

mTOR is a serine-threonine-directed kinase that belongs to the family of phosphatidyl-inositol 3-kinase-related kinases (PIKK). Members of this PIKK family all 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; Abraham 2001; Durocher and Jackson 2001). These latter three kinases play key roles in orchestrating DNA damage checkpoint responses and DNA repair (Zhou and Elledge 2000). 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 (Raught et al. 2001).

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 (Cutler et al. 1999). 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 (Edinger et al. 2003). 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 (Brown et al. 1994; Sabatini et al. 1994).

The mTOR signaling network (Fig. 9.2) 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 receptor (IGFR), can activate PI-3 kinase activity, which, in turn, phosphorylates phosphatidylinositol (PI) on the D-3 position (Grant et al. 2002). 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 (Sekulic et al. 2000) as well as through its effects on the tuberous sclerosis complex-2 (TSC2) protein (Dan et al. 2002; Inoki et al. 2002; Potter et al. 2002; Tee et