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Abstract
Rapamycin and its derivatives (rapalogs), a group of allosteric inhibitors of mammalian target of rapamycin (mTOR), have been actively tested in a variety of cancer clinical trials, and some have been approved by the Food and Drug Administration for the treatment of certain types of cancers. However, the single agent activity of these compounds in many tumor types remains modest. The mTOR axis is regulated by multiple upstream signaling pathways. Because the genes (e.g., PIK3CA, KRAS, PTEN, and LKB1) that encode key components in these signaling pathways are frequently mutated in human cancers, a subset of cancer types may be addicted to a given mutation, leading to hyperactivation of the mTOR axis. Thus, efforts have been made to demonstrate the potential impact of genetic alterations on rapalog-based or mTOR-targeted cancer therapy. This review will primarily summarize research advances in this direction.

Key words Mutation, mTOR, rapalogs, cancer

Mammalian target of rapamycin (mTOR) is a serine-threonine kinase that belongs to the phosphatidylinositol kinase–related kinase family[1] and plays a central role in regulating cell growth, proliferation, and survival. mTOR exerts its effects in part by regulating translation initiation and cell survival signaling through interactions with other proteins, including raptor (forming mTOR complex 1, mTORC1) and rictor (forming mTOR complex 2, mTORC2)[2-4]. The mTOR axis is frequently activated in many types of cancer, largely due to activation of its upstream regulatory signaling pathways, and thus has been considered a promising therapeutic target. Currently, several small molecule drugs, including rapamycin and its analogs (rapalogs), and mTOR kinase inhibitors are being tested in various phases of oncology clinical trials[5-8]. While the rapalogs CCI-779 (tepsirolimus; Torisel™, by Wyeth/now Pfizer) and RAD001 (everolimus; Afinitor™, by Novartis) improved the overall survival of patients with metastatic renal cell carcinoma and advanced pancreatic neuroendocrine tumors[9-13], the single agent activity of these agents in other tumor types remains modest[14-19]. Currently, the Food and Drug Administration (FDA) has approved CCI-779 for the treatment of advanced renal cell carcinoma, and RAD001 for the treatment of advanced renal cell carcinoma, subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC), progressive neuroendocrine tumors of pancreatic origin, and postmenopausal women with advanced hormone-receptor-positive, HER2-negative breast cancer (in combination with exemestane).

As witnessed in some types of cancer, successful targeted therapy relies on careful selection or identification of patients who are most likely to benefit. These patients may often have tumors with specific molecular alterations. An instructive example is the subset of advanced lung cancer patients whose tumors harbor a classic chromosomal translocation involving the echinoderm microtubule associated protein like 4 (EML4) and anaplastic lymphoma kinase (ALK) genes. Crizotinib, a potent inhibitor of the kinase activity of the EML4-ALK fusion protein, showed astounding efficacy and was approved by the FDA for this subset of patients, which accounts for only 4% of all cases of non–small cell lung cancer (NSCLC)[20].

For the past decade, efforts have also been made to
identify genetic alterations that may impact how cancer cells respond to mTOR-targeted therapy. Here we primarily review studies related to this topic.

**Regulation of the mTOR Axis**

The phosphoinositide 3-kinase (PI3K)/Akt survival pathway functions upstream to positively regulate the activity of the mTOR axis\(^{[15]}\). Recent studies show that extracellular signal-regulated kinases 1/2 (ERK1/2) and 90 kDa ribosomal protein S6 kinase 2 (RSK2) also positively regulate mTORC1 through respective phosphorylation of TSC2 and raptor, linking Ras to positive regulation of mTORC1 signaling\(^{[16,17]}\). Thus, mTOR serves as a convergence point of the PI3K/Akt and mitogen-activated protein kinase (MAPK)/ERK signaling pathways, which are often hyperactivated in cancer\(^{[16]}\). In contrast, the tumor suppressors liver kinase B1 (LKB1) and p53 both negatively regulate the mTOR axis. LKB1 inhibits mTORC1 signaling through activation of AMP-activated protein kinase (AMPK) signaling pathways, which are often hyperactivated in cancer\(^{[16]}\). Hence, alteration (e.g., activation and inactivating mutations) of genes whose products regulate the mTOR axis will result in hyperactivation of the axis in certain types of cancers (Figure 1). This category of cancer is expected to be “addicted” to the mTOR axis for survival and growth, hence increasing susceptibility to mTOR-targeted therapy.

Compared with mTORC1 signaling, little is known about the upstream regulators of the mTORC2 axis\(^{[21]}\). Whether the same upstream signals that regulate mTORC1 also regulate mTORC2 is unclear.

**Preclinical Studies**

The tumor suppressor PTEN is a lipid phosphatase that negatively regulates the PI3K signaling pathway. A high frequency of mutation or deletion of PTEN occurs in some types of cancers. Early work by Dr. Sawyers’s group\(^{[22]}\) revealed that PTEN-deficient mouse cells or human cancer cells showed enhanced sensitivity to CCI-779, both in vitro and in vivo. Soon after that study, Shi et al.\(^{[23]}\) reported similar findings in multiple myeloma cells. They found that 3 of 4 PTEN-deficient cell lines with constitutively active Akt were remarkably sensitive to growth inhibition and G1 arrest induced by CCI-779, with ID₅₀ concentrations of <1 nmol/L. In contrast, myeloma cells expressing wild-type PTEN were >1,000-fold more resistant. Acute expression of a constitutively active Akt gene in CCI-779-resistant myeloma cells containing wild-type PTEN and quiescent Akt did not convert them to the CCI-779-sensitive phenotype. Conversely, expression of wild-type PTEN in CCI-779-sensitive, PTEN-deficient myeloma cells did not induce resistance. Thus, the level of PTEN and Akt activity does not regulate sensitivity per se. In a recent study, suppressing PTEN function by expressing a PTEN mutant lacking lipid (G129E) or lipid and protein (C124S) phosphatase activity in MCF-7 cells conferred sensitivity to rapamycin\(^{[24]}\).

PI3Ks are heterodimeric lipid kinases composed of p110 catalytic and p85 regulatory subunit variants encoded by separate genes and alternative splicing. The activity of PI3K is opposed by the action of PTEN.

![Figure 1. mTOR complexes and their regulation by various upstream signaling pathways.](image)

**Figure 1. mTOR complexes and their regulation by various upstream signaling pathways.** Various oncogenes (e.g., Ras and PIK3CA; in blue) or tumor suppressors (e.g., PTEN, LKB1 and p53; in red) positively or negatively regulate the mTOR axis. Mutations in the genes encoding these proteins lead to hyperactivation of the mTOR axis. mTOR, mammalian target of rapamycin; PIK3CA, phosphoinositide-3-kinase, catalytic, alpha polypeptide; PTEN, phosphatase and tensin homolog; LKB1, liver kinase B1; mTORC, mammalian target of rapamycin complex.
Mutations in the p110α catalytic subunit (PI3KCA) occur in certain types of cancer, such as breast cancer, and lead to the activation of PI3K/Akt signaling. In a study with 31 breast cancer cell lines, Weigelt et al. reported that breast cancer cells harboring PI3KCA mutations, but not PTEN loss, were selectively sensitive to RAD001 and the mTOR kinase inhibitor PP242. However, in another study with 31 human cancer cell lines, Meric-Bernstam et al. reported that cell lines with PIK3CA and/or PTEN mutations were more likely to be rapamycin-sensitive. Interestingly, Di Nicolantonio et al. recently reported that human cancer cells carrying alterations in the PI3K pathway were responsive to RAD001, both in vitro and in vivo, except when KRAS mutations occurred concomitantly or were exogenously introduced. In cancer cells with mutations in both PIK3CA and KRAS, genetic ablation of mutant KRAS reinstated response to the drug. These studies clearly suggest that PI3CA mutations seem to predict cell response to rapalogs.

LKB1 is a tumor suppressor that negatively regulates mTORC1 signaling. Mahoney et al. reported that LKB1/KRAS mutant NSCLCs constitute a genetic subset of NSCLC with increased sensitivity to MAPK (CI-1040) and mTOR (rapamycin) signaling inhibition, whereas LKB1 and KRAS mutations alone do not confer similar sensitivity. Contreras et al. reported that LKB1 inactivation-driven endometrial cancer is highly responsive to rapamycin monotherapy. In their study, they found that rapamycin monotherapy not only greatly slowed disease progression, but also led to striking regression of pre-existing tumors.

Clinical Studies

To date, few clinical studies have investigated the impact of genetic alterations on mTOR-targeted cancer therapy. In a cohort of metastatic cancer patients who had received single-agent RAD001, Di Nicolantonio et al. found that the presence of KRAS mutations was significantly associated with lack of benefit (partial response and stable disease) after RAD001 therapy. In this study, 11 of the 12 patients with KRAS mutant tumors had disease progression, whereas 15 of 31 of wild-type cases benefited from treatment (P = 0.0171). Unfortunately, the impact of PIK3CA mutations on patient response to treatment was not analyzed because of limited sample size.

Iyer et al. recently investigated the genetic basis of remission of a patient with metastatic bladder cancer treated with RAD001 using whole-genome sequencing. They found that the mutation of TSC, which occurs in about 8% of bladder cancer cases, correlated with RAD001 sensitivity. After identification of TSC mutation from this responsive bladder cancer case, the authors further analyzed samples from 13 additional bladder cancer patients treated with RAD001 in the same trial. This analysis revealed 3 patients with tumors harboring nonsense mutations in TSC1, including 2 patients who had minor responses to RAD001 (17% and 24% tumor regression). A fourth patient with 7% tumor regression had a somatic missense TSC1 variant of unknown functional consequence. In contrast, tumors from 8 of the 9 patients showing disease progression were TSC1-wild type. Benefit from RAD001 lasted longer in patients with TSC1-mutant tumors than in those with wild-type tumors (7.7 months vs. 2.0 months, P = 0.004), with a significant improvement in time to recurrence (4.1 months vs. 1.8 months; P = 0.001).

SEGA is a benign, slow-growing tumor that usually forms in the walls of fluid-filled spaces in the brain. It is common in patients with TSC, which is caused by the mutation of TSC1 or TSC2 gene. In an early single-arm trial, RAD001 reduced SEGA tumor volume ≧50% in 9 of 28 patients aged 3 to 34 years. In the recently completed double-blind, placebo-controlled phase III trial involving 117 patients with TSC in 24 centers across 10 countries worldwide, 24 weeks of oral RAD001 treatment caused ≧50% reduction in SEGA tumor volume in 35% (27/78) of patients, whereas placebo did not have this effect in any patient (0/39). These results prompted FDA approval of RAD001 for the treatment of pediatric and adult SEGA.

In a prospective clinical trial, Janku et al. sequenced PIK3CA in tumor samples from patients with advanced breast, cervical, endometrial, and ovarian cancers that were refractory to standard therapies. Of the 140 patients analyzed, 25 (18%) had PIK3CA mutations, and 23 were then enrolled in a clinical trial that included a PI3K/Akt/mTOR pathway inhibitor (e.g., CCI-777 alone; CCI-779 plus bevacizumab; rapamycin plus docetaxel; or CCI-779, bevacizumab plus liposomal doxorubicin). Partial response was observed in 30% (7/23) of the patients harboring a PIK3CA mutation. In contrast, only 10% (7/70) of patients with wild-type PIK3CA who were treated on the same protocols and had the same disease types responded to treatment (P = 0.04). Thus, this study suggests that screening for PIK3CA mutations may reveal a subset of patients who are sensitive to treatment regimens that include a PI3K/Akt/mTOR inhibitor.

Summary and Perspectives

Several preclinical studies have consistently suggested that tumors with PIK3CA mutations are likely to be sensitive to rapalog monotherapy. However, the clinical data to confirm these preclinical findings are largely
lacking, although a clinical study has shown that patients with PIK3CA mutations responded better than those without the mutation to treatment regimens with a PI3K/Akt/mTOR inhibitor [25]. Moreover, preclinical data regarding the impact of PTEN mutation or loss on cancer cell response to mTOR inhibition are not consistent and need further clarification or validation, particularly in the clinic.

**KRAS** is a frequently mutated oncogene in many types of cancer. The finding of an association between **KRAS** mutations and cell resistance to rapalogs [26] is intriguing. Thus, the sample size of that clinical study was small. However, further validation trials are urgently needed to confirm this observation. Another important observation is that the concomitant presence of **KRAS** and PIK3CA mutations may confer resistance to RAD001, though mutations in PIK3CA alone predict cell sensitivity to RAD001. It is crucial to fully understand the biological bases or molecular mechanisms for these findings. In this way, we may eventually develop more efficacious, mechanism-driven therapeutic strategies to overcome resistance.

One thing to keep in mind is tumor type. Although mutations in PIK3CA and/or PTEN seem to impact cancer cell sensitivity to mTOR-targeted therapy, this may not be helpful for predicting the sensitivity of cancers in which these genes have low mutation rates, such as NSCLC (<10% for combination of PIK3CA and PTEN) [25,26]. This also applies to the findings that LKB1 mutation or inactivation confers cell sensitivity to rapalogs [35,36], since LKB1 is primarily mutated in NSCLC (up to 30%) but is rarely mutated in other cancers [37].

Nonetheless, we have made considerable progress towards identifying subsets of cancer patients who may benefit from mTOR-targeted therapy, though some studies are preliminary so far. These efforts represent the first step toward personalized mTOR-directed cancer medicine. Considering the limited single-agent activity of rapalogs in the majority of cancers, development of rapalog-based combination regimens should be encouraged. One successful example is the combination of RAD001 and exemestane for the treatment of advanced hormone receptor-positive, HER2-negative breast cancer in postmenopausal women [38]. However, the impact of genetic alterations on tumor response to rapalog-based combination treatments is largely unknown. Demonstration of effective and mechanism-driven rapalog-based combination regimens in patients should be a key effort in this direction.

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