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Review

Current development of the second generation of mTOR inhibitors as anticancer agents

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Abstract

The mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, acts as a "master switch" for cellular anabolic and catabolic processes, regulating the rate of cell growth and proliferation. Dysregulation of the mTOR signaling pathway occurs frequently in a variety of human tumors, and thus, mTOR has emerged as an important target for the design of anticancer agents. mTOR is found in two distinct multiprotein complexes within cells, mTORC1 and mTORC2. These two complexes consist of unique mTOR-interacting proteins and are regulated by different mechanisms. Enormous advances have been made in the development of drugs known as mTOR inhibitors. Rapamycin, the first defined inhibitor of mTOR, showed effectiveness as an anticancer agent in various preclinical models. Rapamycin analogues (rapalogs) with better pharmacologic properties have been developed. However, the clinical success of rapalogs has been limited to a few types of cancer. The discovery that mTORC2 directly phosphorylates Akt, an important survival kinase, adds new insight into the role of mTORC2 in cancer. This novel finding prompted efforts to develop the second generation of mTOR inhibitors that are able to target both mTORC1 and mTORC2. Here, we review the recent advances in the mTOR field and focus specifically on the current development of the second generation of mTOR inhibitors as anticancer agents.

Key words mTOR, inhibitor, rapamycin, rapalogs, cancer

The mammalian target of rapamycin (mTOR), a 289 kDa atypical serine/threonine (S/T) protein kinase, is considered a member of the phosphatidylinositol 3-kinase (PI3K)–related kinase (PIKK) superfamily because its C-terminus shares a strong homology with the catalytic domain of PI3K [1]. Cumulative evidence demonstrates that mTOR plays a central role in the synthesis of key cellular proteins that are important for several aspects of cell growth and proliferation [2-4]. Dysregulation of mTOR and other proteins in its signaling pathway often occurs in a variety of human tumors, and these tumor cells have shown higher susceptibility to inhibitors of mTOR than normal cells [5-9]. Thus, mTOR has emerged as an important target for the development of anticancer agents. mTOR is found in two distinct multiprotein complexes within the cells, mTORC1 and mTORC2, which are evolutionarily conserved from yeast to mammals [10,11]. These two complexes consist of unique mTOR-interacting proteins that determine their substrate specificity. mTORC1 phosphorylates p70 S6 kinase (S6K) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1) and regulates cell growth, proliferation, survival, and motility by integrating growth factors, nutrients, stressors, and energy signals [12,13]. mTORC2 phosphorylates Akt (protein kinase B, PKB), serum- and glucocorticoid-induced protein kinase 1 (SGK1), protein kinase Cα (PKCα), and the focal adhesion proteins, and it controls the activities of the small GTPases (RhoA, Cdc42 and Rac1) and regulates cell survival and the actin cytoskeleton [14-18].

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mTORC1

Currently, mTORC1 is known to consist of mTOR,

regulatory associated protein of mTOR (raptor), mLST8 (also termed G-protein β-subunit–like protein, GβL, a yeast homolog of LST8), and two negative regulators, proline-rich Akt substrate 40 kDa (PRAS40) and DEP domain-containing protein 6 (DEPDC6 or DEPTOR)[19-21]. mTOR is the core component of mTORC1 and mTORC2[11-14]. Structurally, mTOR contains 2549 amino acids. A tandemly repeated HEAT motifs, including Huntingtin, elongation factor 3 (EF3), a subunit of protein phosphatase 2A (PP2A), and TOR, comprises the first 1200 amino acids[22,23]. Immediately downstream of the HEAT repeat region lies a FRAP, ATM, and TRRAP (FAT) domain; an FKBP12-rapamycin binding (FRB) domain; a catalytic kinase domain; an auto-inhibitory (repressor domain or RD domain); and a FAT carboxyterminal (FATC) domain, which is located at the C-terminus of the protein (Figure 1)[22,23]. Rapamycin, the first defined mTOR inhibitor, exerts its action by first binding to the intracellular receptor FKBP12. The FKBP12/rapamycin complex then binds the FRB domain in TOR proteins, thereby exerting its cell growth-inhibitory and cytotoxic effects by inhibiting the functions of TOR signaling to downstream targets such as S6K1 and 4E-BP1[10,24-26]. However, the actual mechanism by which rapamycin inhibits mTOR signaling is still not well understood. Rapamycin-FKBP12 has been proposed to inhibit mTOR function by blocking the interaction of raptor with mTOR and thereby disrupting the coupling of mTORC1 with its substrates[27]. mTORC1 acts to integrate four major regulatory inputs: nutrients, growth factors, energy, and stress (Figure 1)[13,28,29]. The best characterized signaling pathway that regulates mTORC1 activity is the growth factor/PI3K/Akt pathway[13]. PI3K/Akt signaling regulates mTORC1 through phosphorylation/inactivation of tuberous sclerosis complex (TSC) 2 (Figure 2)[30,31]. TSC is a heterodimer composed of the TSC1 and TSC2 subunits, and the TSC1/2 complex acts as a repressor of mTOR function[30,32,33]. TSC2 has GTPase-activating protein (GAP) activity towards the Ras family small GTPase Ras homolog enriched in brain (Rheb), and TSC1/2 antagonizes the mTOR signaling pathway via stimulation of GTP hydrolysis of Rheb[30,33-37]. The TSC1/2 complex can also be activated by energy depletion through the activation of AMP-activated kinase (AMPK) (Figure 2). Under any stress that depletes cellular ATP, such as oxidative stress, hypoxia, or nutrient deprivation, AMPK is activated and phosphorylates unique sites on TSC2, thereby activating the Rheb-GAP activity of TSC, which catalyzes the conversion of Rheb-GTP to Rheb-GDP and thus inhibits mTORC1 activity[30,33-37]. Activation of mTORC1 results in phosphorylation of 4E-BP1 and S6K1, the best studied downstream targets of mTOR[38,39]. Activated S6K1 regulates protein synthesis through phosphorylation of the 40S ribosomal subunit, which has been suggested to increase the translational efficiency of a class of mRNA transcripts with a 5'-terminal oligopolypirymidine[40,41]. Recently, the mechanism by which S6K1 regulates translation has been further proposed to be via phosphorylation of eIF4B at Ser422[42], which causes eIF4B to associate with eIF3 and promotes eIF4F complex formation[43,44]. Phosphorylation of 4E-BP1 by mTOR also stimulates protein synthesis through the release of eIF4E from 4E-BP1, allowing eIF4E to associate with eIF4G and other relevant factors to promote cap-dependent translation[45,46]. Most recently, the growth factor receptor-bound protein 10 (GRB10) was identified as an mTORC1 substrate[47,48]. Both Hsu *et al*.[47] and Yu *et al*.[48] showed that mTORC1 directly phosphorylates and simultaneously stabilizes GRB10,

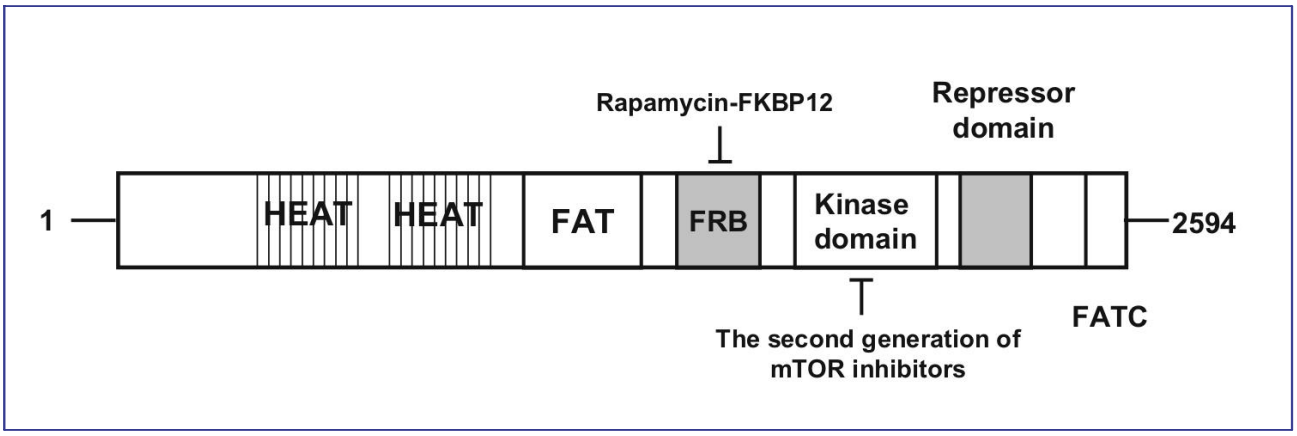

Figure 1. **Schematic structure of mTOR.** The N-terminus of mTOR contains two tandemly repeated HEAT motifs. Downstream of the HEAT repeat region lies a FAT domain, an FRB domain, a catalytic kinase domain, an auto-inhibitory repressor domain, and a C-terminal FATC domain. The first generation of mTOR inhibitors (rapalogs) bind to FRB domain, whereas the second generation of mTOR inhibitors target the kinase domain.

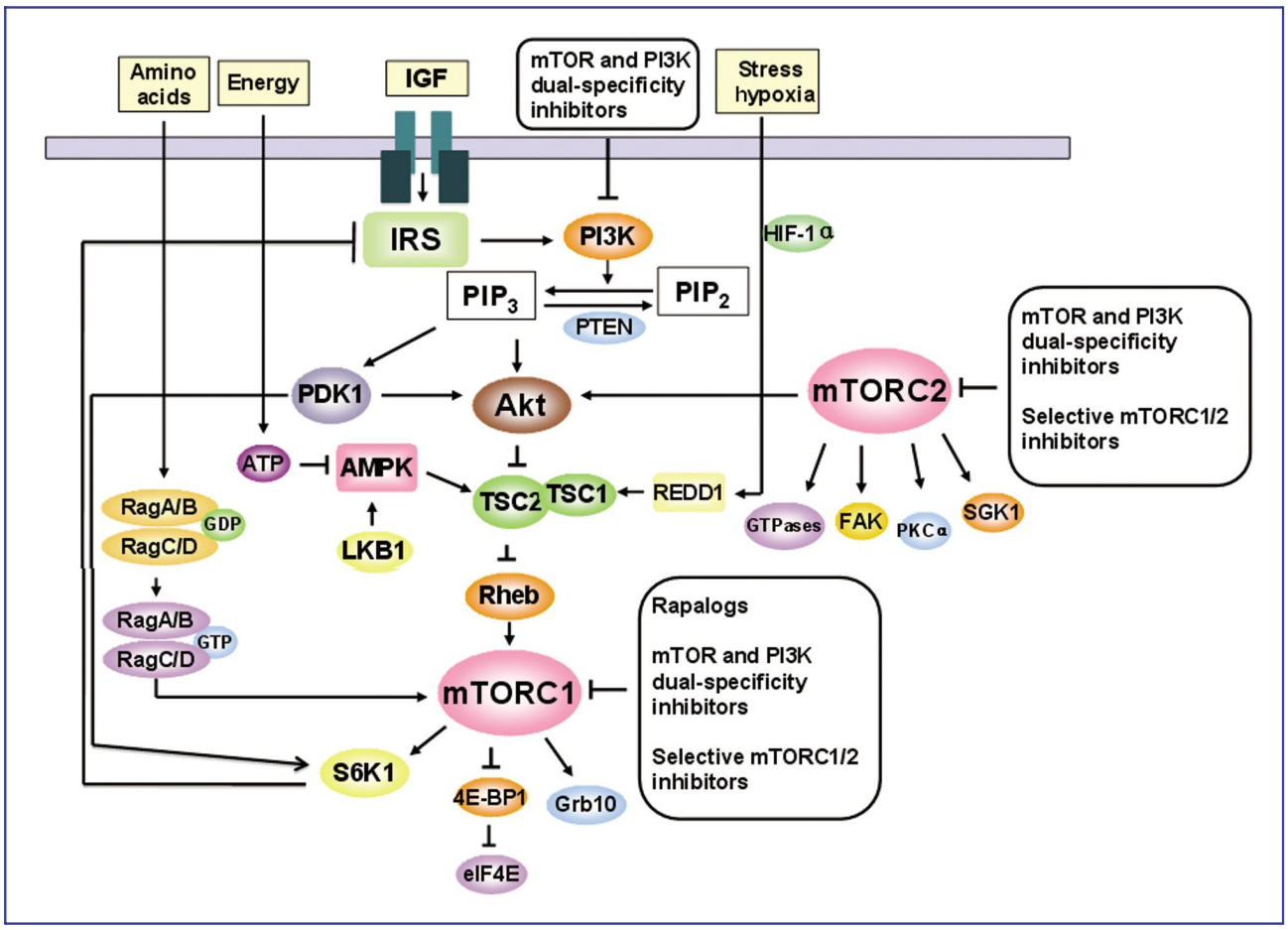

Figure 2. **A model of mTOR signaling network.** mTOR signaling regulates multiple cellular processes by sensing nutrients, growth factors, energy, and stress. Arrows represent activation, whereas bars represent inhibition.

leading to feedback inhibition of the PI3K pathway. Identification of the mTORC1-GRB10 interaction complements a known negative feedback loop in which mTORC1 activation can inhibit the PI3K pathway by S6K1-mediated phosphorylation and degradation of insulin receptor substrate-1 (IRS-1), and it fills an important gap in our understanding the underlying mechanisms by which mTORC1 inhibits PI3K-Akt signaling[49,50].

The first generation of mTOR inhibitors includes rapamycin and its analogues (also known as rapalogs) that specifically inhibit mTORC1 (Table 1). Rapamycin was originally isolated from a bacteria in the soil on Easter Island (Rapa Nui) in 1975 and was used as a fungicide[51,52], and it was later used as an immunosuppressant[53]. Subsequent studies showed that rapamycin is also able to act as a cytostatic agent, slowing or arresting the growth of various cancer cell lines. In addition, rapamycin alone can induce apoptosis in several cancer cell lines and sensitize cells to apoptosis induced by other chemotherapeutic agents[54-56].

Extensive studies have revealed rapamycin's mechanism of action: upon entering the cells, rapamycin binds the intracellular receptor FKBP12, forming an inhibitory complex that binds the FRB domain in the C-terminus of TOR proteins, thereby exerting a cell growth-inhibitory and cytotoxic effect by inhibiting the functions of TOR signaling to downstream targets[10,24-26]. Rapamycin has very poor water solubility and chemical stability, severely limiting its bioavailability[2]. Thus, several rapalogs with improved pharmacokinetic properties and reduced immunosuppressive effects, including temsirolimus (CCI-779), everolimus (RAD001), and deforolimus (AP23573), have been developed[57,58]. The minor chemical modifications to each of these rapalogs preserve their interactions with both FKBP12 and mTOR, and therefore, they share the same action mechanism as rapamycin[59]. Temsirolimus and everolimus were recently approved by the Food and Drug Administration (FDA) for the treatment of advanced/metastatic renal cell carcinoma[60,61]. The preclinical and clinical studies on the anticancer effect of

rapalogs have been extensively reviewed [62-64]. Despite these promising data, however, the collective studies have shown that the single agent activity of rapalogs is modest in other major solid tumors[65]. The emergence of resistance to rapalogs is clearly a critical problem and may limit their utility[65]. One of the mechanisms by which resistance to mTORC1 inhibitors is developed is related to the presence of mTORC1-dependent negative feedback loops [65]. mTORC1 activation is postulated to cause a negative feedback loop through S6K1, which directly phosphorylates IRS-1 and finally induces its degradation [49]. Therefore, mTOR inhibition will increase IRS-1 protein expression, resulting in Akt activation [49]. O'Reilly *et al.* [49] observed that rapamycin up-regulated IRS-1 protein levels and induced Akt activation in a panel of cancer cell lines, partially explaining the general ineffectiveness of rapamycin in the clinic. This study also provides a rationale for the development of effective combination therapy with an mTOR inhibitor and PI3K inhibitor or an inhibitor of the growth factor receptor, such as IGF-IR [49]. As expected, the combination of rapalogs with other anticancer agents, including standard chemotherapies, receptor tyrosine kinase targeted therapies, and angiogenesis inhibitors, has shown greater activity than single agent rapalogs treatment, suggesting that rapalogs may be best utilized in combination therapies[64,66,67].

mTORC2

mTORC2 consists of mTOR, mLST8, rapamycin-insensitive companion of mTOR (rictor) and mammalian stress-activated protein kinase (SAPK) -interacting protein 1 (mSin1) [16,68,69]. In addition, protein observed with rictor (protor), DEPTOR, and Hsp70 are other novel components of mTORC2 [70-72]. Because mTORC2 was discovered only recently, its functions and regulatory mechanisms are less well understood than mTORC1 [16]. In 2005, Akt was identified as the direct substrate of mTORC2 [73]. More specifically, mTORC2 was found to be a long sought-after kinase that phosphorylates Akt on Ser473 [73]. Most recently, SGK1 was identified as a novel substrate of mTORC2 [14,74,75]. mTORC2 phosphorylates SGK1 at its hydrophobic motif site and thereby regulates the activity of SGK1[14]. Unlike mTORC1, mTORC2 is insensitive to acute rapamycin treatment [16]. However, prolonged rapamycin incubation disrupts mTORC2 assembly and inhibits mTORC2 function in certain cells[76]. mTORC2 was suggested to be activated by PI3K because growth factors stimulate mTORC2 activity and low concentrations of wortmannin, a specific PI3K inhibitor, inhibits Akt Ser473 phosphorylation [73]. However, the mechanism by which mTORC2 is activated by PI3K is not understood. The main function of mTORC2 is to regulate reorganization of the actin cytoskeleton via the phosphorylation of PKCα and the focal adhesion proteins, and to control the activities of the small GTPases (RhoA, Cdc42, and Rac1)[15-18,77]. The significant finding that mTORC2 directly phosphorylates Akt, an important survival kinase, provides new insight into the role of mTORC2 in cancer[73]. The phosphorylation of Akt at Ser473 leads to full Akt activation and may regulate different cellular processes, including cell growth, proliferation, apoptosis, and glucose metabolism[78]. In 2009, Sparks *et al.*[79] showed that knocking down rictor, an essential component of mTORC2, impairs the ability of PC-3 cells, a human prostate cancer cell line null for *PTEN*, to form solid tumors *in vivo*. Importantly, rictor is not required to maintain the integrity of a normal prostate epithelium. These data suggest that mTORC2 is important for the development of prostate cancer caused by *PTEN* loss but is not important for normal prostate epithelial cells, thus providing rationale for developing mTORC2-specific inhibitors as promising anti-cancer therapeutic agents. Recently, the second generation of mTOR inhibitors, which target the ATP binding site in the mTOR kinase domain and repress both mTORC1 and mTORC2 activity, have emerged, but none of these inhibitors are specific for mTORC2. This class of mTOR inhibitors includes: (1) mTOR and PI3K dual-specificity inhibitors, which target PI3K in addition to both mTORC1 and mTORC2, and (2) selective mTORC1/2 inhibitors, which target both mTORC1 and mTORC2 (Table 1). The use of the second generation of mTOR inhibitors may overcome some of the limitations of rapalogs [65,79,80]. Single agent rapalogs showed limited activity in the majority of tested cancer types [65]. Mechanistically, rapalogs prevented mTORC1-mediated S6K activation, thereby blocking S6K1-mediated negative feedback loop, leading to activation of Akt and promotion of cell survival[49]. Moreover, treatment with rapalogs has been reported to activate the pro-survival extracellular-signal–regulated kinase (ERK) 1/2 pathway through a S6K-PI3K-Ras–mediated feedback loop[81].

mTOR and PI3K Dual-Specificity Inhibitors

Because the catalytic domain of mTOR is homologous to the p110α subunit of PI3K, mTOR and PI3K dual-specificity inhibitors simultaneously target the ATP binding sites of mTOR and PI3K with similar potency [82-86]. By additionally targeting PI3K, these molecules, including PI-103, GNE-477, NVP-BEZ235, BGT226, XL765, SF-1126, and WJD008 (Table 1), may have unique advantages over single-specific mTORC1 and PI3K inhibitors in certain disease settings [82-87]. For example, inhibition of mTORC1 activity alone by

Table 1. mTOR inhibitors

mTOR inhibitors	Origination	Development status	Potential use for the tumor types	Action mechanism
First generation of mTOR inhibitors				
Rapamycin	Wyeth, USA	FDA approved	(Renal transplantation)	Bind to the intracellular receptor FKBP12, and the rapamycin/FKBP12 complex then binds to the FKBP-rapamycin binding (FRB) domain of mTOR kinase
Temsirolimus (CCI-779)	Wyeth, USA	FDA approved	Renal cell carcinoma	
Everolimus (RAD001)	Novartis, Switzerland	FDA approved	Advanced kidney cancer and progressive or metastatic pancreatic neuroendocrine tumors	
Deforolimus (AP23573)	ARIAD, USA	FDA approved	Designated by the FDA as an orphan drug for treatment of soft-tissue and bone sarcomas	
Nab-rapamycin (ABI 009)	Abraxis BioScience, USA	Phase I	Breast cancer, colon cancer	
Second generation of mTOR inhibitors				
mTOR and PI3K dual-specificity inhibitors				
PI-103	Merck, Germany	Preclinical	Acute myeloid leukemia, glioblastoma, melaloma	Target the ATP binding sites of mTOR and PI3K
NVP-BEZ235	Novartis, Switzerland	Phase I/II	Breast cancer, multiple myeloma, glioblastoma, sarcoma, pancreatic cancer	
WJD008	Chinese Academy of Sciences, China	Preclinical	Breast cancer, colon cancer, prostate cancer, glioblastoma, lung cancer	
XL765	Exelixis, USA	Phase I/II	Breast cancer, lung cancer, ovarian cancer, prostate cancer, gliomas	
SF-1126	Semafore, USA	Phase I	Gastrointestinal stromal tumor, colorectal cancer, ovarian cancer, breast cancer, prostate cancer, haematological cancer	
Selective mTORC1/2 inhibitors				
Torin1	Gray Laboratory, Harvard, USA	Preclinical	–	Target the active site of mTOR in both mTORC1 and mTORC2
PP242	University of California, USA	Preclinical	Multiple myeloma, leukemia, breast cancer	
PP30	University of California, USA	Preclinical	–	
Ku-0063794	Kudos, UK	Preclinical	–	
WYE-354	Wyeth, USA	Preclinical	Breast cancer, prostate cancer, glioblastoma, colon cancer, renal cell carcinoma	
WAY-600	Wyeth, USA	Preclinical	Breast cancer, prostate cancer, glioblastoma, colon cancer, renal cell carcinoma	
WYE-687	Wyeth, USA	Preclinical	Breast cancer, prostate cancer, glioblastoma, colon cancer, renal cell carcinoma	
INK128	Intellikine, USA	Phase I	Multiple myeloma, breast cancer, prostate cancer, non-Hodgkin's lymphoma,	
AZD8055	AstraZeneca, UK	Phase I	Gliomas, breast cancer, renal cell carcinoma	
OSI-027	OSI, USA	Phase I	Lymphoma, colorectal cancer, melanoma, neuroendocrine tumors, endometrial cancer, renal cell carcinoma, cervical cancer	

rapalogs may result in the enhanced activation of the PI3K axis because of the mTOR-S6K1-IRS-1 negative feedback loop[49]. Therefore, the mTOR and PI3K dual-specificity inhibitors might be sufficient to avoid PI3K pathway reactivation.

PI-103

PI-103, a dual class I PI3K/mTOR inhibitor, is a small synthetic molecule of the pyridofuropyrimidine class[88]. PI-103 potently and selectively inhibited

recombinant PI3K isoforms, p110α, p110β, and p110δ, and suppressed mTOR and DNA-PK, which belong to the PIKK family [88]. PI-103 showed inhibitory effects on cell proliferation and invasion in a wide variety of human cancer cells *in vitro*[88]. *In vivo*, PI-103 exhibited therapeutic activity against a range of human tumor xenografts, showing inhibitory effects on tumor growth, angiogenesis, invasion, and metastasis [88,89]. In human leukemia cells and primary blast cells from acute myelogenous leukemia (AML) patients, PI-103 suppressed constitutive and growth factor-induced activation of PI3K/Akt and mTORC1 [90]. In human leukemia cell lines, PI-103 inhibited cell proliferation and induced cell cycle arrest in the G_1 phase. In blast cells, PI-103 induced apoptosis and inhibited the clonogenicity of AML progenitors, indicating the therapeutic value of PI-103 in AML [90]. In addition, PI-103 was able to enhance the efficacy of radiotherapy and sensitize cells to chemotherapy-induced apoptosis [91,92]. In primary glioblastoma cells derived from patients, PI-103 significantly increased doxorubicin- and etoposide-induced apoptosis, further verifying its clinical relevance[91]. Obviously, these findings may have implications for rational design of drug combination regimens to overcome the frequent chemoresistance of glioblastoma[91].

NVP-BEZ235

NVP-BEZ235 (Novartis), a novel, dual class I PI3K/mTOR inhibitor, is an imidazoquinoline derivative currently in phase I/II clinical trials. NVP-BEZ235 binds the ATP-binding clefts of PI3K and mTOR kinase, thereby inhibiting their activities [83]. Increasing evidence shows that NVP-BEZ235 is able to effectively and specifically reverse the hyperactivation of the PI3K/mTOR pathway, resulting in potent antiproliferative and antitumor activities in a broad range of cancer cell lines and experimental tumors[93-95]. In breast cancer cells, NVP-BEZ235 blocked the activation of the downstream effectors of mTORC1/2, including Akt, S6, and 4E-BP1[93]. Meanwhile, NVP-BEZ235 showed greater antiproliferative activity than the allosteric selective mTOR inhibitor everolimus in all cancer cell lines tested[93]. In a xenograft model of BT474-derived breast cancer cells overexpressing either the p110α H1047R oncogenic mutation or the empty vector, NVP-BEZ235 significantly inhibited tumor growth of both xenografts[93]. Consistently, NVP-BEZ235 at nanomolar concentrations suppressed phosphorylation of Akt, S6K, and 4E-BP1 and inhibited cell growth in a panel of cancer cells, including myeloma cells [95,96], as well as human glioma [97], osteosarcoma, Ewing's sarcoma, and rhabdomyosarcoma [98]. Recently, a phase I, multicenter, open-label, single-agent, dose-escalation trial of NVP-BEZ235 showed that NVP-BEZ235 is active in patients, especially in those with PI3K pathway dysregulated tumors, and is well tolerated with a favorable safety profile [99]. However, pharmacokinetic studies showed that the area under the curve and C_{max} increased non-proportionally with dose and were variable within and among patients, so future studies will use a new formulation of NVP-BEZ235 with improved pharmacokinetic properties. In combining treatments, NVP-BEZ235 together with melphalan, doxorubicin, and bortezomib showed synergistic and additive effects on cell growth inhibition in multiple myeloma cells [95]. In a xenograft model with TC-71 Ewing's sarcoma cell line, treatments with NVP-BEZ235 in combination with vincristine effectively inhibited tumor growth and metastasis [98]. These data suggest potential clinical activity of NVP-BEZ235 combined with chemotherapeutic agents.

WJD008

WJD008, another novel dual PI3K/mTOR inhibitor, was recently synthesized and exhibited potent inhibition on the kinase activity of both p110α and mTOR [86]. In *PIK3CA*-mutant transformed cells and a panel of tumor cells, including liver cancer, lung cancer, stomach cancer, glioblastoma, prostate cancer, rhabdomyosarcoma, colon cancer, ovarian cancer, squamous carcinoma, and breast cancer, WJD008 showed potent antiproliferative activity[86].

Selective mTORC1/2 Inhibitors

Recently, several selective mTORC1/2 inhibitors have been developed. These molecules, including Torin1, PP242, PP30, Ku-0063794, WAY-600, WYE-687, WYE-354, INK128, AZD8055, and OSI-027 (Table 1), showed potent, selective inhibition on mTOR. Unlike PI3K family inhibitors, they inhibit both mTORC1 and mTORC2 without inhibiting other kinases [100]. These molecules were shown to potently inhibit both mTORC1 and mTORC2 at nanomolar concentrations, as evidenced by inhibition of S6K1 phosphorylation at Thr389 and Akt phosphorylation at Ser473, respectively [100-103].

Torin1

Torin1, a pyridinonequinoline compound discovered from a biochemical screen for mTOR inhibitors, was identified as a potent and selective mTOR kinase inhibitor [101]. *In vitro* kinase assays showed that Torin1 inhibited both mTORC1 and mTORC2 with half maximal inhibitory concentration (IC_{50}) values between 2 nmol/L and 10 nmol/L [101]. In mouse embryonic fibroblasts (MEFs), Torin1 potently suppressed the phosphorylation

of the downstream substrates of mTORC1 and mTORC2, S6K1 at T389 and Akt at S473, with IC_{50} between 2 nmol/L and 10 nmol/L as well[101]. Meanwhile, the study showed that Torin1 was at least 200-fold selective for mTOR over other PIKK kinases, including PI3K and the DNA-damage response kinases ATM and DNA-PK, suggesting that Torin1 is a highly selective inhibitor of mTOR[101]. Moreover, Torin1 exhibited a greater inhibitory effect on cell growth and proliferation than rapamycin[101]. Surprisingly, Thoreen *et al.*[101] argued that these effects of Torin1 are not caused by the inhibition of mTORC2, but by the suppression of rapamycin insensitive functions of mTORC1. These results suggest that mTOR kinase domain inhibitors are useful not only in the study of mTORC2, but also for revealing rapamycin-resistant functions of mTORC1[100,101].

PP242 and PP30

PP242 and PP30, two novel and specific mTOR kinase domain inhibitors against both mTORC1 and mTORC2, were reported by Feldman *et al.*[100]. In biochemical assays, these two compounds inhibited mTOR in both mTORC1 and mTORC2 with IC_{50} values of 8 nmol/L and 80 nmol/L, respectively[100]. Compared with rapamycin, PP242 has a much higher antiproliferative effect in primary MEFs[100]. One might expect that this is due to the inhibition of both mTORC1 and mTORC2. However, the study surprisingly showed that the suppression of mTORC2 by PP242 did not result in a total blockade of Akt, and moreover, the inhibition of 4E-BP1 phosphorylation by PP242 was more complete than rapamycin. These results suggest that additional mTORC1 inhibition by PP242 could be the basis for its superior antiproliferation activity[100]. In this regard, further study revealed that the inhibition of translational control and the antiproliferative effects of PP242 require inhibition of 4E-BP1 phosphorylation and eIF4E activity[100]. However, a recent study showed that the superior antitumor effect of PP242 over rapamycin in multiple myeloma (MM) cells was not due to a greater inhibition on 4E-BP1 phosphorylation, but to its additional inhibitory effects on mTORC2[104]. Another more recent study showed that knockdown of rictor with prevention of mTORC2 assembly inhibited cell growth and induced apoptosis, further supporting mTORC2 as a therapeutic target in MM[104].

Others

Ku-0063794 (Kudos Pharmaceuticals), WAY-600 (Wyeth), WYE-687 (Wyeth), WYE-354 (Wyeth), INK128 (Intellikine), AZD8055 (Astra Zeneca), and OSI-027 (OSI Pharmaceu ticals) (Table 1), which have been most recently reported as ATP-competitive mTOR inhibitors, effectively inhibited both mTORC1 and mTORC2[102,103,105-107]. Ku-0063794 is a potent and highly selective inhibitor of mTOR with an IC_{50} of 10 nmol/L[103]. It did not significantly inhibit a panel of 76 protein kinases nor 7 lipid kinases tested[103]. Three pyrazolopyrimidine ATP-competitive mTOR inhibitors, WAY-600, WYE-687 and WYE354, potently inhibited recombinant mTOR enzyme with IC_{50} values of 9, 7, and 5 nmol/L, respectively[102]. Moreover, they were highly selective for mTOR over PI3K families (>100 fold to PI3Kα and >500 fold to PI3Kγ) and did not significantly affect a panel of 24 protein kinases tested[102]. These inhibitors induced G_1 cell cycle arrest and exhibited antiproliferative effects against several cancer cell lines[102]. In nude mice bearing PTEN-null PC3MM2 tumors, WYE-354 inhibited both mTORC1 and mTORC2 and suppressed tumor growth in a dose-dependent manner[102]. INK128, which was developed by Intellikine, is another potent and selective mTOR inhibitor[107]. The IC_{50} for INK128 toward mTOR kinase is at the sub-nanomolar level, and it showed a high selectivity against a panel of more than 400 kinases. In multiple xenograft models, administration of INK128 alone or in combination with other standard targeted therapy or chemotherapy resulted in antiangiogenic and tumor growth inhibitory effects[107]. The preclinical evidence of pharmacologic activity with AZD8055, a first-in-class, orally available, potent, and specific inhibitor of mTOR kinase, has recently been reported[105]. AZD8055 exhibited potent inhibitory activity against mTOR with an IC_{50} of 0.8 nmol/L and showed at least 1000-fold differential in potency against all class I PI3K isoforms and other members of the PI3K-like kinase family[105]. In the H383 and A549 non–small cell lung cancer cell lines, AZD8055 potently inhibited cell proliferation and induced autophagy[105]. In glioblastoma (U87-MG) and lung cancer (A549) xenografts, a single oral administration of AZD8055 decreased the phosphorylation of S6 at Ser235/236 and Akt at Ser473[105]. AZD8055 also induced significant growth inhibition and/or regression in a broad range of tumor xenografts[105]. Most recently, Jiang *et al.*[108] reported that a combination of AZD8055 and αCD40 agonistic antibody induced synergistic antitumor responses in a model of metastatic renal cell carcinoma. Currently, AZD8055 is being evaluated in phase I clinical trials. A recent study reported the preclinical characterization of OSI-027, a selective and potent dual inhibitor of mTORC1 and mTORC2 with biochemical IC_{50} values of 22 nmol/L and 65 nmol/L, respectively[109]. OSI-027 exhibited high selectivity for mTOR relative to PI3Kα, PI3Kβ, PI3Kγ, and DNA-PK (>100-fold)[109]. In several tumor cell lines with activated PI3K-Akt signaling, OSI-027 potently inhibited cell proliferation and induced cell death. OSI-027 was well-t olerated *in vivo* and demonstrated potent anti-tumor activity in multiple tumor xenografts models. Moreover, OSI-027 showed significantly greater inhibition of tumor growth in GEO

and COLO 205 colorectal cancer xenografts compared to rapamycin [109]. Currently, OSI-027 is in phase I clinical trials in cancer patients [106]. Recently, a first-in-human phase I trial exploring three schedules of OSI-027 in patients with advanced solid tumors and lymphoma has been presented [106]. OSI-027 was reported to be well tolerated at the doses and schedules tested. Preliminary evidence of the pharmacological activity of OSI-027 was also observed in this study[106].

Summary

mTOR plays a pivotal role in the control of cell growth and proliferation and is an important anti-cancer drug target. mTOR is found in two distinct multiprotein complexes within the cells, mTORC1 and mTORC2. Rapamycin is widely accepted as selective inhibitor of mTORC1. Rapalogs with improved pharmacokinetic properties and reduced immunosuppressive effects have demonstrated preclinical and clinical therapeutic efficacy in certain types of cancer. However, single agent activity of rapalogs is modest in most tumor types. Mechanistically, the specific inhibition of mTORC1 by rapalogs may induce multiple pro-survival feedback loops, including PI3K-Akt and PI3K-Ras-Erk pathways, leading to the attenuation of the therapeutic effects of the rapalogs. Thus, combination therapy or the use of the second generation of mTOR inhibitors, which include mTOR and PI3K dual-specificity inhibitors and selective mTORC1/2 inhibitors, may overcome some of the limitations of rapalogs and exhibit improved antitumor activity. As expected, the second generation of mTOR inhibitors, which can block PI3K-mediated or mTORC2-mediated Akt activation, have demonstrated improved efficacy, particularly in cancer cells with activating PI3K mutations. Data from early phase of clinical trials have recently shown significant clinical activity and good tolerability for most of these inhibitors. The development of this class of mTOR inhibitors marks the beginning of an exiting new phase in mTOR-based therapeutic strategies. An important requirement to improve the clinical outcomes is the identification of predictive biomarkers that can define the tumor subtypes and patient populations that are most likely to respond to the use of mTOR inhibitors. As these mTOR inhibitors are still in the early stage of evaluation, their therapeutic efficacy and potential toxicity still need to be further investigated. Meanwhile, we are also looking forward to the development of a new generation of mTOR inhibitors that specifically target mTORC2. mTORC2-specific inhibitors might have substantial clinical value in treating cancers by not perturbing the feedback activation of the PI3K-Akt pathway that occurs with mTORC1 inhibition.

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