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mTOR Inhibitors at a Glance

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Abstract

Mechanistic target of rapamycin (mTOR) is a conserved threonine and serine protein kinase that was identified more than two decades ago as the target of immunosuppressive drug rapamycin. Since then considerable amount of information has been learned about the function of this kinase. It is now well-established that mTOR plays a pivotal role in governing cell growth and proliferation, hence making mTOR a therapeutic target for disease conditions caused by deregulated cell proliferation, such as cancer. In the past decade, numerous mTOR inhibitors have been developed and many are currently in clinical trials for cancer treatment. This commentary is to provide a brief summary of these mTOR inhibitors.

Keywords

mTOR; Phosphatidylinositol-3-kinase; Akt; Rapamycin; Kinase inhibitors

Introduction

mTOR belongs to the family of phosphatidylinositol-3-kinase-related kinases (PIKKs). Members in this family are large in size (>2,500 amino acids) and harbor a kinase domain at their C-terminals that shares sequence similarity to phosphatidylinositol-3-kinase (PI3K) (1). Despite having the sequence signature of a lipid kinase, mTOR is a protein kinase that phosphorylates threonine and serine residues in its substrates. In cells mTOR serves as the catalytic subunits of two multi-protein complexes termed as the mTOR complex 1 (mTORC1) and complex 2 (mTORC2) (2–4). TORC1 is a major downstream component of the PI3K/AKT pathway that relays the signals from tumor suppressors PTEN, LKB1 and TSC1/2, and oncoproteins PI3K and AKT (Figure 1) (5). Downstream mTORC1 controls cellular biogenesis through regulation of protein synthesis and turnover. It phosphorylates eIF4E binding protein 1 (4EBP1) and ribosomal protein S6 kinase (S6K), two factors involved in translation initiation (6). Its activity controls protein turnover through repressing autophagy (7). mTORC2 is also involved in the PI3K/AKT pathway but its function is

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Conflicts of Interest

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independent of mTORC1. It phosphorylates and stimulates AKT activation, and hence plays a critical role in AKT mediated cell survival (8).

Rapamycin is the prototype of the first generation of mTOR inhibitors (9). It is a macrocyclic lactone that contains two binding moieties that are essential for its action (Figure 2). One moiety binds to FKBP12, a small cytosolic protein that displays peptidylprolyl isomerase activity. At pharmacological relevant concentration, rapamycin imposes no significant effect on the function of FKBP12. But binding with FKBP12 allows it to interact with its mechanistic target, mTOR, to form a ternary complex (10). The rapamycin-FKBP12 dimer binds to mTOR in a region (FKBP12-rapamycin binding domain) that is outside of the kinase domain. As such, the binding itself does not inhibit the kinase activity of mTOR. It is believed that the binding interferes with the association of the kinase with its substrates, although the precise mechanism remains to be elucidated (11). Despite the presence of mTOR in both mTORC1 and mTORC2, rapamycin only inhibits mTORC1 activity. The unique components of mTORC2 may exclude the drug from binding with the kinase. However, in some cells, prolonged incubation with the drug also affect mTORC2, presumably by binding the newly synthesized mTOR, hence preventing it from assembly into the complex (8).

Rapalogs, the first generation of mTOR inhibitors

Rapamycin was first developed into an immunosuppressive drug for its potent action in blocking T-cell activation. It was approved by the FDA in 1997 for use in transplantation to prevent allograft rejection, and in 2003 for use in coronary-artery stents to prevent restenosis (9). Although the anti-cancer activity of rapamycin was documented in early 1980s, its application in cancer therapy was not exploited until late 1990s, when several analogs of the drug, which often termed as rapalogs, were developed. Rapamycin is poorly water soluble, which affects its bioavailability. The development focused mainly on improving its pharmacokinetics and stability. However, because the drug requires two sides for binding with FKBP12 and mTOR, there is not much room for further modification. All rapalogs are created by replacing the hydrogen at C-40-O position with different moieties (Figure 2). For Temsirolimus (CCI-779), it is a dihydroxylmethyl propionic acid ester (12), Everolimus (RAD001), a hydroxylethyl group (13), and Ridaforolimus (AP23573), a dimethyl phosphate group (14) (Figure 2). In 2007 Temsirolimus became the first rapalog approved by FDA for cancer treatment (15).

Given the specificity of the drug and its potency in anticancer activity in many preclinical models, it was hard to image the need for new types of mTOR inhibitors. However, the clinical application of rapalogs in cancer treatment has so far met with limited success. Rapalogs are effective in treating a few cancers, including renal cell carcinoma and mantle cell lymphoma, but not in the majority of solid tumors (16). The mechanisms underlying the rapalog resistance are complex. Genetic variations of the factors associated with the mTOR signaling pathway in cancer cells contribute to *de novo* resistance to drug (17), however, the main reason is likely due to the fact that the drug does not directly cause cell death. Inhibition of mTORC1 by rapamycin actually induces stress responses, including reduction in protein synthesis and induction of autophagy, which are protective mechanisms for the

cells to survive under stress conditions (18). Consequently, the direct effect of the drug is cytostatic rather than cytotoxic. In addition, in many types of cancer cells, inhibition of mTORC1 turns off a S6K-dependent negative feedback loop that downregulates upstream signaling of PI3K/AKT (Figure 1), resulting in enhanced PI3K/AKT activity that promotes cell survival (19).

mTOR kinase inhibitors, the second generation of mTOR inhibitors

Two strategies have been explored to circumvent the limitation of rapalogs in cancer therapy (20). The first involves pairing the drugs with cytotoxic agents. For instance, rapalogs in combination with chemotherapeutic drugs such as paclitaxel and carboplatin are currently used in trials for treating advanced ovarian cancer and metastatic melanoma (NCT01196429 and NCT00976573). In addition, the drugs are also used in conjunction with hormonal therapy in endocrine cancers as a way to sensitize the cancer cells to the treatment (21–22). The second strategy involves developing inhibitors that target both PI3K and mTOR or selectively, mTOR (23–25). In the latter case, because mTOR serves as the catalytic subunits for both mTORC1 and mTORC2, a drug inhibiting the kinase activity is expected to affect both complexes, and consequently, block the mTORC2 dependent activation of AKT. In the second half of the last decade, many pharmaceutical companies and academic laboratories were actively engaged in development of this new generation of mTOR inhibitors and numerous compounds were discovered that possess potent inhibitory effect on PI3K and/or mTOR.

The development of PI3K and mTOR kinase inhibitors exploits the structure of the ATP binding pocket of the kinases with small molecules that compete for the binding pocket with ATP. Hence, these inhibitors are collectively called ATP competitive inhibitors. Because the sequence similarity of mTOR with PI3K, many ATP competitive PI3K inhibitors were found to display various degrees of mTOR inhibitory activity. These inhibitors were often used as prototype compounds for the PI3K/mTOR dual inhibitors. One of such compounds is PI-103, a pyrimidine derivative, which was originally developed as a pan-PI3K inhibitor but was subsequently found to inhibit PI3K-related kinases, including mTOR. PI-103 inhibits several isoforms of PI3K with an IC₅₀ of 2–3 nM but is less selective for mTORC1 and mTORC2 with IC₅₀ of 20 nM and 83 nM, respectively (26). Several other inhibitors were developed based on the structure of PI-103, including GDC-0980, which possess a similar IC₅₀ for both PI3K and mTOR (27), and GNE-493, GNE-477 and PF-04691502, which are more selective for PI3K than for mTOR (28–30). BEZ-235, an imidazoquinoline derivative, represents another group of the inhibitors that also include BGT226 and GKS2126458. BEZ-235 inhibits several isoforms of PI3K and mTOR with IC₅₀ of ~5 nM (31). GKS2126458 is one of the most potent PI3K/mTOR dual inhibitors. It exhibits an IC₅₀ of 0.04 nM for PI3K and 0.18 and 0.3 nM for mTORC1 and mTORC2, respectively (32). XL765, a quinoxaline derivative, is another non-pyrimidine derivative that exhibits high potency for PI3K and mTOR (33) (Figure 3).

While simultaneously targeting PI3K and mTOR circumvents the limitation of rapalogs in blocking PI3K/AKT signaling, the potential toxicity associated with this type of inhibitors presents a big concern, owing to the diverse functions of different isoforms of PI3K. It is

generally believed that inhibitors more selective for mTOR may be better tolerated than the dual inhibitors (34). In addition, because mTORC2 is required for activation of AKT, inhibiting this complex by mTOR kinase inhibitors is also expected to dampen the pro-survival function of AKT (35). These considerations fueled the development of inhibitors that are more specific to mTOR than to PI3K. In 2009, a series of selective mTOR inhibitors were reported. These selective mTOR inhibitors include pyrimidine derivatives PP242 and PP30 (36), morpholino-linked pyrimidine derivatives, WAY-600, WYE-687 and WYE354 (37), and KU0063794 and AZD8055 (38–39) (Figure 3). In comparison with many PI3K/mTOR dual inhibitors, these selective mTOR inhibitors exhibit 10–100 fold selectivity toward mTOR than to isoforms of PI3K. Additional mTOR inhibitors were also developed subsequently that are in general more potent and selective for mTOR inhibition. For instance, OSI-027, a triazine derivative (40), and AZD2014, a pyrimidine derivative similar to AZD8055 (41), both have IC₅₀ below 4 nM and exhibit >300 fold selectivity for mTOR than for PI3K. INK128, a pyrimidine derivative similar to PP242 and PP30, has an IC₅₀ of 1 nM and is >200 fold more selective for mTOR (42–43). Torin 1, a quinoline derivative developed based on BEZ-235, has an IC₅₀ less than 10 nM and is >1,000 fold more specific (44). The high potency and selectivity of these new inhibitors ensure effective and specific downregulation of mTORC1 and mTORC2, while leave PI3K unaffected.

As expected, in both cell based and animal studies, the dual inhibitors, such as BEZ-235 and GSK2126458, are effective in inhibiting PI3K, mTORC1 processes and mTORC2-dependent processes (31–32, 45), while the mTOR selective inhibitors, such as OSI-027 and INK128, specifically reduce mTORC1 and mTORC2 activity (40, 46–47). In comparison with rapalogs, PI3K/mTOR and mTOR selective inhibitors are more potent in blocking cell proliferation and induction of apoptosis in many cultured cancer cells and in tumor xenograft models, including some of rapamycin resistant tumors (33, 40, 47–49). Several of this new generation of inhibitors have successfully made into clinical trials, among which BEZ235, INK128, AZD2014 and XL765 are currently in phase II trials for efficacy studies in treatment of various cancers. In phase I studies, these inhibitors exhibited toxicity that was tolerable and manageable (34), which, to certain degree, alleviates the safety concern association with the new generation drugs.

Conclusion

The past decade has seen an influx of many new mTOR inhibitors which have been proven to be valuable biological and pharmacological tools. The recent clinical success of some of these inhibitors validates the initial expectation for this new generation of mTOR inhibitors. However, a glance at NCI clinical trial website reveals that despite their superior potency, the new generation drugs are still not as favored as rapalogs, presumably over safety concern. As for many other drugs targeting intracellular signaling pathways, the challenges for the new mTOR inhibitors remain the identification of cancers that are sensitive to these inhibitors and optimization of the treatment strategy to obtain the maximal benefit of the drugs.

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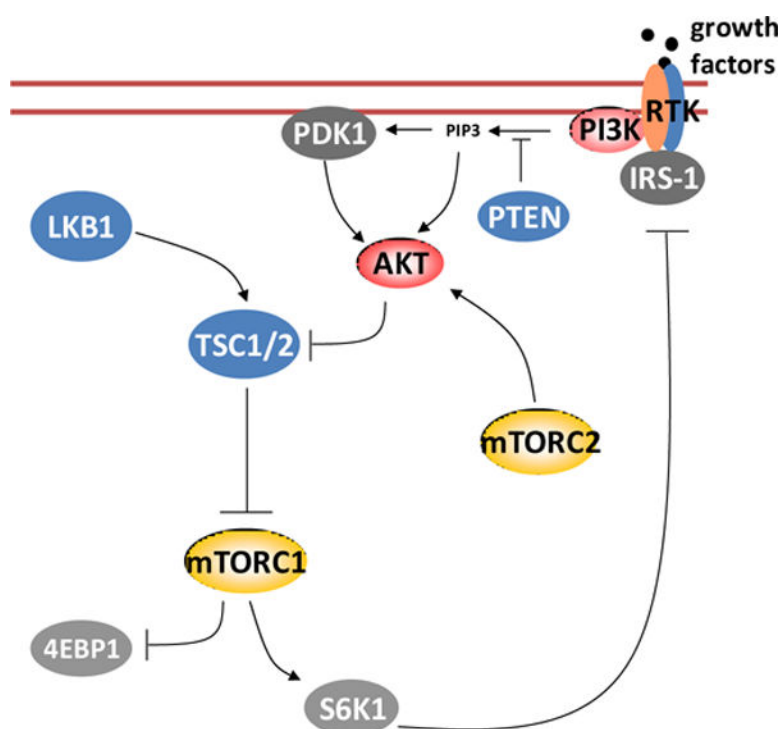


Figure 1.
The mTOR pathway.

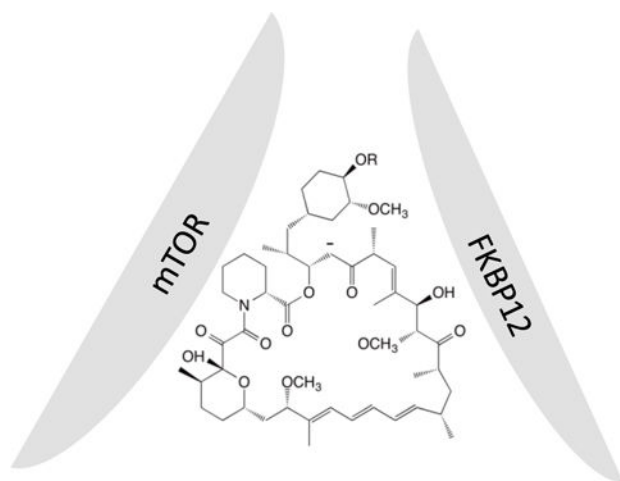
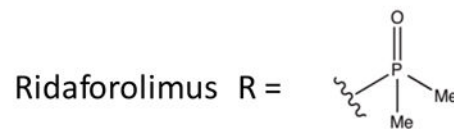
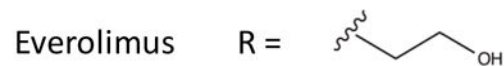
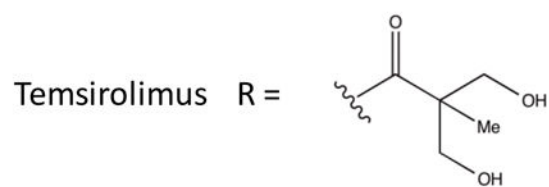


Figure 2.
Structures of rapalogs.

Rapamycin R = H



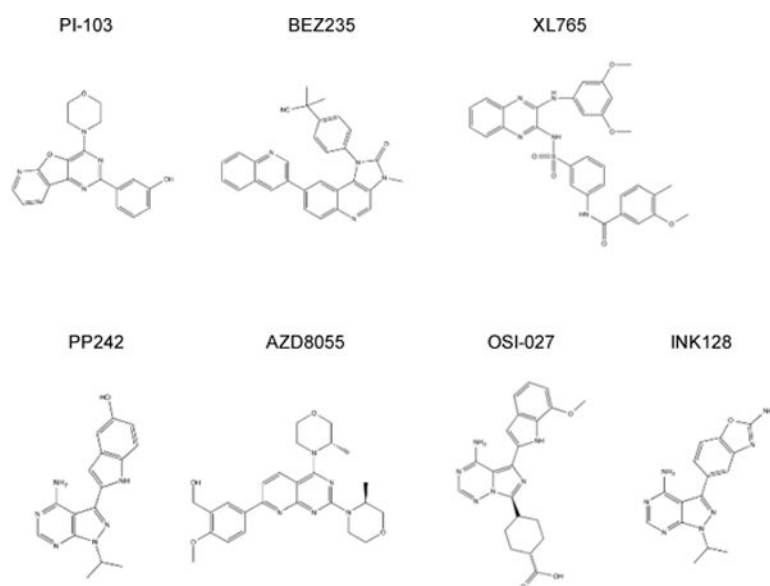


Figure 3.
Structures of mTOR kinase inhibitors.