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Targeting mTOR: up-to-date mTOR inhibitors

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Abstract: mTOR, a member of the phosphatidylinositol 3-kinase-related kinase family, controls major physiological cellular processes such as growth and metabolism in response to nutrients, growth factors, and cellular energy levels. Deregulation of the mTOR activity has been associated with various pathological conditions such as cancer, diabetes, obesity, neurological diseases, and genetic disorders. Therefore, research on the mTOR signalling pathway and the development of effective molecules on this pathway has been continuing intensively in recent years. In the past decade, numerous mTOR inhibitors have been developed and many are currently in clinical trials. These molecules are classified as Rapamycin and its analogs (all are termed rapalogs), ATP-competitive dual PI3K/mTOR inhibitors, mTOR kinase inhibitors, and Rapa Links. In this review, we aimed to summarize current findings on mTOR signalling network and molecules acting on this signalling pathway.

Keywords: mTOR; mTOR inhibitors; rapalogs; dual inhibitors; kinase inhibitors; rapaLinks ©2019 ACG Publications. All rights reserved.

1. Introduction

As the name implies, the history of the target of rapamycin (TOR) is closely linked to the discovery of the rapamycin. Rapamycin, a secondary lipophilic macrolide metabolite, with the chemical formula of C51H79NO13, produced by Streptomyces hygroscopicus, was first isolated in the 1970s in the soil of Easter Island (Rapa Nui). Rapamycin was first discovered as a potent antifungal agent, but it has also been shown to exhibit antitumor and immunosuppressant effects ¹. mTOR plays a central role in the regulation of cell growth, metabolism, and proliferation ²⁻⁴. The presence of two rapamycin targets (TOR1 and TOR2) in yeast cells in 1991 clarified the underlying causes of the antiproliferative activity of rapamycin. In 1994, the mammalian analogs of the yeast TOR complexes were found as mTORC1 and mTORC2 ^{3,5}. Thus, it was understood that TOR was evolutionarily conserved from yeasts to mammals. The brief history of mTOR is shown in Figure 1.

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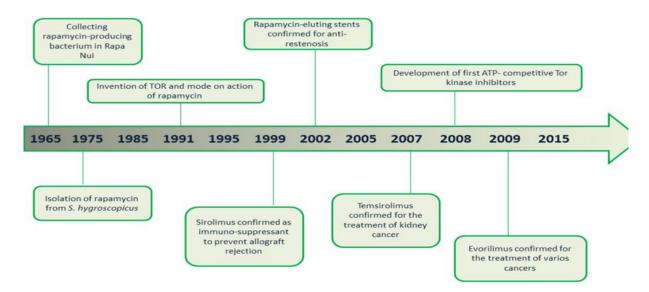


Figure 1. The timeline of key discoveries of rapalogs.

1.1. Structure and Features of mTOR

The mammalian or mechanistic target of rapamycin (mTOR) is a 290 kDa, intracellular, atypical serine/threonine kinase that belongs to the family of phosphatidylinositol 3-kinase (PI3K) ^{6,7}. The N terminus possesses 20 tandem HEAT repeats and this is present in many proteins and is implicated in protein-protein interactions ⁸. The C-terminal includes half of mTOR the kinase domain, which has sequence similarity with the catalytic domain of phosphatidylinositol 3-kinase (PI3K) 9. Instantly upstream of the catalytic domain is the FRB domain and, mTOR includes a comparatively large FAT (for FRAP, ATM, TRAP) domain. The FATC domain is essential for mTOR activity, and the deletion of a single amino acid from this domain cancels the activity 10,11. It has been suggested that the FATC and FAT domains interact to yield a configuration that evinces the catalytic domain. mTOR besides contains a putative negative regulatory domain (NRD) between the catalytic and FATC domains 12. mTOR forms the catalytic core of two functionally distinct complexes, mTORC1 and mTORC2 which have different functions and signal networks. These complexes play a critical role in the fundamental cellular processes ^{13,14}. In comparison with mTORC1, comparatively than little is known about the function of mTORC2 ¹⁵. mTORC1 was composed of mammalian lethal with SEC13 protein 8 (mLST8), proline-rich Akt substrate of 40 kDa (PRAS40), Dep domain-containing mTOR-interacting protein (Deptor) and the regulatory associated protein of mTOR (Raptor) fraction ¹⁶. Raptor and mLST8 positively regulate mTOR's activity and functions, while PRAS40 and Deptor are the negative regulators of the mTORC1 ^{17,18}. mTORC1 comprehensively senses nutrients, growth factors, mitogens, and stress signals, hence being generally associated with cell growth by regulating important cellular processes, involved the translation of mRNAs into the synthesis of key proteins for proliferation, lipid synthesis, mitochondrial biogenesis, and autophagy ^{2,19}. mTORC1 beside suppresses an important catabolic process, autophagy, both by inhibiting its activation and by suppressing the production of lysosomes, the organelles in which autophagy occurs ^{13,20}. Another complex is mTORC2 which is composed of, a rapamycin-insensitive companion of mTOR (Rictor), mLST8, stress-activated protein kinase-interacting protein 1 (SIN1) and protein observed with Rictor (Protor) 1 and 2 fractions ²¹. mTORC2 regulates cell survival, cytoskeleton organization, lipogenesis and gluconeogenesis 2. Threedimensional structure of mTORC1 and structure of mTOR are shown in Figure 2.

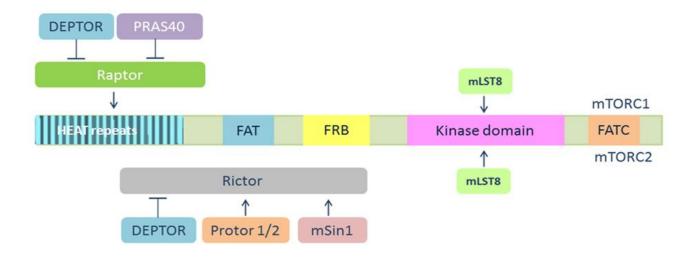


Figure 2. Structure of mTOR. DEPTOR, Dep domain-containing mTOR-interacting protein; mLST8, mammalian lethal with SEC13 protein 8; mSIN1, mammalian stress-activated protein kinase-interacting protein1; PRAS40, proline-rich Akt substrate of 40 kDa; Protor1/2, protein observed with Rictor 1 and 2 fractions; Raptor, regulatory assosicated protein of mTOR; Rictor, rapamycin-insensitive companion of mTOR.

1.2. mTOR Signalling Network

The mTOR signalling network is a potent regulator of anabolic and catabolic processes including protein synthesis, cell proliferation as well as autophagy and, a central controller of cell growth. The mTORCs differ from in terms of upstream modulators, substrate specificity, functional outputs, and susceptibility to inhibitors ²². mTORC1 signalling is activated by several intracellular and extracellular inputs, including the Ras/Raf/MEK/ERK network and the PI3K/AKT network, the intracellular energy levels, and nutrients ^{23,24}. The essential targets of mTORC1 are S6K and a eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4EBP1) ²⁵. Especially, mTORC1 signalling positively regulates the main component of the cell's protein synthesis machinery, eukaryotic initiation factor eIF4E ²⁶. mTOR inhibits 4EBP1 and actives S6K to activate protein synthesis, ribosome biogenesis, nutrient transport and lipid synthesis in return for nutrients, growth factors and cellular energy ²⁷. The main inhibitor and negative upstream regulator of mTORC1 are TSC (tuberous sclerosis complex) 1 and TSC2 heterodimer protein complex ²⁸. The TSC1/2 heterodimer inhibits mTOR signalling by acting as a GTPase activating protein (GAP) towards the small GTPase Ras homolog enriched in brain (Rheb) ^{29,30}. Numerous signalling networks regulate the TSC1/2 heterodimer function as a central coordinator of mTOR signal transduction. Growth factors, nutrients, cytokines, hormones, and cellular energy levels activate many networks, for example, PI3K-Akt and RAS-mitogen-activated protein kinase (MAPK) activates mTORC1 signalling network (via impairment of TSC1/2 function) while leading to the inhibition of the TSC1/2 function. In contrast to the mitogenic network, the energysensing AMP-dependent protein kinase (AMPK) inhibits mTOR signal transduction by phosphorylating and activating TSC2 31. As a result, activated mTORC1, further through S6 kinase 1 (S6K1), 4E-binding protein-1 (4EBP1), cyclin-dependent kinases (CDKs), and the hypoxia-inducible factor 1α (HIF1α), promotes energy metabolism, protein synthesis and lipogenesis, proliferation, and growth ³². However, there is a relative paucity of information regarding the mTORC2 function. The most well-known functions of mTORC2 are the organization of the actin cytoskeleton and maintenance of cell viability. Unlike with mTORC1, the guanosine triphosphate-binding protein Rheb (Ras homolog enriched in brain) is not an upstream activator of mTORC2 and indeed, upstream regulators of mTORC2 have not

been defined. mTORC2 can be activated directly by phosphatidylinositol 3,4,5-trisphosphate and insulin. mTORC2 activates multiple PKA, PKC, and PKG family kinases, including, PKC α , and serum/glucocorticoid regulated kinase 1 (SGK1) $^{33-36}$. Significantly, mTORC2 signalling is also regulated by mTORC1 due to a negative feedback loop between mTORC1 and insulin/PI3K signalling. Moreover, Akt seems to have a complex dual role on mTOR, as of upstream regulator of mTORC1 and a downstream target of mTORC2. The schematic representation of the mTOR signalling network is shown in Figure 3.

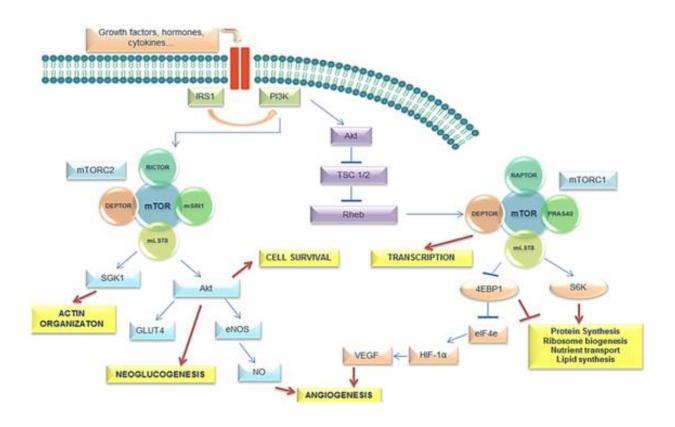


Figure 3. The shematic representation of the mTOR signalling network. 4EBP1, eukaryotic initiation factor 4E-binding protein 1; Akt, protein kinase B; DEPTOR, dep domain-containing mTOR-interacting protein; eIF4e, eukaryotic initiation factor 4E; eNOS, endothelial nitric oxide synthase; GLUT4, glucose transporter type 4; HIF-1α, hypoxia-inducible factor 1α; IRS, insulin receptor substrate; mLST8, mammalian lethal with SEC13 protein 8; mSIN1, mammalian stress-activated protein kinase-interacting protein1; mTORC, mammalian target of rapamycin complex; NO, nitric oxide; PI3K, phosphatidylinositol 3-kinase; PRAS40, proline-rich Akt substrate of 40 kDa; Protor1/2, protein observed with Rictor 1 and 2; RAPTOR, regulatory assosicated protein of mTOR; Rheb, Ras homolog enriched in brain; RICTOR, rapamycin-insensitive companion of mTOR; S6K, S6 kinase; VEGF, vascular endothelial growth factor; SGK1, serum/glucocorticoid regulated kinase 1; TSC, tuberous sclerosis complex.

1.3. Current mTOR Inhibitors

In the past decade, numerous mTOR inhibitors have been developed and many are currently in clinical trials for various diseases treatment ³⁷. These are 1) rapamycin and its derivatives (rapalogs), 2) ATP-competitive dual PI3K/mTOR inhibitors, 3) ATP-competitive mTOR kinase inhibitors (TORKIs), and 4) RapaLink-1.

1.3.1. Rapamycin and Rapalogs (First-Generation mTOR inhibitors)

The best-known mTOR inhibitor rapamycin, macrocyclic antibiotic produced by the bacterium Streptomyces hygroscopicus, is the first inhibitor discovered ^{38,39}. Rapamycin was discovered as a potent antifungal agent, but it also exhibited antiproliferative, antitumoral, and immunosuppressive effect. Rapamycin was approved due to immunosuppressive activity by FDA firstly 40-42. By the reason of their important antiproliferative, neuroprotective/neuroregenerative and cellular effects, during the last two decades, researchers worldwide have developed new semisynthetic rapamycin analogs with similar and more specific pharmacological properties 43,44. Rapamycin is an allosteric inhibitor of mTORC1 and binds to the FRB domain, outside the ATP-binding pocket, together with FKBP12 45,46. This effect reduces the interaction between mTOR and Raptor, resulting in a decrease in mTORC1 activity ⁴⁷. Some studies have shown that although there is no mTORC2 interaction with rapamycin, this molecule changes mTORC2 activity depending on dose, time, and cell type. Therefore, rapamycin can inhibit mTORC2 activity during chronic treatment or long-term exposure ^{22,48,49}. Rapamycin analogs are called as rapalogs which are temsirolimus, everolimus, ridaforolimus (previously name as deforolimus), zotarolimus, WYE-592, ILS-920 50-53. Rapamycin has been clinically confirmed for prophylaxis of organ rejection for renal transplant patients ⁵⁴. Temsirolimus, dihydroxymethyl propionic acid ester of rapamycin, was formulated to increase the solubility of rapamycin and so it can be used to orally and intravenously 55. Everolimus is an O-(2-hydroxyethyl) substitution at position C-40 on the rapamycin structure was designed an orally proper rapamycin analog. It was developed to improving the oral bioavailability of rapamycin. ^{56,57}. Ridaforolimus, a phosphorus-containing analog of rapamycin, was designed depending on computational modelling studies. According to rapamycin, ridaforolimus has more suitable pharmacological properties, including aqueous solubility, chemical stability and bioavailability ⁵⁸. The chemical structures of some of these molecules are shown in Figure 4.

1.3.2. ATP Competitive Dual PI3K/mTOR Inhibitors

mTOR is a member of PIKK-related family sharing a high degree of similarity/sequence homology within the catalytic domain with PI3K, for this reason, the next logical approach was the development of ATP-competitive dual PI3K/mTOR inhibitors ^{59,60}. As mentioned above, rapamycin and rapalogs only partially inhibit mTORC1-dependent translation and cause feedback activation of several signalling networks, including PI3K/Akt. Therefore, several mTOR/PI3K dual inhibitors have been developed ^{61,62}. The development of these agents has benefited from previous attempts with PI3K-selective inhibitors ^{63,64}. The therapeutic advantage of these dual-acting inhibitors is their high efficacy and low risk of drug resistance development, so they are considered to be superior to first-generation mTOR inhibitors ⁶⁵. The prototype molecule of dual PI3K/mTOR inhibitors is pyridofuropyrimidine PI-103. It was never clinically used because of its rapid in vivo metabolism. Over the next few years, other dual PI3K/mTOR inhibitors were developed; the imidazoquinoline derivative NVP-BEZ235 (dactolisib), GDC-0980 (apitolisib), and PKI-587 (gedatolisib) ^{66,67}. The chemical structures of these molecules are shown in Figure 5.

Rapamycin HO чΟН **Everolimus Temsirolimus** HO, H₃CO H₃C ČH₃ ĊH₃ CH₃ CH₃ осн •CH₃ ÇH₃ ÇH₃ OCH₃ Ridaforolimus Zotarolimus CH₃ CH₃ ĊН3 OH H OCH3 **WYE-592** ILS-920

Figure 4. Chemical structures of rapamycin and rapamycin analogs.

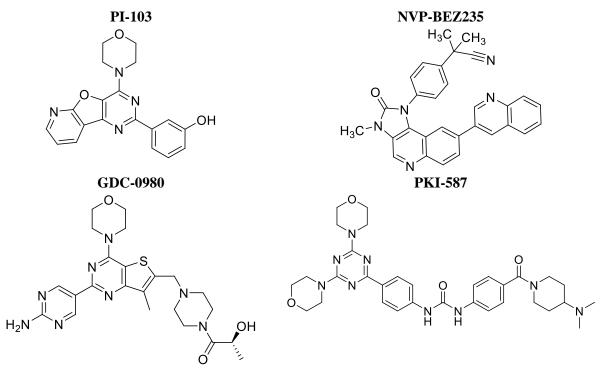


Figure 5. Chemical structure of ATP competitive dual PI3K/mTOR inhibitors.

1.3.3. mTOR Kinase Inhibitors (Second-Generation mTOR Inhibitors)

mTOR kinase inhibitors, also known as ATP-competitive inhibitors, block only the catalytic domain of mTOR resulting in widespread inhibition of the mTOR signalling ^{68,69}. This new generation of mTOR inhibitors is ATP analogs that inhibit mTOR kinase activity by competing with ATP for binding to the kinase domain in mTOR²². mTOR kinase inhibitors exhibit a much lower half-maximal inhibitory concentration (IC50) against mTOR than PI3K ⁵⁹. A small molecule designed to compete with ATP in the catalytic kinase domain of mTOR would be expected to inhibit all of the kinase-dependent functions of mTORC1 and mTORC2. Unlike rapalogs that only target mTORC1, the mTOR kinase inhibitors inhibit both mTORC1 and mTORC2 activity. Because of the similarity between the kinase domains of mTOR and the PI3Ks, several mTOR kinase inhibitors may also cause PI3K activity inhibition as well as mTOR inhibition. Moreover, the antiproliferative and anticancer activity of these compounds has been superior to the first-generation of mTOR inhibitors in cell models ^{70,71}. Torin 1, Torin 2, Ku-0063794, AZD8055, AZD2014 (Vistusertib), CZ415, INK128/MNL0128 (now referred to as TAK-228), OSI-027, WYE354, WYE312, WYE687, WAY600, Palomid 529, GDC-0349, CC223, and XL388 are known mTOR kinase inhibitors ⁷². The chemical structures of some of these molecules are shown in Figure 6.

Figure 6. Chemical structure of mTOR kinase inhibitors.

1.3.4. Rapa Links (Tird-Generation mTOR Inhibitors)

Recently, the third generation of mTOR inhibitors has been reported. Because of the poor efficacy, resistance mechanisms and serious side effects of the previously mentioned drugs, third-generation mTOR inhibitors have been developed. The RapaLink is the representative of this generation and it synchronously associates with and allosterically inhibits mTORC1 by the way of FRB domain while blocking the catalytic activity of this mTOR complex by binding to the ATP-binding pocket of mTOR itself ⁷³. Its chemical structure is shown in Figure 7. This molecule is a bivalent mTOR inhibitor and has been generated by binding rapamycin to MLN0128 molecules that effectively inhibit mTOR kinase activity. Especially, RapaLink-1 also leads to inhibition of both mTORC1 and mTORC2 by acting on their downstream targets. Further studies are needed to clarify the effects of RapaLink on the immune system, autophagy and feedback mechanisms ^{73,74}.

Figure 7. Chemical structure of RapaLink 1.

2. Conclusion

Although the discovery of mTOR in the last two decades, the importance and function of the mTOR signalling pathway is just beginning to be understood. It is not surprising, given its importance in normal physiology and in several diseases, that so much attach importance to has been dedicated to understanding mTOR signalling pathways and to developing agents that interfere with signalling through mTOR. Many aspects of the mechanisms of action of mTOR inhibitors and their clinical effects remain unknown. While the pharmacological profiling of rapalogs is be clarified, much less is known about the other mTOR inhibitors. Therefore, a great deal of research has focused on mTOR inhibitors and elucidating the mechanisms linking mTOR signalling. New inhibitors may be developed because of the side effects or limited effects of existing drugs. Besides, due to the physiological importance of mTOR, new indications for inhibitory molecules targetting this signalling pathway may arise. Consequently, studies on the characterization of the mTOR signalling pathway and the development of new molecules targeting this pathway will also continue in the future.



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