

Mechanisms of Rapamycin Inhibition on mTOR and Drug Design

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Abstract. The phosphoinositide 3-kinase related kinases (PIKKs) are a family of serine/threonine protein kinases that play important roles in multiple significant signaling pathways and some of them have close relationships with a variety of human diseases. Several structures of PIKKs determined by cryo-electron microscopy and X-ray crystallography have been published recently. Among these structures, mTOR-related complexes, especially mTOR complex 1 (mTORC1) is best studied. mTOR-related structures suggest that the binding of rapamycin to mTOR can generate steric hindrance to narrow the access to the recessed active site and thus block the recruitment of substrates, which induces the inhibition of mTOR activity. mTOR signaling is often hyper-active in multiple cancer types and inhibition of mTOR is an available strategy in tackling cancers. Rapamycin is the natural inhibitor of mTOR, but it has not been taken into cancer therapy because of its poor pharmacokinetic properties. Therefore, rapamycin analogues (called rapalogues) are developed and some rapalogues have been clinically applied to the treatment of diseases including soft tissue and bone sarcoma, breast cancer and renal cancer. Unfortunately, these rapalogues have some drawbacks both in research and cancer therapy. Hence, novel types of inhibitors are being explored, such as ATP-competitive inhibitors. There is still a long way to the development of mTOR-targeted drugs that have significant effects on diseases, and combination of several therapeutic methods will help to improve cancer therapy. Design of novel and selective inhibitor drugs based on mTOR-related structures awaits more structural and functional information of mTORC1 and mTORC2.

Introduction

The phosphoinositide 3-kinase (PI3K) related kinases (PIKKs) are a family of serine/threonine protein kinases that participate in a variety of significant signaling pathways, including nutrition metabolism, DNA damage responses and cell growth. PIKKs are evolutionally conserved from yeast to human beings [1] and mediate multiple biological processes [2]. In human, there are six PIKKs, namely ATM, ATR, mTOR, SMG-1, TRRAP and DNA-PKcs [1, 3]. ATM, ATR and DNA-PKcs are responsible for transducing the signals of DNA damage; SMG-1 is associated with nonsense-mediated mRNA decay; mTOR transduces signals of nutrient metabolism and so on, while TRRAP mediates transcription [3]. However, the precise mechanisms that how these PIKKs function in different pathways remain unclear.

Several members of PIKK family have close relationships with many human diseases. Germline mutations of genes encoding ATM, ATR and DNA-PKcs induce

ataxia-telangiectasia, Seckel syndrome or severe combined immunodeficiency, respectively [1]. The disorder of mTOR signaling leads to tumor growth [4].

Human mTOR can be divided into four main domains: N-terminal HEAT repeats, FAT domain, kinase domain and FATC domain at the C terminus. The kinase domain consists of N-lobe and C-lobe. A FK506 binding protein 12 (FKBP12)- rapamycin-binding (FRB) domain is contained in the N-lobe of the kinase domain (Fig. 1, upper panel). The FRB domain is responsible for binding FKBP12 and rapamycin, while ATP binds within the kinase domain [2].

mTOR forms two distinct complexes, mTOR complex 1 (mTORC1) with a positive regulatory subunit Raptor and mTOR complex 2 (mTORC2) with a regulatory subunit Rictor. Both complexes also contain mLST8 and Deptor subunits, but they differ in other subunits that interact with the regulatory subunit (Fig. 1, lower panel). However, there may exist some other subunits that participate in the assembly of mTOR complexes but have not been identified. mTORC1 is rapamycin sensitive, while mTORC2 is rapamycin insensitive. mTORC1 is involved in protein synthesis and autophagy while mTORC2 participates in regulating kinases of the AGC family [5]. The two mTOR complexes have distinct roles in cell growth control. mTORC1 mediates cell growth in part by phosphorylating its substrates, two key regulators responsible for protein synthesis, the eIF4E-binding protein 1 (4E-BP1) and ribosomal S6 kinases (S6K) [6]. mTORC2 regulates cell proliferation in response to growth factors by phosphorylating its downstream effectors including the serine/threonine protein kinases AKT, SGK and PKC [2].

As the indispensable roles PIKKs play in human, determination of three-dimensional structures of these kinases can implicate many functional properties. Among all the PIKKs, the structure of mTORC1 is best studied. Here, we review structural data of mTOR-related complexes determined by cryo-electron microscopy (cryo-EM) and X-ray crystallography recently and the potential mechanisms of rapamycin inhibition of mTORC1. In the end, the status quo and tendency of mTOR-targeted drug design are concluded concisely.

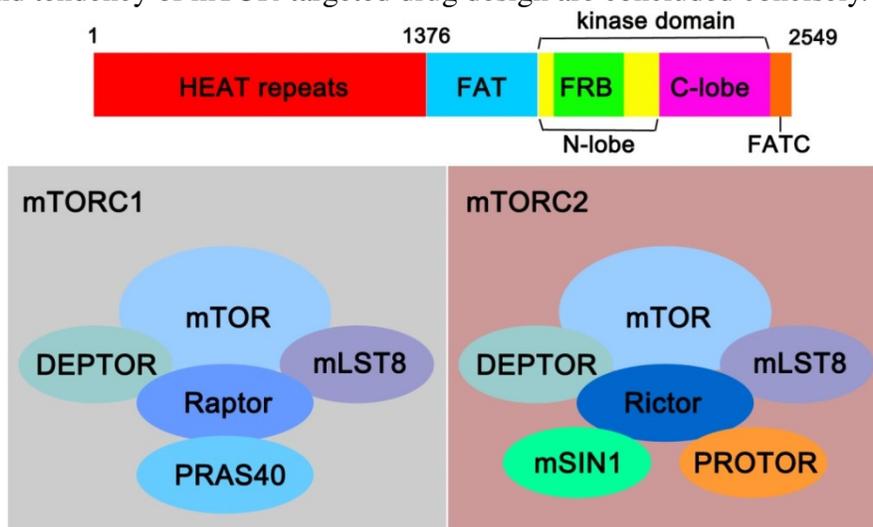


Figure 1. The schematic diagrams of mTOR domain organization and two mTOR complexes, mTORC1 and mTORC2. Besides mTOR, mTORC1 contains Raptor, PRAS40 and DEPTOR, and the requisite protein of unknown function called mLST8. mTORC2 also contains mTOR, mLST8 and DEPTOR, as well as other unique subunits, Rictor, mSIN1 and PROTOR.

Structures of mTOR-related Complexes

The low resolution cryo-EM structure of mTORC1 shows a two-fold symmetric dimer architecture at 26 Å, which contains mTOR, mLST8, PARS40 and Raptor. The two heterotetramers assemble into a rhomboid shape with a central cavity [7].

As the rapid development of cryo-EM and eukaryotic expressing systems in recent years, the structure of mTORC1 is more and more precise combining multiple biochemical and biophysical technologies. The first high resolution structure of mTOR is that of a complex of N-terminally truncated human mTOR (FAT, kinase and FATC domains) and mLST8 at 3.2 Å determined by X-ray crystallography [2]. The structure shows that the kinase domain adopts a classical protein kinase conformation. Binding of rapamycin–FKBP12 to FRB domain is predicted to narrow the active-site cleft and directly block substrate recruitment, thus inhibiting the kinase, which suggests that rapamycin inhibition of mTOR is induced by steric hindrance [2]. This was followed by the structure of human mTORC1 which consists of mTOR, mLST8 and Raptor, bound to FKBP–rapamycin, by combining cryo-EM at 5.9 Å with the crystal structure of *Chaetomium thermophilum* Raptor at 4.3 Å [8]. And subsequently, a higher resolution cryo-EM structure of human mTORC1 at 4.4 Å was reported [9]. This mTORC1 structure comprises a dimer of heterotrimer (mTOR-Raptor-mLST8) mediated by the mTOR protein.

The 5.9 Å and 4.4 Å mTORC1 structures show that the dimer is formed between the two full-length mTOR molecules with the N-terminal HEAT repeats packing against one another and a small part of HEAT repeats buried against the base of the adjacent mTOR FAT domain, thus completing an interlocking interaction between the two mTOR molecules. The structure of mTOR dimer is similar to the previously reported human DNA-PKcs dimeric architecture [10] and butterfly-shaped ATM/Tel1 dimeric organization [11, 12].

Previous studies have characterized that FKBP12-rapamycin binds to mTOR and inhibits its kinase activity. The crystal structure of the ternary complex of human FKBP12, rapamycin, and the FRB domain of FRAP was resolved at 2.7 Å [13]. The FRB domain protrudes from the kinase domain and acts as a gatekeeper of the active site in the crystal structure of N-terminally truncated human mTOR [2]. Combining the above-mentioned mTORC1 structures, it is proposed that the binding of FKBP12-rapamycin to the FRB domain of mTOR can generate steric hindrance to narrow the access to the recessed active site and block the recruitment of substrates [2, 8, 9]. The conformational changes of mTOR or mTORC1 induced by FKBP12-rapamycin binding result in decreased interactions between mTOR and Raptor [14], which may lead to the inhibition of the phosphorylation and activation of the major mTORC1 downstream targets including 4E-BP1 and S6K. Higher resolution structure of FKBP12-rapamycin in complex with mTOR or mTORC1 will help to explain the detailed mechanisms of rapamycin inhibition on mTOR.

mTORC2 is less studied than mTORC1. Up to now, mTORC2 still has many secrets to reveal. For example, prolonged treatment using rapamycin inhibits mTORC2, however, the mechanism is unclear [15]. Therefore, the structure of mTORC2 is urgent to be solved, and the upstream of mTORC2 remains to be uncovered in depth.

Analysis of these mTOR-related structures provides important structural and functional foundation for other PIKKs and contributes to the elucidation of pathological mechanisms and relevant drug designs.

mTOR-related Diseases and mTOR-targeted Drugs

mTOR complexes regulate numbers of cellular events, so dysfunction of mTOR signaling is associated with cancers and considered to be oncogenic. mTOR signaling is often hyper-active in multiple diseases including tuberous sclerosis, renal cancer and Alzheimer's disease. Hyper-activation of mTOR complexes will result in an increased amount of protein synthesis and an increased inhibition of autophagy, which benefits the growth of tumor cells. Coincidentally, it is found that tumor progression is accelerated and the rate of patient survival decreases when mTOR signaling is over activated [16, 17].

It has been shown that inhibition of mTOR is an available strategy in tackling cancers, because it can slow down tumor growth and limit the spread of cancers. Rapamycin is the natural inhibitor of mTOR [5], however, it has not been taken into cancer therapy because of its poor pharmacokinetic properties, including its low solubility [18]. Hence, some analogues of rapamycin (called rapalogues) have been developed. Rapalogues predominantly inhibit mTORC1 activity through an allosteric mechanism that is similar to rapamycin. Some rapalogues have been clinically applied to the treatment of soft tissue and bone sarcoma, breast cancer and renal cancer patients, such as everolimus, temsirolimus, ridaforolimus and zotarolimus [5]. Unfortunately, rapalogues have some drawbacks both in research and cancer therapy. First, rapalogues often activate feedback pathways that counter their usefulness. Second, cancer cells with the activation of mTOR signaling will produce resistance to multiple drugs, which has been detected in breast cancer. Third, inhibitor drugs have side effects that may lead to some severe diseases. For example, interstitial lung disease is an mTOR inhibitor-induced adverse event that is asymptomatic or mildly symptomatic, but can lead to severe morbidity and even mortality [19].

It has been reported that several alterations and mutations lying upstream of mTOR lead to an increased activation of mTOR signaling. Commonly, many cancers present alterations or mutations in PI3Ks, which are pivotal activators of mTOR in PI3K/Akt/mTOR signaling [20]. Activating mutations in PI3K α are common in tumors of the brain, breast, colon, liver, stomach, lung as well as ovary, and the mutations usually distribute in kinase and helical domains. These studies indicate that inhibitors that are specific for kinase and helical domains of PI3K α are essential to block the activating signals to mTOR complexes.

Novel types of inhibitors are being explored, including inhibitors that are ATP-competitive with mTOR [5]. Unlike rapalogues, ATP-competitive inhibitors can block ATP binding and lower the activity of both mTOR complexes. Due to the sequence similarity of kinase domains of mTOR and PI3Ks, the ATP-competitive inhibitors may be able to inhibit mTOR complexes as well as PI3Ks. Ultimately, these inhibitors decrease signaling of the entire PI3K/Akt/mTOR axis and may resolve the problems of feedback activation to PI3K signaling or mTORC2 activation.

There is still a long way to develop mTOR-targeted drugs that have significant effects on diseases. Considering the capacity of the access to the recessed active site, ATP-competitive inhibitors should have a small size, so that they can enter into the recessed active cavity to inhibit mTOR activity. Besides, combination of several therapeutic methods will help to improve cancer therapy. Design of specific inhibitors that prevent the regulatory subunit Raptor or Rictor from assembling with the mTOR protein may be a fresh idea. The popular nano-drugs and antibody-drugs provide a new tendency for developing mTOR-targeted drugs. Design of novel and selective inhibitor drugs based on mTOR-related structures awaits more structural and functional information of mTORC1 and mTORC2.

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