Review

TOR protein: Pieces of Information in a Logic Puzzle

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Abstract: Target of rapamycin (TOR) is a master hub of a complex signaling pathway involved in crucial cellular functions. Its signaling dysregulation has been associated with many diseases and health problems in humans. Numerous studies have greatly improved our understanding of many aspects of the TOR signaling mechanisms. This short review is trying to summarize some of the recent, remarkable, findings related to TOR signaling cascade.

Keywords: target of rapamycin; TOR complexes; autophosphorylation; E3 ligases

1. Introduction

Target of rapamycin (TOR) has been comprehensively studied in the last two decades. The serine-threonine kinase TOR was initially identified in the Saccharomyces cerevisiae in screening for drug rapamycin target protein [1-3]. TOR is a multidomain protein with a highly conserved primary sequence among eukaryotes. It is categorized as a nutrient- and energy-sensor kinase implicated in proper cellular and organismal growth. TOR can be involved in the assembly of, at least, two distinct protein complexes (TORCs) that underlie a variety of biological functions [4, 5].

2. TOR redundancy, does it exist?

TOR protein is an essential regulator in a complex signaling pathway and is encoded by a single gene in most organisms. Deletion of TOR is accompanied by lethality at early stages of development in eukaryotic model organisms [3, 6-10]. Surprisingly, additional TOR genes, up to four paralogues, have been characterized in obligate parasites Leishmania major and Trypanosoma brucei. Contrary to classical TORs, L. major and T. brucei TOR3 and TOR4 are involved in distinct TOR-related mechanisms to cope with their parasitic mode of life [11-14].

The existence of an alternative spliced “truncated” form of the mammalian target of rapamycin (mTOR), termed mTORβ, with distinct physiological functions and cytoplasmic localization is another remarkable finding [15]. Careful analysis of alternative splicing and gene duplication events might add new layer(s) in the complexity of TOR/mTOR signaling cascade that may not be attributed to simple genomic sequence redundancy.

3. TOR kinase complexes

TOR proteins are relatively large (∼ 300 kDa), and contain, in addition to the characteristic C-terminal kinase domain, an N-terminal HEAT repeats (Huntington elongation factor 1A-protein phosphatase 2A-A subunit-TOR) that are thought to mediate protein-protein interactions and specific subcellular localization [16]. TOR kinase shuttles between the cytoplasm and the nucleus. Several studies have reported ‘dynamic’ localization of TOR, and its physical and functional connection with the different compartments of the endomembrane system [17, 18]. The targeting mechanism of this trafficking process is not yet fully understood.

The overall functions of TOR have been largely attributed to its physical interaction with specific protein binding partners. Two distinct TOR signaling complexes, TOR complex1 (TORC1) and TOR complex2 (TORC2), are functionally conserved from budding yeast to humans. TORC1 components are not equally conserved among eukaryotes. Only TOR and LST8 (lethal with SEC13 protein 8) are the common proteins in both TORC1 and TORC2 [16].

Interestingly, mTORβ with only 23 amino acids of its N-terminal mTOR full length, has the ability to interact with mTORC1 part raptor (regulatory-associated protein of mTOR) and mTORC2 specific subunit rictor (rapamycin
-insensitive companion of mTOR) [15]. Yet, mTORβ related signaling pathway remains to be further investigated.

Little is known about the architecture of TOR complexes. Fully assembled human mTORC1, ∼1 MDa, (including raptor, mLST8, and PRAS40, proline-rich AKT substrate of 40 kD) has been examined by cryo-electron microscopy (cryo-EM). The cryo-EM structure information (26.0 angstroms ‘Å’ resolution) revealed an obligate dimeric organization of the human mTORC1, with an overall rhomboid shape and a central cavity. The mTORC1 subunits organize in a way that control substrates interaction and specificity [19].

TOR complexes are functionally segregated pathways, the existence of cross-talk between the two TOR complexes have been suggested. TORCs activate a number of different effector cascades in response to a variety of upstream inputs. The assigned functions of TORCs have been extensively discussed in several reviews [5, 20-24].

Activation of mTORC1 and mTORC2 involves changes in their subcellular localization. It has been reported that mTORC1, but not mTORC2, localizes to the late endosomes/lysosomes that are consistent with its recognized ability to sense internal nutrients availability [25, 26]. mTORC2 is localized mainly at the cytosol and can be recruited to specific areas of the cell membrane, namely lipid rafts [27].

4. TOR/TORCs and post-translational modifications

Limited data are available on TOR/TORCs signaling pathways and post-translational modifications. Four phosphorylation sites have been characterized in mTOR kinase itself [16, 28]. mTOR Ser-2481 autophosphorylation has been recommended as a simple screening biomarker for assessment of mTORC1 and/or mTORC2 activation state [29]. mTOR complex subunits phosphorylation on various sites has been detected that has been suggested to alter the TORCs stability and activity [16].

mTOR kinase is ubiquitously expressed in most cells and tissues. Its depletion is lethal at early stages of development. Ubiquitin-dependent proteasomal degradation of mTOR is mediated, largely/exclusively, by binding to E3 ubiquitin ligase Fbxw7 [30]. How the biological systems in the cell can handle “partial/temporary” mTOR degradation and the intimate relationship between ubiquitin proteasome system and mTOR signaling cascade are under intensive investigations, with special focus upon E3 ubiquitin ligases Pami (protein associated with Myc), MID1 (midline-1) and CUL4A (cullin 4A) [31-33].

5. Second generation/structure based mTOR inhibitors

mTOR signaling pathway is deregulated in many human diseases, including cancer, obesity, type 2 diabetes and neurodegeneration [34]. Initially, rapamycin and its related analogs (rapalog) were considered reliable mTOR inhibitors. Limited clinical efficacy of rapamycin and its rapalogs can refer to conceptual gaps in our understanding of mTOR signaling pathway. Rapamycin and its rapalogs bind FRB (FKBP12-rapamycin-binding) domain that is adjacent to the catalytic site of mTOR and inhibit mainly the mTORC1. Second-generation mTOR inhibitors bind to ATP-binding site in the mTOR kinase domain can globally inactivate the mTOR complexes [35] and hence are clinically promising.

Recently, a high resolution diffraction data (3.2 Å) derived from N-terminally truncated human mTOR, in complex with full length human mLST8, has been collected. The structure indicates an intrinsically active kinase conformation that is controlled by restricted access to the active site. Co-crystal structures of truncated mTOR-mLST8 bound to an ATP transition state analogue and ATP-competitive inhibitors have elucidated the blocking mechanism of the kinase domain by the rapamycin-FKB12 complex. Undoubtedly, such studies can pave the way to develop novel, structure-based, mTOR inhibitors that are efficient [36].

6. Perspective

Extensive efforts have been made in improving our understanding of the complexity of TOR/mTOR signaling network. Most of these functions have been revealed by utilizing rapamycin as a TOR kinase specific inhibitor. Use of unicellular green alga, Chlamydomonas, lower eukaryotes and rapamycin insensitive green plants will continue to increase our understanding of mammalian TOR signaling pathway. Additionally, structure-based rational design of mTOR kinase inhibitors should be a very selective tool that will have therapeutic value.

Conflict of interest: No conflict of interest.

References

mTOR complex 2 integrates cell movement during chemotaxis, which is essential for growth and proliferation in early mouse embryos and embryonic stem cells. Mol. Cell Biol. 2004; 24:6710-18.


