Regulation of the mTOR signaling pathway: from laboratory bench to bedside and back again

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Abstract

Recent publications have moved us significantly closer to a complete understanding of the mammalian target of rapamycin (mTOR) signaling pathway, which plays a central role in the control of growth and metabolism and is dysregulated in a broad spectrum of human diseases, including cancer, tuberous sclerosis, diabetes, and cardiovascular and neurodegenerative diseases. Rapamycin-related mTOR inhibitors have shown clinical efficacy in several of these diseases, and novel inhibitors currently in development will be valuable tools for further dissections of the mTOR signaling network in human health and disease.

Introduction and context

Nearly 15 years have passed since the discovery of mammalian target of rapamycin (mTOR) as the protein kinase whose signaling functions are modulated by the natural product rapamycin [1-3]. The mTOR mRNA transcript encodes a relatively large (~300 kDa) polypeptide with an extended amino-terminal domain and a carboxy-terminal kinase domain that bears considerable sequence similarity to the catalytic domains of the phosphoinositide-3-kinases (PI3Ks) [4]. In spite of the similarity to lipid kinases, the mTOR kinase domain phosphorylates protein substrates on serine or threonine residues. Mammalian cells express several additional mTOR-related kinases, which collectively comprise a family of large signaling proteins named the PI3K-related kinases [4,5].

Studies of mTOR signaling functions have been immeasurably facilitated by the availability of the natural product rapamycin, a potent and highly selective inhibitor of a subset of mTOR signaling functions. As a chemical probe, rapamycin enabled the identification of the mTOR polypeptide, the cloning of its cognate cDNA, and numeous fundamental insights into the physiologic and pathologic roles of mTOR in a broad array of cells, tissues, and organ systems. Rapamycin also possesses established clinical utility as an immunosuppressant/anti-inflammatory agent in organ transplantation and coronary arterial diseases [6,7]. More recently, rapamycin-related mTOR inhibitors (collectively termed 'rapalogs') have shown activity in patients with renal cancer and other malignant diseases, and many drug companies are aggressively developing second-generation mTOR inhibitors with the hope that these compounds will show broader efficacy and an improved safety profile relative to the rapalogs. These new mTOR inhibitors, like rapamycin, will undoubtedly serve as valuable chemical probes for more detailed dissections of mTOR signaling in healthy and diseased cells.

Two mTOR-containing protein complexes are expressed in mammalian cells (Figure 1). The mTOR complex (mTORC)1 contains the 'signature' subunit, Raptor, together with LST8 and PRAS40, and the activity of this multi-subunit complex is acutely sensitive to rapamycin [1,8]. The salient function of mTORC1 is to coordinate growth factor and nutrient availability with the translation of a subset of mRNAs into proteins needed for cell-cycle progression and mitotic cell division [2,9]. mTORC2 bears a distinct subunit composition, with

Figure 1. Regulation of mammalian target of rapamycin (mTOR) signaling functions
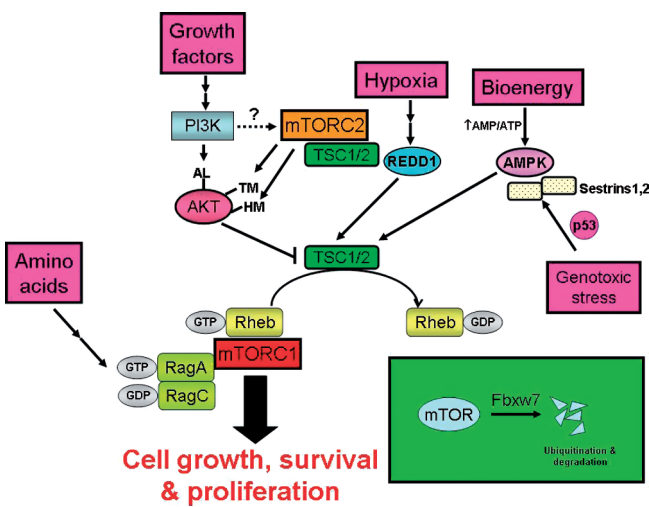
mTOR complex (mTORC)1 regulates protein synthesis in cycling cells by promoting cap-dependent mRNA translation, and the assembly of actively translating poly-ribosomes (polysomes). The signaling functions of mTORC1 are tightly controlled by growth factors, amino acids, and bioenergetic molecules, including oxygen and ATP. Growth factor receptors communicate with mTORC1 by activating phosphoinositide-3-kinase (PI3K). A key target for PI3K is the AGC family kinase AKT, which is phosphorylated at Thr308 in the activation loop (AL) by PI3K-dependent kinase-1. Full activation of AKT requires a second phosphorylation event at Ser473 in the hydrophobic motif (HM), which is carried out by mTORC2. mTORC2 is also responsible for a third phosphorylation event at Thr450 in the turn motif (TM), which promotes AKT signaling by increasing the stability of this protein kinase. The signaling functions of mTORC2 are stimulated by direct binding to the tuberous sclerosis complex (TSC)1 and TSC2 heterodimer (TSC1/2), and possibly by an undefined signal generated by PI3K. Once activated, AKT phosphorylates TSC2, which inhibits TSC1/2-associated GTPase-activating protein (GAP) activity, leading to increased levels of mTORC1-activating, GTP-bound Rheb. Conversely, hypoxia and ATP depletion (revealed by an increase in the AMP:ATP ratio) stimulate TSC1/2 GAP activity via induction of REDD1 (Regulated in Development and DNA Damage Responses-1) and AMPK activities, respectively. Genotoxic stress-induced p53 activity also stimulates TSC1/2 GAP activity via induction of Sestrins, which bind to and stimulate AMPK. Finally, amino acids activate mTORC1 by inducing the assembly of Rag heterodimers (e.g., the RagA-RagC heterodimers shown in the figure). Deregulated mTORC1 activity promotes the synthesis of proteins that drive inappropriate cell survival and growth, and supports tumor progression. The inset box shows the newly defined, Fbxw7-dependent mechanism that mediates mTOR ubiquitination and subsequent proteasomal degradation. Loss of the tumor suppressor Fbxw7 stabilizes mTOR and promotes elevated mTORC1 and mTORC2 activities in cancer cells.

Rictor and mSin1 serving as the signature subunits, and LST8 and Protor as additional components [1,8]. It plays a key role in the phosphorylation of AKT at Ser473, which is located in the 'hydrophobic motif' of AKT and

other members of the protein kinase A, G, and C (AGC) family [10,11]. Remarkably, mTORC2 is not directly inhibited by rapamycin [12,13]; however, long-term exposure to the drug disrupts the assembly of functional mTORC2 [14,15]. Recent studies indicate that mTORC1 and mTORC2 are found in both the cytoplasm and the nucleus; however, they show only partially overlapping sub-compartmental localizations [15,16].

In mammalian cells, mTORC1 activity is stimulated by growth factors and amino acids, and inhibited by hypoxia and depletion of the main source of metabolic energy, ATP (Figure 1). The mechanism through which growth factors activate mTORC1 has been intensively studied, and the PI3K-activated protein kinase AKT is clearly a major player in the transmission of growth factor-derived signals to mTOR. A nodal point of convergence for mTORC1 regulatory signals delivered by growth factors, hypoxia, and ATP is a heterodimeric protein complex consisting of tuberous sclerosis complex 1 (TSC1) and TSC2 subunits (hereafter termed TSC1/2), which functions as a GTPase-activating protein (GAP) for the Ras-related GTPase Rheb [17,18]. In its active GTP-bound state, Rheb binds to and activates mTORC1 [18]; hence, TSC1/2 acts as a suppressor of mTORC1 activity by limiting the amount of GTP-bound Rheb available to stimulate mTORC1. The signals that converge on TSC1/2 largely target the TSC2 subunit, and directly or indirectly modulate the Rheb-GAP activity of TSC1/2. Given that protein synthesis and mitotic cell division are extremely energy-dependent processes, it is not surprising that mTORC1 activity is strongly influenced by cellular bioenergetics. A key player in the energy-sensing pathway upstream of mTORC1 is AMP-dependent protein kinase (AMPK), which is activated by an increase in the AMP to ATP ratio (a sensitive indicator of intracellular energy depletion) [19]. AMPK phosphorylates TSC2, thereby increasing the repressive effect of TSC1/2 on Rheb-dependent mTORC1 signaling [20,21]. Collectively, this elegant regulatory network ensures that mTORC1 activity is elevated when supplies of growth factors and metabolic precursors are sufficient to support cell growth and proliferation. Disruption of any of the mechanisms responsible for repressing mTORC1 activity (for example, impaired TSC1/2 function) leads to persistent mTORC1 activation, and favors inappropriate cell growth (hyperplasia) and proliferation.

Recent studies have addressed several major conceptual gaps in our understanding of mTOR regulation and function. These studies have forged new connections between growth factor receptor- and metabolism-related signals and mTORC1 activity. A second major area of uncertainty surrounds mTORC2 regulation and activity,

and exciting progress has been made here as well. Finally, several recent investigations have underscored the importance of mTOR pathway deregulation in cancer development, and shed new light on mechanisms of tumor resistance to mTOR inhibitor-based therapies.

Major recent advances

Our perception of the regulatory controls that govern mTORC1 activity continues to evolve. As described above, growth factors are known to signal to mTORC1 via PI3K-AKT-dependent repression of TSC1/2. A recent report indicates that mTORC1 also responds to mitogenic signals delivered through the Ras pathway [22]. The authors demonstrate that the Ras-activated, extracellular signal-regulated kinase (ERK) phosphorylates TSC2, thereby dampening the repressive effect of TSC1/2 on Rheb-mTORC1 activity [22]. These studies also implicate mTORC1 deregulation in Ras-dependent oncogenesis in humans. Our view of the influence of cellular bioenergetics on mTORC1 activity was enhanced with the discovery that AMPK directly modulates mTORC1 through phosphorylation of Raptor [21]. Finally, a particularly noteworthy advance during the past year involved the identification of a family of small GTP-binding proteins as key players in the transmission of amino acid-derived stimulatory signals to mTORC1 [23,24]. Mammalian cells express four Rag GTPases (RagA-D), which form hetero-oligomeric complexes in response to unidentified, amino acid-derived signals. These active Rag hetero-oligomers bind to mTORC1 via Raptor, and stimulate both the redistribution of mTORC1 to a different cytoplasmic sub-compartment, and an increase in mTORC1-associated kinase activity.

Several recent reports have also provided important insights into the regulation and functions of mTORC2. Remarkably, TSC1/2, which functions as a repressor of mTORC1 activity, was identified as a positive regulator of mTORC2 activity [25]. The stimulatory effect of TSC1/2 on mTORC2 is independent of its Rheb-GAP activity; instead, TSC1/2 interacts directly with mTORC2, and activates the complex through an allosteric mechanism. Two reports indicate that mTORC2 exerts a broader than previously appreciated influence on AKT and certain other AGC family protein kinases [26,27]. In addition to its well-characterized functions as an effector of Ser473 phosphorylation in the hydrophobic motif of AKT, mTORC2 phosphorylates conserved threonine residues located in the 'turn motifs' of AKT and the α, β, γ, and ε isoforms of protein kinase C. Surprisingly, turn motif phosphorylation serves a constitutive, 'housekeeping' function that is required to maintain steady-state expression levels of these particular AGC kinase family

members. Interestingly, loss of mTORC2 function leads to reduced AKT expression, and renders the remaining AKT molecules more highly dependent on the molecular chaperone, HSP90, for protection from proteasomal degradation [27].

New mechanisms of mTOR pathway deregulation during the evolution of cancer cells continue to be elucidated. Two reports demonstrated that Rheb over-expression supports the development of more aggressive B-cell lymphomas, as well as the survival of dormant epithelial tumor cells [28,29]. Conversely, tumor development is suppressed by a newly described, p53-dependent mechanism involving Sestrin-1 and Sestrin-2. These proteins bind to and activate AMPK, which promotes TSC1/2-dependent inhibition of Rheb and mTORC1 [30]. Yet another avenue whereby tumor cells acquire mTOR pathway deregulation was unveiled with the discovery that the F-box protein Fbxw7 targets the mTOR polypeptide for ubiquitination and subsequent degradation by the proteasome. Fbxw7 represents the targeting subunit of an SCF-type ubiquitin E3 ligase, and is a known tumor suppressor that counts cyclin E and c-Myc among its other known substrates [31]. Loss of Fbxw7 during tumorigenesis leads to elevated steady-state expression of mTOR and enhanced mTOR signaling. Interestingly, Fbxw7-deficient cancer cells are sensitive to mTOR inhibitors, suggesting that Fbxw7 might be a useful biomarker for therapeutic responsiveness to these drugs in cancer patients.

Future directions

The drive to fully understand the signaling network that surrounds the two mTOR complexes has intensified with the entry of first-generation mTOR inhibitors into the oncology clinics. From the translational perspective, we urgently need to understand whether the new, second-generation mTOR kinase inhibitors, which target both mTORC1 and mTORC2 [1], will have a clear therapeutic advantage over the mTORC1-selective rapamycin analogs already in the clinic. We predict that additional genetic alterations leading to deregulated signaling through mTORC1 and/or mTORC2 will be uncovered in human cancers, and that these impending insights will enable clinicians to predict which cancer patients are most likely to respond favorably to mTOR inhibitor-based therapeutic regimens. On a more basic level, we eagerly await the results of ongoing studies that build on the new knowledge that Rag GTPases relay amino acid-derived signals to mTORC1. Finally, clinical experience with mTOR inhibitors in cancer patients will undoubtedly raise new questions to be addressed by the basic research laboratories. From the bench to the bedside and

back again, mTOR and its signaling partners will undoubtedly hold the interest of biologists and clinicians alike, for a long time to come.

Abbreviations

AGC family, protein kinase A, G, and C family; AMPK, AMP-dependent protein kinase; GAP, GTPase-activating protein; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; PI3K, phosphoinositide-3-kinase; TSC, tuberous sclerosis complex.

Competing interests

The author declares a competing interest due to his employment at Wyeth, a pharmaceutical company involved in the development and commercialization of mTOR pathway inhibitors.

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