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mTOR Signaling for Biological Control and Cancer

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Abstract

Mammalian target of rapamycin (mTOR) is a major intersection that connects signals from the extracellular milieu to corresponding changes in intracellular processes. When abnormally regulated, the mTOR signaling pathway is implicated in a wide spectrum of cancers, neurological diseases, and proliferative disorders. Therefore, pharmacological agents that restore the regulatory balance of the mTOR pathway could be beneficial for a great number of diseases. This review summarizes current understanding of mTOR signaling and some unanswered questions in the field. We describe the composition of the mTOR complexes, upstream signals that activate mTOR, and physiological processes that mTOR regulates. We also discuss the role of mTOR and its downstream effectors in cancer, obesity and diabetes, and autism.

The mammalian target of rapamycin (mTOR, also known as the mechanistic target of rapamycin) is a 289 kDa serine/threonine kinase that belongs to the family of phosphatidylinositol 3-kinase-related kinases (PIKK). It was discovered as a target for a molecule called rapamycin, an anti-fungal macrolide produced by the bacterium *Streptomyces hygroscopicus* (Vezina et al., 1975). Rapamycin binds to FK506-binding protein of 12 kDa (FKBP12) and inhibits mTOR by interacting with the FKBP12-rapamycin binding domain (FRB) of mTOR (Brown et al., 1994; Sabatini et al., 1994). Rapamycin was found to strongly inhibit cell cycle progression, implicating mTOR in regulation of cell growth and proliferation (Brown et al., 1994; Sabers et al., 1995). Since then, mTOR has been a primary focus of many research groups that discovered a variety of other critical cellular mTOR-regulated processes such as metabolism, survival, protein and lipid synthesis, and autophagy. The best characterized targets of mTOR are the eukaryotic initiation factor 4E binding protein 1 (4E-BP1) and the p70 ribosomal S6 kinase 1 (S6K1). The mTOR pathway is dysregulated in a number of human diseases such as cancers, proliferative diseases, autism spectrum disorders, and type 2 diabetes. mTOR interacts with other proteins to form two distinct complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2; Laplante and Sabatini, 2009). These complexes differ from each other in their protein composition as well as in their sensitivity to rapamycin, with mTORC1 being acutely rapamycin-sensitive and mTORC2 being rapamycin-insensitive.

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mTORC1

mTORC1 components and their function

mTORC1 is regulated by different signals including growth factors, genotoxic stress, oxygen levels, amino acids, and energy status of the cell; it integrates these signals to regulate anabolic (protein and lipid synthesis, and nutrient storage) and catabolic (autophagy and use of stored energy) processes of the cell (Sengupta et al., 2010). mTORC1 is largely sensitive to inhibition by rapamycin, which is used to treat solid tumors, organ rejection after transplantation, coronary restenosis, and rheumatoid arthritis (Kreis et al., 2000; Koehl et al., 2004; Molina et al., 2011; Kohno et al., 2013).

mTORC1 is composed of five proteins: mTOR, regulatory-associated protein of mTOR (Raptor), proline-rich AKT substrate 40 kDa (PRAS40), mammalian lethal with Sec13 protein 8 (mLST8, also known as GβL), and DEP-domain-containing mTOR interacting protein (DEPTOR). Raptor is a 150 kDa protein that binds to mTOR, allowing it to bind and phosphorylate downstream proteins such as the S6Ks, 4EBPs, and STAT3 (Hara et al., 2002; Kim et al., 2002; Schalm and Blenis, 2002; Schalm et al., 2003; Gulati et al., 2009). Additionally, raptor is important for sensing amino acids and regulating the subcellular localization of mTORC1 (Sancak et al., 2008). The role of mLST8 in mTORC1 function is not clear, as deletion of this protein does not affect mTORC1 activity (Guertin et al., 2006).

PRAS40 and DEPTOR are negative regulators of mTORC1. When mTORC1 is activated, it is able to directly phosphorylate PRAS40 and DEPTOR, which reduces their physical interaction with mTORC1 (Oshiro et al., 2007; Peterson et al., 2009). PRAS40 binds to raptor, but upon stimulation with insulin dissociates from mTORC1, thereby relieving its negative effect (Sancak et al., 2007; Wang et al., 2008). DEPTOR is highly overexpressed in multiple myelomas, where it maintains cell survival through high phosphoinositide 3-kinase (PI3K) and AKT activity levels (Peterson et al., 2009).

mTORC1 regulation

All signaling pathways that activate mTORC1 (with the exception of amino acids) act through the tuberous sclerosis complex (TSC) proteins TSC1 and TSC2. Mutation or loss of heterozygosity of TSC1/2 cause tuberous sclerosis, a disease characterized by numerous benign tumors. TSC1/2 complex negatively regulates mTORC1 by converting small Ras-related GTPase (Rheb) into its inactive GDT-bound state (Tee et al., 2002). When Rheb is in its GTP-bound form, it directly interacts with and activates mTORC1, but the exact mechanism by which Rheb activates mTOR is unclear. When TSC is phosphorylated, the repressive function is removed so that GTP-bound Rheb can activate mTORC1 (Inoki et al., 2003; Tee et al., 2003).

Upstream signals that regulate mTORC1

Growth factors—When nutrients are high, levels of growth factors such as insulin and IGF-1 are plentiful and promote anabolic processes of the cell through mTORC1. When insulin binds to its tyrosine kinase receptor, insulin substrate receptor 1 (IRS1) is recruited to the plasma membrane-bound receptor and activates PI3K, which produces phosphoinositol

(3,4,5)-triphosphate (PIP3) and recruits Akt to the membrane for full activation. Akt in turn phosphorylates and inactivates TSC2, a negative regulator of mTORC1 (Potter et al., 2002; Roux et al., 2004); however, it is unclear how phosphorylation of TSC2 leads to its inactivation. Growth factors also activate mTORC1 through the Ras signaling pathway effectors ERK1/2 and p90 ribosomal S6 kinase 1 (RSK1; Roux et al., 2004; Ma et al., 2005). Downstream of mTORC1, AKT specifically phosphorylates and inhibits 4E-BP1, a translational repressor, but is sometimes dispensable for S6K1 phosphorylation and activation (Gingras et al., 1998; Dufner et al., 1999). Growth factors can also activate mTORC1 in a TSC-independent manner through phospholipase-D (PLD)-dependent accumulation of PA (phosphatidic acid), which directly binds to the FRB domain of mTORC1 and activates its downstream signaling (Fang et al., 2003). However, PA alone is not sufficient to activate mTORC1, and it is not entirely clear whether this process is truly TSC-independent (Sun et al., 2008). Loss of growth factor signaling represses mTORC1 activity, which leads to lower energy and nutrient consumption and an increase in the lifespan of an organism. Conversely, hyperactivation of growth factor signaling or loss of TSC1 or TSC2 leads to inappropriate activation of mTORC1, causing various pathologies, including cancer, cardiac hypertrophy, and neuronal dysfunction.

Energy levels—mTORC1 also senses energy status of the cell; specifically when intracellular energy levels are high, mTORC1 is activated and when energy levels are low, mTORC1 is inactivated. AMP-activated protein kinase (AMPK) relays the intracellular energy status of the cell to mTORC1. When energy is low, AMPK phosphorylates TSC2, which increases its affinity for the inactive, GDP-bound Rheb, which in turn blocks mTORC1 activity (Corradetti et al., 2004; Shaw et al., 2004). AMPK can also block mTORC1 activity directly by phosphorylating raptor (Gwinn et al., 2008).

Oxygen levels—Hypoxia, or low oxygen levels, is sensed by low ATP levels, which blocks signaling to mTORC1 through AMPK activation of TSC as well as inhibition and inactivation of raptor (Liu et al., 2006; Gwinn et al., 2008). Hypoxia also leads to activation of transcriptional regulation of DNA damage response 1 (REDD1), which releases TSC2 from interacting with 14-3-3 proteins. This allows TSC1/2 complex to inhibit mTORC1 (Brugarolas et al., 2004; DeYoung et al., 2008). Recent studies have called into question whether REDD1 directly binds to 14-3-3 (Vega-Rubin-de-Celis et al., 2010). Additionally, tumor suppressor promyelocytic leukemia (PML) and BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) disrupt mTORC1 interaction with Rheb, its positive regulator, and block mTORC1 signaling (Bernardi et al., 2006; Li et al., 2007).

Amino acids—Amino acids, particularly leucine, strongly activate mTORC1 and are transported into the cell in a glutamine-dependent fashion (Nicklin et al., 2009). Withdrawal of amino acids causes dephosphorylation of S6K1 and 4E-BP1, while addition of amino acids to the media restores their phosphorylation (Hara et al., 1998). This activation of mTORC1 by amino acids is TSC-independent because amino acid signaling to mTOR occurs even in cells that lack TSC2 (Smith et al., 2005). Moreover, overexpression of Rheb activates mTORC1 even in the absence of amino acids (Long et al., 2005). Rag proteins bind to Raptor in an amino acid-sensitive manner and activate mTORC1 by localizing it to

the cytosolic side of the lysosomal membranes, where it interacts with Rheb (Smith et al., 2005; Sancak et al., 2010).

Genotoxic stress—Genotoxic stress reduces mTORC1 activity. When DNA is damaged, p53 is activated, which in turn activates AMPK, leading to activation of TSC2 and inhibiting mTORC1 activity (Budanov and Karin, 2008). However, it is unclear how p53 activates AMPK. p53 also negatively regulates mTORC1 by activating two negative regulators of the pathway: TSC2 and phosphatase and tensin homolog deleted on chromosome 10 (PTEN), which opposes the activity of PI3K (Feng et al., 2005).

Inflammation—Inflammation causes activation of pro-inflammatory cytokines such as TNFα, which activates IκB kinase-β (IKKβ). IKKβ physically interacts and inactivates TSC1, thereby activating mTORC1 (Lee et al., 2007). This pathway is thought to be important in tumor angiogenesis and insulin resistance.

mTORC1 inhibitors

Rapamycin is a specific inhibitor of mTORC1. It remains unknown how rapamycin blocks mTORC1 activity as it does not affect complex formation or autophosphorylation of mTORC1; however, it may affect the affinity of raptor interaction with the complex (Kim et al., 2002). Recent reports suggest that prolonged treatment with rapamycin causes disintegration of mTORC1 (Yip et al., 2010). It also remains unclear why some mTORC1 downstream targets are rapamycin-sensitive, while others are rapamycin-insensitive (Peterson et al., 2000; Kudchodkar et al., 2004; Thoreen et al., 2009). Although rapamycin primarily inhibits mTORC1 and not mTORC2, prolonged treatment of rapamycin can also inhibit mTORC2 by blocking its assembly and causing disintegration of mTORC2 (Sarbassov et al., 2006; Barilli et al., 2008; Rosner and Hengstschlager, 2008; Hong et al., 2013).

Downstream pathways of mTORC1

mTORC1 regulates a number of downstream processes including protein synthesis, lipid synthesis, cholesterol synthesis, mitochondrial metabolism, and autophagy.

Protein synthesis—mTORC1 activates protein synthesis by phosphorylating 4E-BP1 and S6K1, the two most studied downstream targets of mTORC1. S6K1 regulates cell size, protein translation, and cell proliferation (Holz, 2012). Activation of S6K1 by mTORC1 leads to increased protein synthesis through regulation of several substrates: S6K1 aly-REF-like target (SKAR), programmed cell death 4 (PDCD4), eukaryotic initiation factor 4B (eIF4B), eukaryotic elongation factor 2 kinase (eEF2K), and ribosomal protein S6 (Wang et al., 2001; Raught et al., 2004; Richardson et al., 2004). eEF2K negatively regulates translation elongation by repressing eukaryotic elongation factor 2 (eEF2). S6K1 directly phosphorylates eEF2K, relieving its negative regulatory effect on protein synthesis. eIF4A is an RNA helicase that unwinds 50UTR secondary structures that may impede ribosome movement. S6K1 phosphorylates PDCD4, an inhibitor of eIF4A, leading to its degradation, thereby promoting efficient translation (Dorrello et al., 2006). eIF4B is an accessory factor that activates eIF4A. Phosphorylation by S6K1 increases the recruitment of eIF4B into the

translation initiation complex (Raught et al., 2004; Holz et al., 2005). Phosphorylation of SKAR by S6K1 promotes translation efficiency of spliced mRNAs (Ma et al., 2008).

Transgenic mice with mutations in *S6K1* or *4E-BPs* show severe metabolic changes. *S6K1*-null mice are hypoinsulinemic but are also hypersensitive to insulin signaling. These mice do not accumulate fat as they have increased lipolysis of triglycerides from stored adipose tissue and have an increased lifespan (Pende et al., 2000;Um et al., 2004; Selman et al., 2009). In contrast, mice deficient in both *4E-BP1* and *4E-BP2* exhibit a completely opposite phenotype of an increase in adipose tissue (Le Bacquer et al., 2007).

S6, a component of the 40S ribosomal subunit, is phosphorylated by S6K1, which under growth-promoting conditions causes increased rates of protein synthesis. However, when a nonphosphorylatable version of S6 is expressed, cells also exhibit high rates of protein synthesis, hence the function and mechanism of S6 in protein synthesis remains unclear (Ruvinsky et al., 2005). S6 is also phosphorylated by RSK in an mTOR-independent manner (Roux et al., 2007).

4E-BP1 represses eIF4E, a rate limiting protein of cap-dependent translation. When mTORC1 phosphorylates 4E-BP1, it disassociates from eIF4E and allows it to recruit protein translation machinery to the 50 cap of eukaryotic mRNAs (Gingras et al., 2001), leading to activation of cap-dependent translation. 4E-BP1 is phosphorylated by mTOR on four sites, two of which are acutely rapamycin-sensitive. However, rapamycin inhibition of 4E-BP1 is not as potent and permanent as that of S6K1 (Choo et al., 2008). Recent studies have shown that high-dose treatment of rapamycin causes apoptosis in human cancer cell lines, which is associated with inhibition of 4E-BP1 phosphorylation and dissociation of raptor from mTORC1 (Yellen et al., 2011).

Metabolic pathways—mTORC1 regulates metabolic pathways on transcriptional, translational, and posttranslational levels. When rapamycin inhibits mTORC1 in lymphoma cells, it induces changes in the levels of mRNAs encoding metabolic enzymes involved in glycolysis, amino acid, sterol, lipid, and nucleotide metabolism (Peng et al., 2002). mTORC1 activates SREBP-1, transcription factor belonging to the sterol regulatory element binding proteins (SREBPs) family, as inhibition of mTORC1 by rapamycin blocks expression of SREBP target genes (Porstmann et al., 2008). SREBP-1 is found in an inactive state on the Endoplasmic Reticulum and is escorted to the Golgi in response to low sterol, insulin, or fatty acid levels, where it is processed into the active form. Once active, SREBP-1 translocates to the nucleus where it binds to promoters of genes containing sterol regulatory elements and Enhancer Box sequences. mTORC1 is thought to activate SREBP-1 through S6K1 by promoting posttranslational processing (Duvel et al., 2010).

Lipid and cholesterol synthesis—mTORC1 promotes lipid and cholesterol synthesis by activation of SREBP-1 and peroxisome proliferator-activator-γ (PPARγ; Kim and Chen, 2004; Porstmann et al., 2008). Rapamycin treatment reduces PPARγ expression as well as phosphorylation of lipin-1, a phosphatidic acid (PA) phosphatase that is involved in glycerolipid synthesis. The exact mechanism of SREBP1 activation by mTORC1, as well as the importance of lipin-1 phosphorylation on lipid synthesis, remain unclear.

Mitochondrial metabolism—mTORC1 regulates mitochondrial number and function, because inhibition of mTORC1 by rapamycin lowers mitochondrial membrane potential, oxygen consumption, and cellular ATP, while hyperactivation of mTORC1 increases mitochondrial DNA copy number (Schieke et al., 2006). Additionally, studies in mice found that lack of raptor reduces expression of genes involved in mitochondrial biogenesis (Bentzinger et al., 2008).

Autophagy—Autophagy is a process through which cells are able to create and use intracellular energy by degrading cytoplasmic proteins and organelles in lysosomes during times of nutritional deprivation. The intracellular components targeted for degradation are sequestered inside autophagosomes and are degraded through fusion with lysosomes. Autophagy is inhibited in the presence of nutrients and growth factors and is upregulated during starvation, growth factor withdrawal, oxidative stress, and infection (Codogno and Meijer, 2005). This process is also important for general cellular housekeeping. Autophagy is regulated by mTORC1, and correspondingly, mTOR was found to localize to the lysosomal surface (Sancak et al., 2010). When mTORC1 is activated under high nutrient conditions, autophagy is inhibited; conversely, mTORC1 inhibition stimulates autophagy, which leads to the release of amino acids through protein degradation, which in turn activates mTORC1. Autophagy mediator ULK forms a complex with Atg13 and FIP200, promoting autophagy induction. mTORC1 phosphorylates ULK and Atg13, but the role of this phosphorylation is unclear (Ganley et al., 2009). While autophagy is negatively regulated by mTORC1, it remains unclear why this process could be either activated or inhibited by rapamycin, with S6K1 having a positive role in autophagy induction (Armour et al., 2009).

Negative feedback loops

mTORC1 activation by growth factors leads to activation of several negative feedback loops, which modulate growth factor signaling pathway activation. Akt signals via TSC1/2 to activate mTORC1 and its downstream target S6K1. S6K1, in turn, reduces IRS1 activity by phosphorylating it, preventing its binding to the insulin receptor and causing its subsequent degradation (Harrington et al., 2004; Shah and Hunter, 2006). S6K1 interacts with IRS1 through raptor, phosphorylating and inactivating it (Tzatsos, 2009). S6K1 similarly suppresses activity of platelet-derived growth factor receptor (PDFR; Zhang et al., 2003). S6K1 also leads to mTORC1 phosphorylation on Ser2448 (Holz and Blenis, 2005). While the role of this event is not understood, it may act to modulate negative inhibition within the “repressor domain” of mTOR (Sekulic et al., 2000). Growth factor receptor-bound protein 10 (Grb10) is a putative tumor suppressor that is frequently downregulated in a number of cancers. Recently, it has been characterized as a substrate of mTORC1, with mTORC1 stabilizing Grb10 and inhibiting PI3K (Hsu et al., 2011; Yu et al., 2011). DEPTOR, the negative regulator of mTORC1, was found to promote adipogenesis by blocking the mTORC1-mediated negative feedback inhibition of insulin signaling (Laplante et al., 2012).

mTORC2

mTORC2 components and their function

mTORC2 is comprised of six proteins: mTOR, rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated protein kinase interacting protein (mSIN1), protein observed with rictor-1 (Protor-1), mLST8, and DEPTOR. Very little is known about mTORC2, the function of its components, the upstream signals it integrates and its downstream targets. Deletion of mTORC2 protein components in mice causes early embryonic lethality phenotype (Guertin et al., 2006; Shiota et al., 2006), which makes it challenging to study its function, compounded by the fact that there are no known specific inhibitors of mTORC2.

mTORC2 is characterized by association with rictor, a binding partner for mTOR, whose interaction is mutually exclusive with that of raptor, a binding partner of mTORC1 (Jacinto et al., 2004; Sarbassov et al., 2004). Rictor is essential for mTORC2 catalytic activity and may have a function in recruitment of other substrates to mTORC2 (Sarbassov et al., 2004). Rictor also binds to mSIN1 and these proteins help to stabilize each other (Frias et al., 2006; Jacinto et al., 2006); however, other functions of mSIN1 are unknown.

Protor-1 (aka PRR5) specifically binds to rictor; however, this interaction is independent of mTOR and is not required for mTORC2 activation (Thedieck et al., 2007; Woo et al., 2007). Thus the function of protor-1 or the significance of its binding to rictor is unknown. mLST8 is required for mTORC2 activity, as knockdown of mLST8 severely reduces the stability and activity of mTORC2 (Guertin et al., 2006). DEPTOR negatively regulates mTORC2 through the FRAP-ATM-TTRAP (FAT) domain of mTORC2 and is the only characterized endogenous inhibitor of mTORC2 (Peterson et al., 2009).

When active, mTORC2 is phosphorylated on Ser2481 (Copp et al., 2009) and is important for cell survival, metabolism, proliferation, and cytoskeletal organization. mTORC2 phosphorylates Protein Kinase Cα (PKC), which regulates cell proliferation, differentiation, apoptosis, and telomerase activities and is constitutively active in hematopoetic cancers, where it promotes cell proliferation and survival (Ikenoue et al., 2008; Yamada et al., 2010; Zhang et al., 2012). Knockdown of mTORC2 components disrupts cell morphology and actin polymerization (Loewith et al., 2002; Jacinto et al., 2004). While mTORC2 is rapamycin-insensitive, studies suggest that it can be sensitive to rapamycin in some cancer cell lines, using Ser2481 phosphorylation as a read-out (Copp et al., 2009).

The serine/threonine kinase AKT is activated downstream of PI3K signaling. Akt is important for cell survival, metabolism, and proliferation, and its full activation requires phosphorylation at Ser308 by PDK1 and at Ser473 by mTORC2 (Sarbassov et al., 2005). The importance of mTORC2 in Akt activation is also demonstrated by the fact that deletion of rictor, mLST8, and SIN1 components of mTORC2 blocks AKT phosphorylation at Ser473 (Guertin et al., 2006; Jacinto et al., 2006; Shiota et al., 2006). Research suggests that TSC1 and TSC2 physically interact with mTORC2 and positively regulate its activity, as cells lacking TSC1/2 have impaired phosphorylation of AKT by mTORC2 (Huang et al., 2008).

Another target of mTORC2 is serum- and glucocorticoid-induced protein kinase 1 (SGK1). SGK1 is activated by growth factors and phosphorylates proteins involved in growth and ion transport and is regulated by mTORC2 through phosphorylation at Ser422 (Garcia-Martinez and Alessi, 2008). Together, these data seem to indicate that growth factors might be one of the upstream signals that regulate mTORC2.

mTORC1 and mTORC2 regulation of each other

Within the mTOR signaling network, there exist unexplained questions regarding the nature of the relationship and the extent of the interplay between mTORC1 and mTORC2. mTORC1 and mTORC2 contain raptor or rictor, respectively, and these two proteins are mutually exclusive in their association with mTOR. Additionally, we know that mTORC1 is largely sensitive to rapamycin treatment, while mTORC2 is acutely rapamycin-insensitive. However, studies have shown that prolonged treatment of rapamycin does inhibit mTORC2 assembly and function (Guertin et al., 2006; Sarbassov et al., 2006). Recent studies suggest that low doses of rapamycin affect mTORC1 signaling, while higher doses primarily affect mTORC2 (Chen et al., 2010). Additionally, S6K1 was found to phosphorylate rictor on Thr1135 in a rapamycin-sensitive manner, suggesting that there exists important cross-talk between mTORC1 and mTORC2 complexes (Julien et al., 2010). More studies are needed to further understand this relationship and its implications for regulation of downstream targets.

Is there another mTOR complex?

Studies of 4E-BP1 phosphorylation utilizing the ATP-competitive mTOR inhibitor Torin 1 revealed that while the inhibitory effects of this compound were greater than rapamycin treatment alone and similar to results obtained with raptor knock-down, there was no appreciable difference when Torin1 was used in cells lacking rictor (Thoreen et al., 2009). These data provide rationale for the possible existence of a distinct rapamycin-insensitive mTOR complex, which may contain raptor. Another piece of evidence for the existence of additional mTOR complexes comes from studies of 5′-terminal oligopyrimidine tract (5′ TOP), a feature of proteins involved with the translational machinery. Translation of the TOP mRNAs is regulated by the PI3K/mTOR pathway; however, the mechanism is still unclear. TOP mRNA synthesis was initially thought to be regulated by S6K1 but this mechanism was subsequently challenged by other groups, because activation of S6K1 did not relieve translational repression of TOP mRNAs (Tang et al., 2001), and S6K1/S6K2 double knockout mice were still able to translate TOP mRNA in a rapamycin-sensitive manner (Pende et al., 2004). Rapamycin treatment has a broad range of effects on TOP mRNA translation, and knockdown of either raptor or rictor has only a slight effect (Patursky-Polischuk et al., 2009; Conn and Qian, 2011). Together, these data support the hypothesis that there exists an additional mTOR complex, a putative “mTORC3.” This complex might have additional binding proteins that regulate its specificity towards a potentially different set of downstream proteins.

mTOR in cancer and disease

mTOR is an important mediator of cancer progression and this pathway is frequently deregulated during tumor formation through the loss of PTEN, activation of PI3K, or

overexpression of AKT (Depowski et al., 2001; Kirkegaard et al., 2005). TSC1/2 is a tumor suppressor complex, mutations in which lead to tuberous sclerosis (TSC), a syndrome characterized by benign tumors composed of large cells some of which become malignant, or lymphangioleiomyomatosis (LAM), a lung disease characterized by abnormal proliferation of smooth muscle cells. Since TSC inhibition leads to hyperactivation of mTOR, drugs that inhibit the mTOR pathway would benefit TSC and LAM patients. Inactivation of the tumor suppressor PTEN leads to hyperactivation of AKT and mTOR, and PTEN is often mutated in human cancers (Neshat et al., 2001). These patients would benefit from rapamycin-like inhibitors of mTORC1; however inhibition of mTORC1 using rapamycin also relieves the S6K1-mediated negative feedback loop, upregulating PI3K/AKT signaling and promoting cell growth, survival, and migration. Under homeostatic conditions, this negative feedback loop may be a powerful way of impeding oncogenesis following growth factor stimulation. This may explain why rapamycin treatment is not an effective monotherapy in cancer patients. Thus, current therapeutic strategies seek inhibition of both the mTOR and PI3K/AKT pathways.

Amplification of chromosomal region containing the gene for S6K1 occurs frequently in breast cancer, which is associated with poor prognosis in patients (Perez-Tenorio et al., 2011). It has been shown that S6K1 regulates estrogen receptorα (ERα), and allows cells to proliferate under low-serum conditions (Yamnik et al., 2009). S6K1 specifically phosphorylates ERα on Ser167, contributing to endocrine therapy resistance in breast cancer (Yamnik and Holz, 2010). Estrogen also activates S6K1 expression through ERα, while S6K1 in turn phosphorylates and activates ERα, creating a positive regulatory loop (Holz, 2012; Maruani et al., 2012). This positive co-regulatory loop between the mTORC1/S6K1 and ERα signaling explains the success of the combination therapy targeting these two pathways in hormone-positive breast cancer (Baselga et al., 2009; Baselga et al., 2012).

Type 2 diabetes is caused by chronic overfeeding and insulin resistance which leads to accumulation of high levels of glucose and amino acids in the blood. mTORC1 appears to be involved in the pathogenesis of type 2 diabetes. Obese rats have high levels of mTOR and S6K1 and reduced IRS-1 expression, which is reverted by rapamycin treatment (Khamzina et al., 2005). Recent studies suggest that mTORC2 disruption also contributes to insulin resistance (Lamming et al., 2012; Ye et al., 2012).

Autism spectrum disorder (ASD) is characterized by impaired social and intellectual abilities and restricted, repetitive behavior, affecting about 1% of children. Mutations of various components of mTOR signaling pathway have been associated with ASD (Ehninger and Silva, 2011; Veenstra-VanderWeele and Blakely, 2012). Heterozygous mutation of TSC1 or TSC2 is associated with ASD and epilepsy, possibly because Tsc1 and Tsc2 are important for axon formation and growth (Choi et al., 2008). Mouse models heterozygous for Tsc2 mutations show memory and learning defects, and some of the behavioral defects are reversed upon treatment with rapamycin (Ehninger et al., 2008). Additionally, loss of PTEN also causes ASD, and mouse models also showed that rapamycin treatment can prevent as well as reverse some of the neuronal defects (Zhou et al., 2009). Aberrant synaptic translation has been implicated in the etiology of Fragile X Syndrome (FXS), the leading inherited cause of autism and intellectual disability. Removal of S6K1 in mice was

found to correct the molecular, synaptic, and behavioral phenotypes in FXS (Bhattacharya et al., 2012). EIF4E, a downstream effector of mTOR, is also implicated in ASD, as this gene is deregulated in patients with ASD (Neves-Pereira et al., 2009).

Calorie restricting lifestyle extends lifespan and delays onset of age-related diseases in many different species from yeast to humans, and the mTOR signaling pathway is highly involved in the regulation of this process (Fontana et al., 2004, 2010; Harrison et al., 2009). Specifically, S6K1 and 4E-BP1 are implicated in modulation of aging because deletion of S6K1 in mice increases lifespan (Selman et al., 2009), while loss of 4E-BP in flies reduces lifespan (Zid et al., 2009). In mice, mTOR activity is increased in hematopoietic stem cells (HSC) of animals with conditional deletion of Tsc1 in the HSCs. Notably, rapamycin was able to increase lifespan and restore self-renewal of HSCs in old animals (Chen et al., 2009).

Future directions

Over the past 30 years much progress has been made in understanding the significance of mTOR as a central regulator of multiple cellular functions as well as its involvement in the pathogenesis and progression of several diseases. There are numerous current challenges ahead: to identify other cellular functions connected to the mTOR signaling pathway, to discover novel downstream components of the pathway, to elucidate how mTOR pathway dysregulation leads to some pathologies, and determine which mTOR pathway components are viable candidates for therapeutic intervention.

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