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mTOR: ON TARGET FOR NOVEL THERAPEUTIC STRATEGIES IN THE NERVOUS SYSTEM

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Abstract

The mammalian target of rapamycin (mTOR), the key component of the protein complexes mTORC1 and mTORC2, plays a critical role in cellular development, tissue regeneration, and repair. mTOR signaling can govern not only stem cell development and quiescence, but also cell death during apoptosis or autophagy. Recent studies highlight the importance of both traditional and newly recognized interactors of mTOR, such as p70S6K, 4EBP1, GSK-3 β , REDD1/RTP801, TSC1/TSC2, growth factors, wingless, and forkhead transcription factors that influence Alzheimer's disease, Parkinson's disease, Huntington's disease, tuberous sclerosis, and epilepsy. Targeting mTOR in the nervous system can offer exciting new avenues of drug discovery, but crucial to this premise is elucidating of the complexity of mTOR signaling for robust and safe clinical outcomes.

Keywords

apoptosis; autophagy; neurodegeneration; mTORC1/2; tuberous sclerosis; stem cells

The mammalian target of rapamycin (mTOR)

The mammalian target of rapamycin (mTOR), also termed the mechanistic target of rapamycin and FK506-binding protein 12-rapamycin complex-associated protein 1 (FRAP1), is a 289 kDa protein serine/threonine protein kinase that plays a critical role in protein synthesis, cytoskeletal organization, cellular differentiation, development, survival, and aging [1] (Box 1). In mammals, a single gene *FRAP1* encodes mTOR and mTOR is ubiquitously expressed as a protein throughout the body including the nervous system, vascular system, and immune system [1, 2], three systems crucial for maintaining neuronal health. The kinase was first isolated in *Saccharomyces cerevisiae* through the analysis of rapamycin toxicity using rapamycin-resistant TOR mutants in yeast that resulted in the

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Conflicts

There are no conflicts to disclose.

identification of the yeast genes *TOR1* and *TOR2* that encode two isoforms in yeast Tor1 and Tor2 [3].

Changes in cellular metabolism or the exposure to growth factors, hormones, mitogens, or amino acids can influence mTOR signaling [4]. mTOR signaling is influential in many aspects of homeostasis that are critical for cellular health, including the health of cells that compose the nervous system. Here we highlight recent advances in understanding the effects of mTOR signaling in the context of systems important for neurodegeneration, including stem cell development and protection against cellular oxidative stress. Insights into the signal transduction pathways of mTOR can open exciting new therapeutic perspectives for several neurodegenerative disorders.

Phosphorylation Domains of mTOR

The carboxy-terminal domain of mTOR consists of a conserved sequence with homology to the catalytic domain of phosphoinositide 3-kinase (PI 3-K) (the catalytic PI 3/PI 4-related kinase domain) as well as other domains that include FKBP12 (FK506 binding protein 12) - rapamycin-binding domain (FRB) that is the docking site for FKBP12- rapamycin complex, FAT domain [FKBP associated protein (FRAP), ataxia-telangiectasia (ATM), transactivation/transformation domain-associated protein (TRRAP)], and FATC domain (FRAP, ATM, TRRAP, Carboxy terminal) [5]. The FAT domain resides adjacent to the FKBP12- rapamycin binding domain (FRB) that allows interaction between mTOR and FKBP12 protein when bound to rapamycin. The N-terminal portion of mTOR contains at least a 20 HEAT (Huntingtin, Elongation factor 3, A subunit of Protein phosphatase-2A, and TOR1) repeat. This region facilitates binding with two important, and mutually exclusive, regulatory proteins, Raptor (regulatory-associated protein of mTOR) and Rictor (rapamycin-insensitive companion of mTOR) [1, 6]. It is the association with either Raptor or Rictor that determines whether mTOR is a component of mTOR complex 1 (mTORC1) or mTORC2 (see below).

Post-translational phosphorylation of mTOR occurs at several levels (Table 1). The C-terminal domain with sequence homology to the catalytic domain of the phosphoinositide 3-kinase (PI 3-K) family [7] contains several phosphorylation sites that regulate mTOR activity (Table 1). Serine²⁴⁴⁸ is a target of protein kinase B (Akt) and the p70 ribosomal S6 kinase (p70S6K) [8, 9]. Of note, serine²⁴⁸¹ is a rapamycin insensitive autocatalytic site of mTOR phosphorylation [10], while threonine²⁴⁴⁶ is phosphorylated by AMP activated protein kinase (AMPK) and p70S6K. In addition, combined phosphorylation at serine²¹⁵⁹ and threonine²¹⁶⁴ increases mTOR activity and promotes autophosphorylation of serine²⁴⁸¹. Within the HEAT domain, serine¹²⁶¹ can be phosphorylated by insulin signaling, both in mTORC1 and mTORC2, through the PI 3-K pathway to increase mTOR activity [7]. Phosphorylation of serine¹²⁶¹ also leads to mTOR serine²⁴⁸¹ autophosphorylation [7].

Complexes, Targets, and Regulation of mTOR

The distinct signaling pathways of mTOR rely upon the formation of two protein complexes, mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2) [1, 6] (Figure 1). mTORC1 relies upon the regulatory-associated protein of mTOR (Raptor) protein to allow mTORC1 to bind to its complex constituents (Table 2), and phosphorylation of Raptor, and consequent regulation of mTORC1 activity, can occur through multiple pathways. One Raptor phosphorylation pathway involves the protein Ras homologue enriched in brain (Rheb). Rheb can increase phosphorylation on Raptor residue serine⁸⁶³ as well as on five other identified residues that include serine⁸⁵⁹, serine⁸⁵⁵, serine⁸⁷⁷, serine⁶⁹⁶, and threonine⁷⁰⁶ (Table 1). The inability to phosphorylate serine⁸⁶³ reduces mTORC1 activity, as demonstrated using a site-direct mutation of serine⁸⁶³ [11]. mTOR, once

activated, also controls Raptor activity and phosphorylates Raptor that can be stimulated by insulin and inhibited by rapamycin [11]. In addition to containing Raptor, mTORC1 consists of the (proline rich Akt substrate 40 kDa), Deptor (DEP domain-containing mTOR interacting protein), and mLST8/G β L (mammalian lethal with Sec13 protein 8, termed mLST8 in this review).

The second protein complex, mTORC2 controls cell size and actin cytoskeleton organization, endothelial cell survival and migration, and cell cycle progression and is similar to mTORC1 because it also contains mTOR, mLST8, and Deptor (Table 2). However, the HEAT domains of mTORC2 bind Rictor, rather than Raptor, and the complex contains the additional proteins mSIN1 (mammalian stress-activated protein kinase interacting protein) and Protor-1 (protein observed with Rictor-1). Similar to Raptor, Rictor fosters the activity of mTORC2.

The components of mTORC1 and mTORC2 influence the activity and function of these protein complexes (Table 2). For example, PRAS40 (Akt1s1) can block mTORC1 activity through its association with Raptor [12] and the maintenance of mTORC1 activity occurs via the inhibitory phosphorylation of PRAS40 by protein kinase B (Akt). Two accessory proteins are present in both complexes, mLST8 and Deptor. mLST8 is a 36 kDa peripheral membrane protein that is a component of both complexes. It comprises 7 repeats of tryptophan and aspartate residues (WD-40 repeats) and localizes to the endosomal or Golgi membranes [13]. The presence of mLST8 promotes mTOR kinase activity toward p70S6K and 4EBP1 [14], controls insulin signaling through the transcription factor FoxO3 [15], is required for the phosphorylation of Akt and protein kinase C- α (PKC α) [15], and is necessary to maintain the association between Rictor and mTOR [15]. Deptor is an inhibitory subunit of the mTOR complexes; in the absence of Deptor, the activity of Akt, mTORC1, and mTORC2 increase [16]. mTORC1 and mTORC2 can inhibit Deptor expression [16], but in some forms of cancer, Deptor expression is necessary for Akt signaling [16, 17].

Other components are specific to each complex, such as PRAS40 and Raptor (mTORC1) or Rictor, mSIN1, and Protor-1/PRR5 (mTORC2). Rictor and mSIN1 form the structural basis of mTORC2. Rictor allows mTORC2 to activate and phosphorylate Akt at Ser⁴⁷³, facilitating threonine³⁰⁸ phosphorylation by phosphoinositide-dependent kinase 1 (PDK1) [18]. mSIN1 enables TORC2 to phosphorylate Akt and is necessary for TORC2 to activate Akt. mTOR has also been shown to phosphorylate mSIN1, preventing the lysosomal degradation of mSIN1 [19]. Protor-1 is a Rictor-binding subunit of mTORC2 that does not appear to alter other mTORC2 components in a way that leads to the phosphorylation of Akt or PKC α . However, Protor-1 may function to activate serum and glucocorticoid induced protein kinase 1 (SGK1). In murine experimental models, loss of Protor-1 reduces the hydrophobic motif phosphorylation of SGK1 and its substrate NRDG1 (N-Myc downregulated gene 1 in the kidney) [20].

mTORC1 and mTORC2 oversee multiple cellular functions [4, 6] (Figure 1). mTORC1 regulates the activity of the eukaryotic initiation factor 4E-binding protein 1 (4EBP1) and the serine/threonine kinase ribosomal protein p70S6K, via interactions between these proteins and Raptor. Binding of 4EBP1 and p70S6K to Raptor can be prevented during activation of PRAS40. When 4EBP1 is hypophosphorylated, it can block protein translation by binding to eukaryotic translation initiation factor 4 epsilon (eIF4E) through eIF4 gamma (eIF4G), a protein that shepherds mRNA to the ribosome. mTORC1 phosphorylation of 4EBP1 leads to the dissociation of 4EBP1 from eIF4E, allowing eIF4G to begin mRNA translation [21]. mTORC1 also fosters mRNA biogenesis, translation of ribosomal proteins, and cell growth through p70S6K phosphorylation, and the presence of amino acids, such as

glutamate and leucine, have been shown to activate the phosphorylation of p70S6K. Through amino acid activation of the mTOR-p70S6K pathway, glutamate may control neuronal synaptic signaling [22] and leucine can decrease food intake [23].

mTORC2 controls cytoskeleton remodeling through the phosphorylation and activation of protein kinase C alpha (PKC α) [24], and Rho signaling pathways through mTORC2 also control cell-to-cell contact [25]. Expression of the constitutively active forms of the Rho GTPases with mTORC2 activation promote cell-to-cell contact and decrease remodeling of the actin cytoskeleton. The guanine nucleotide exchange factors P-Rex1 and P-Rex2, which interact with the Rho GTPase Rac, are also targets of mTORC2, are phosphorylated by Akt through mTORC2 acting as a catalytic complex, and are linked to Rac activation and cell migration [26].

One of the more important upstream modulators of mTOR activity may be the tuberous sclerosis complex (TSC1, hamartin/ TSC2, tuberin), whose activity can inhibit mTORC1 activity. Although several regulatory phosphorylation sites have been identified in TSC1, phosphorylation of TSC2 by Akt, extracellular signal-regulated kinases (ERKs), activating protein p90 ribosomal S6 kinase 1 (RSK1), AMP activated protein kinase (AMPK), or glycogen synthase kinase -3 β (GSK-3 β) may be more relevant for inhibiting the TSC1/TSC2 complex. TSC2 is a GTPase-activating protein (GAP) for the active form of the small G protein Rheb (Rheb-GTP) to the inactive GDP-bound form (Rheb-GDP). Rheb-GTP can directly interact with Raptor and activate the mTORC1 complex. In addition, Rheb regulates 4EBP1 binding to mTORC1 and reduces mTOR activity by controlling the association of mTOR with mTORC1 through FKBP38. FKBP38 is structurally related to FKBP12 and is an endogenous inhibitor of mTOR. Akt phosphorylates TSC2 on multiple sites, disrupting the interaction of TSC2 with TSC1, leading to the sequestration of TSC2 by 14-3-3, and subsequently activating Rheb and mTORC1 [27].

In contrast to mTORC1, the TSC1/TSC2 complex appears to promote the activity of mTORC2 [28]. Loss of a functional TSC1/TSC2 complex can lead to the loss of mTORC2 kinase activity *in vitro* [28]. The TSC1/TSC2 complex can physically associate with mTORC2 to enhance the activity of mTORC2 using a mechanism that is independent of its GAP activity toward Rheb but which involves the N-terminal region of TSC2 and the C-terminal region of Rictor.

It is important to note that in addition to phosphorylation of the TSC1/TSC2 complex, other mechanisms exist to control mTORC1 activity (Figure 1). For example, AMPK phosphorylates TSC2 on serine¹³⁸⁷ (human) or serine¹³⁴⁵ (rat), promoting its GAP activity to turn Rheb-GTP into Rheb-GDP and thereby inhibiting the activity of mTORC1 [29]. However, AMPK also can modulate TSC1/2 activity through RTP801 (REDD1/ product of the *Ddit4* gene) [30]. During periods of hypoxia, AMPK activity may promote REDD1 expression that can suppress mTORC1 activity by releasing TSC2 from its inhibitory binding to protein 14-3-3 [30].

Role of mTOR in Stem Cell Development, Proliferation, and Quiescence

mTOR can have a significant impact upon stem cell development, proliferation, and quiescence (Table 3). For example, deletion of the C-terminal six amino acids of mTOR, which are essential for kinase activity, decreases in proliferation of embryonic stem cells [31]. Complete ablation of *mTOR* leads to lethality and the arrest of embryonic stem cell proliferation [32].

The activity of mTOR is essential for the long-term undifferentiated growth of human embryonic stem cells, as demonstrated by the fact that inhibition of mTOR impairs

pluripotency, prevents cell proliferation, and enhances mesoderm and endoderm activities in embryonic stem cells [33]. Stem cell quiescence is necessary to maintain stem cell proliferation properties and protect against premature differentiation or senescence, and decreased levels of phosphorylated and active p70S6K, a downstream target of mTOR, appears to be necessary to maintain hematopoietic stem cell dormancy [34]. In addition, expression of constitutively active p70S6K, or the siRNA-mediated knockdown of both TSC2 and Rictor to increase p70S6K activation, results in the differentiation of human embryonic stem cells [35]. In the nervous system, mTOR signaling is necessary for insulin-induced neuronal differentiation in neuronal progenitor cells [36], and mTORC1-associated pathways with REDD1 can regulate neuronal migration and cortical patterning [37]. Nevertheless, sustained activation of the mTOR pathway can lead to premature differentiation of neuronal stem cells and impaired maturation [38].

Stem cell proliferation and homeostasis regulated by mTOR may require other molecules or pathways, such as growth factors, PI 3-K, and Wnt proteins. Growth factors, such as erythropoietin (EPO), rely upon mTOR pathway signaling for neuronal and endothelial cell survival [39]. EPO also utilizes mTOR signaling for microglia survival during oxidative stress [40] and for osteoblastogenesis and osteoclastogenesis [41]. Furthermore, a loss of growth factor signaling that limits mTOR activity leads to endothelial progenitor cell death [42]. Inhibition of either PI 3-K or mTOR alone also results in attenuated growth factor induced proliferation of neural stem cells without affecting their capacity to self-renew, suggesting that both PI 3-K and mTOR are involved in the maintenance of neural stems [43].

Wnt protein signaling is critical to multiple cell process that involve stem cell proliferation, cell development, and cellular survival [44]. The Wnt pathway can increase the activity of mTOR by inhibiting GSK-3 β through phosphorylation. In the absence of Wnt activity, GSK-3 β can phosphorylate TSC2 on serine¹³³⁷ and serine¹³⁴¹, following a necessary priming phosphorylation by AMPK of TSC2 on serine¹³⁴⁵, resulting in the inhibition of mTOR activity [45]. Recent work has shown that a balance between Wnt and GSK-3 β activation is required for hematopoietic stem cell self renewal and lineage commitment [46].

Oxidative Stress Pathways of mTOR during Apoptosis and Autophagy

Although diseases that affect various systems of the body may have multiple etiologies, oxidative stress can be a primary component that leads to the onset and progression of both acute and chronic disorders in the nervous system. Disorders associated with aging, cognitive loss, immune system impairment, or metabolic disorders may be the result of the release of reactive oxygen species (ROS) that lead to oxidative stress [47] and recent studies link ROS to DNA damage in diabetic patients [48], mitochondrial injury and aging mechanisms [49], and nutritional impairment [50]. ROS can be produced in excessive quantities through different sources, such as superoxide free radicals, hydrogen peroxide, singlet oxygen, nitric oxide (NO), and peroxynitrite. Detrimental effects of ROS are usually prevented by endogenous antioxidant systems including the activity of superoxide dismutase, glutathione peroxidase, catalase, and vitamins that include C, D, E, and K [51].

Oxidative stress leads to injury in the nervous system through pathways that involve apoptosis as well as autophagy [52] (Table 3). For example, apoptotic DNA fragmentation and caspase activation has been observed in the brains of patients with Alzheimer's disease (AD) [53] and Parkinson's disease (PD) [54]. During periods of oxidative stress, autophagy can also lead to cell death in purkinje neurons [55], cerebral astrocytes [56], cortical neurons [57], and spinal cord motor neurons [58]. However, activation of autophagy may be beneficial, as suggested by models of Parkinson's disease (PD) [59], Alzheimer's disease

(AD) [60], and prion protein mediated neurotoxicity [61]. Autophagy may be necessary for neuronal protection to process toxic protein accumulation, such as α -synuclein toxicity in Parkinson's disease [59].

Apoptosis consists of two distinct components that involve genomic DNA degradation and the loss of plasma membrane lipid asymmetry [52]. The early energy dependent phase of apoptosis involves the externalization of phosphatidylserine (PS) residues on the surface of cells that can be a signal for inflammatory cells to engulf and dispose of injured. PS-labeled cells. The late phase of apoptosis usually does not allow for the repair or recovery of cells because it involves nuclear DNA degradation. Loss of mTOR activity during periods of oxidative stress can lead to apoptotic cell death. For example, exposure to the oxidant hydrogen peroxide impairs mTOR kinase activity and leads to apoptotic cell death in neuronal cells [62]. During periods of serum deprivation that prevent mTOR activation, insulin, a cytoprotective growth factor, does not appear able to rescue cell survival unless mTOR activity is restored [63]. Other growth factors, such as EPO, have also been shown to be dependent upon mTOR activity, and p70S6K, for cytoprotection [40, 41, 64]. Inflammatory cells also can succumb to the toxic effects of oxidative stress if deprived of mTOR activation [64, 65].

mTOR relies upon the modulation of p70S6K and BAD to avert apoptotic cell death. Activation of p70S6K promotes the phosphorylation of BAD in astrocytes to limit apoptotic cell injury [66]. Insulin also prevents apoptosis in rat retinal neuronal cells against serum deprivation, and this may be through the activation of mTOR and p70S6K [63]. Over-expression of wild type p70S6K or expression of a rapamycin resistant form of the kinase enhances neuroprotection by insulin [63].

The inhibition of apoptosis through mTOR relies upon Akt activation. Cytoprotection through Akt can occur at several levels: fostering cell survival by maintaining mitochondrial membrane potential, preventing caspase activation, and blocking inflammatory cell activation that can lead to the disposal of functional cells [1]. mTOR has been shown to require Akt activation to block apoptotic cell death and requires the inactivation of forkhead transcription factors, such as FoxO3a [15]. Inflammatory cells also succumb to apoptosis during oxidative stress if deprived of Akt and mTOR activation [64, 65]. Apoptotic cell death in dopaminergic neurons can be prevented by applying agents that increase Akt and mTOR activity [67]. Akt controls apoptosis by phosphorylating PRAS40, promoting its dissociation from mTORC1 and leading to the association of PRAS40 with cytoplasmic 14-3-3 proteins [68, 69].

In contrast to apoptosis, autophagy allows cells to recycle cytoplasmic components and dispose of defective organelles, but preserve essential cytoskeletal structures during development, cell differentiation, and tissue remodeling [52]. In some scenarios, progression of apoptosis may conversely require the inhibition of autophagy [70, 71], suggesting that autophagy may not be a principal component of cell death in some models of neuronal injury [72]. During acute injury, mTOR activation can prevent cell injury in the nervous system, as seen in dopamine neurons during oxidative stress – degeneration can be prevented by activating mTOR and inhibiting autophagy [67]. By contrast, loss of mTOR activity can lead to autophagic cell death [73]. Nevertheless, chronic diseases, such as AD, may benefit from the inhibition of mTOR to allow the progression of autophagy [60]. Early in the progression of chronic disorders such as AD, AMPK activation, which can inhibit mTOR to foster autophagy, may be required to regulate tau protein phosphorylation and amyloidogenesis, but prolonged AMPK activity can have detrimental effects and lead to neuronal demise [74]. The degree of mTOR activation may be a significant variable in cases such as this. During the early phases of autophagy mTOR activity can be inhibited [75], but

re-activation of mTOR appears to be required to continue with autophagy, so long as elevated levels of mTOR activity do not eventually lead to a blockade of autophagy [75]. This modulation of mTOR with autophagy may be a conserved response in multi-cellular systems that is governed by nutrient availability [76].

mTOR appears to modulate autophagy through the regulation of autophagic genes. mTOR can phosphorylate the mammalian homologue of Atg13 (autophagy related gene 13) and the mammalian Atg1 homologues ULK1 (UNC-51 like kinase 1) and ULK2 to prevent the progression of autophagy [77]. The focal adhesion kinase family interacting protein of 200 kDa (FIP200) has been identified as a ULK binding protein, and both FIP200 and Atg13 are critical for the stability and activation of ULK1. Mammalian Atg13 binds to ULK1/2 and FIP200 to activate ULKs and facilitate FIP200 phosphorylation by ULKs [77]. As a result, it is suggested that mTOR activation prevents autophagy in mammalian cells by phosphorylating Atg13 and ULKs, thus inhibiting the ULK–Atg13–FIP200 complex. During inhibition of mTOR, dephosphorylation of ULKs and Atg13 ensues, leading to the induction of autophagy [77, 78]

mTOR based Therapeutic Strategies in Neurodegenerative disease

Alzheimer's disease

mTOR may be necessary for synaptic plasticity and memory formation in the hippocampus, given that mTOR inhibition has been shown to impair memory consolidation [79] (Table 3). Yet as the data below illustrates, the degree of activity in mTOR pathways needed to be therapeutic in disorders such as AD has not been determined. An increase in phosphorylated levels of substrates, such as mTOR, GSK-3 β , and tau protein, have been observed in AD, indicating that these substrates may promote AD progression [80]. p70S6K activation has also been associated with hyperphosphorylated tau formation and potential neurofibrillary accumulation in AD patients [81]. Furthermore, mTOR inhibition that is associated with increased autophagy in murine models of AD improves memory and reduces amyloid (A β) levels [60]. Other investigations support the premise that activating mTOR to some degree is necessary to prevent pathology during AD, and decreased mTOR activity in peripheral lymphocytes appears to correlate with AD progression [82]. Loss of mTOR signaling has been shown to impair long-term potentiation and synaptic plasticity in models of AD [83]. In addition, A β is toxic to cells and can block the activation of mTOR and p70S6K in neuroblastoma cells and in lymphocytes of patients with AD [84]. Activation of mTOR and p70S6K has been shown to prevent cell death during A β exposure in microglia, cells necessary for A β sequestration to prevent toxicity of A β exposure [64]. Other work suggests that blockade of mTOR activity may lead to neuronal atrophy in AD [85]. For example, insufficiency of retinoblastoma tumor suppressor (RB1) inducible Coiled-Coil 1 (RB1CC1) has been observed in the brains of AD patients. In these patients, RB1CC1 appears to be necessary for neurite growth and to maintain mTOR signaling, but the reduced expression of RB1CC1 leads to reduced mTOR activity, neuronal apoptosis, and neuronal atrophy [85].

Parkinson's disease

Activation of mTOR signaling pathways and inhibition of autophagy may be a critical component for the treatment of PD, a movement disorder characterized by resting tremor, rigidity, and bradykinesia with the loss of dopaminergic neurons in the substantia nigra (Table 3), but as with AD, tuning the levels of activity may be crucial. Blockade of mTOR activity in cell culture models during oxidative stress can lead to dopaminergic neuronal cell death as a result of autophagy activation [67]. Loss of mTOR activity and the chronic activation of the mTOR pathway 4EBP1 by leucine-rich repeat kinase 2 (LRRK2), a site for dominant mutations PD, can alter protein translation and result in the loss of dopaminergic

neurons [86]. However, the role of 4EBP1 is not entirely clear because the activation of 4EBP1 can suppress pathologic experimental phenotypes of PD, including the degeneration of dopaminergic neurons in *Drosophila* [87].

REDD1(RTP801) is another signaling pathway for therapeutic consideration and is an inhibitor of mTORC1 activity [30]. REDD1 is up-regulated in dopaminergic neurons in PD patients [88] and is highly expressed in cellular models of PD [88]. REDD1 appears to lead to neuronal death through the blockade of mTOR activity. As a result, restoring mTOR activity is neuroprotective against REDD1 [88]. However, mTOR activation may require a fine level of modulation during PD because other studies suggest that inactivating mTOR and promoting autophagy may preserve dopaminergic neurons in PD, possibly by protecting against α -synuclein accumulation and toxicity [59]. When mTOR is inhibited and autophagy is promoted, the accumulation of toxic α -synuclein in transgenic mice is reduced and neurodegeneration is decreased [89]. In addition, treatment with derivatives of dopamine, such as L-DOPA, can lead to dopamine D1 receptor-mediated activation of mTORC1, resulting in dyskinesias and disability for patients [90].

Huntington's disease

Inhibition of mTOR pathways that foster autophagy may be critical for developing strategies against Huntington's disease (HD) (Table 3). Autophagy is considered important for HD because it is responsible for clearing the intracellular aggregates of huntingtin protein that can lead to neuronal degeneration. Inhibiting mTOR activity with the agent rapamycin can increase the autophagic clearance of proteins with long polyglutamine or polyalanine expansions [91], attenuating huntingtin accumulation and cell death in cell models of HD and protecting against neurodegeneration in fly and murine models [92]. In addition, small molecules that enhance mTOR inhibition have been shown to be beneficial for the clearance of huntingtin protein in *Drosophila* while eliminating the unwanted immunosuppressive side effects of agents such as rapamycin [93]. However, in some experimental models of HD, inhibition of mTORC1 alone, as opposed to inhibition of both complexes, does not affect autophagy or huntingtin accumulation. By contrast, the combined inhibition of mTORC1 and mTORC2 is required for autophagy and reducing huntingtin accumulation, suggesting that multiple components of the mTOR pathway may modulate the pathology observed in HD [94]. As evidence for this suggestion, other studies show that decreased activity of p70S6K protects against early decline in motor performance with beneficial effects on muscle, without affecting mutant huntingtin levels in the brain [95]. Neuroprotection by the mTOR pathway may also require the molecule growth arrest and DNA damage protein 34 (GADD34). GADD34 function leads to the dephosphorylation of TSC2 and induction of autophagy in cell models of HD, and consequently increased cell survival is observed when GADD34 is overexpressed [96].

Tuberous Sclerosis and Epilepsy

Activation of the mTOR signaling pathway has been reported as an underlying mechanism in tuberous sclerosis (TS), a disorder in which epilepsy occurs in over 80% of patients [97] (Table 3). In both healthy and lesioned skin biopsies of TS patients, increased mTOR activity occurs with the up-regulation of p70S6K [98]. Mutations within TSC1 and TSC2 that lead to hyperactive mTOR result in a high incidence of epilepsy in experimental models [99], and inhibition of mTOR signaling with rapamycin in animal models of TS early in the course of the disease can prevent astrogliosis, neuronal disorganization, and seizures, suggesting that the aberrant mTOR activation interferes with normal brain function and leads to epilepsy [100]. Chronic hippocampal infusion of rapamycin also limits mossy fiber sprouting in a rat pilocarpine model of temporal lobe epilepsy [101]. In addition, mTOR activation has been linked to acquired epilepsy [102]. For example, inhibition of mTOR

activity during the development of kainate-induced chronic epilepsy decreases neuronal cell death, neurogenesis, mossy fiber sprouting, and the development of spontaneous epilepsy [102]. In clinical studies of patients with TS, the United States Food and Drug Administration (FDA) has approved the use of everolimus (RAD-001), an analogue of rapamycin and an inhibitor of mTOR, for the treatment of subependymal giant cell astrocytoma. Everolimus has been shown in TS to limit giant cell astrocytoma cell growth [103, 104] and decrease seizure frequency in almost 60% of patients examined, but patients also experienced treatment-related oral and respiratory infections, stomatitis, and leukopenia [104]. Increased seizure frequency was observed in a minority of patients treated with everolimus [104].

Concluding Remarks and Future Perspectives

mTOR and its signaling pathways have a significant impact on multiple disorders of the nervous system, including Alzheimer's disease, Parkinson's disease, Huntington's disease, tuberous sclerosis, and epilepsy. Therefore, targeting mTOR for the development of novel therapeutic avenues to treat neurodegenerative disorders is viewed with great enthusiasm. Both newly recognized pathways of growth factors, wingless, and forkhead transcription factors and traditional known pathways of mTORC1 and mTORC2 that involve p70S6K, 4EBP1, PI 3-K, Akt, AMPK, GSK-3 β , REDD1, and the TSC1/TSC2 complex can significantly influence the biological and clinical outcome of mTOR signaling. Furthermore, the increased expression of mTOR signaling pathways, such as p70S6K and 4EBP1, may be biomarkers of disease progression. Presently, the FDA has approved rapamycin (sirolimus) and several rapamycin derivative compounds ("rapalogs") for the treatment of renal cancer (everolimus, temsirolimus), allograft rejection (everolimus, sirolimus), subependymal giant cell astrocytoma associated with tuberous sclerosis (everolimus), and neuroendocrine pancreatic tumors (everolimus), as well as the prevention of vascular re-stenosis (sirolimus, zotarolimus, umirolimus) [4, 6]. Current clinical trials investigating everolimus for the treatment of advanced neuroendocrine tumors [105] and for subependymal giant cell astrocytoma and epilepsy [103, 104] suggest that progression-free survival can be improved with agents that inhibit mTOR signaling.

However, for the effective translation of mTOR and its signaling pathways into robust clinical agents directed against disorders of the nervous system, several current hurdles must be overcome (Box 2). For example, therapies that employ mTOR inhibition to treat subependymal giant cell astrocytoma or epilepsy can have a limited response in patients or only be effective for a subpopulation of patients [103, 104]. Disorders such as nervous system tumors can develop resistance to agents that inhibit mTOR signaling, and some tumors may have an abnormal increased basal activity of the PI 3-K, Akt, mTOR axis [106]. As a result, inhibiting only the mTOR pathway without addressing the activity of PI 3-K or Akt can lead to a poor clinical response and ineffective blockade of cell tumor cell proliferation [106]. Other studies suggest that blockade of the mTORC1 pathway can result in the feedback activation of PI 3-K, Akt, and Ras-mitogen activated protein kinase (MAPK) signaling that can lead to further neoplastic growth, therefore advocating the inhibition of multiple pathways that involve PI 3-K, Akt, mTOR, and MAPK signaling [107, 108]. In some clinical studies with chronic myelogenous leukemia [70] and colorectal cancer metastases [25], targeting both mTORC1 and mTORC2 pathways has been proposed. In addition, it may be necessary to broaden cellular targets to focus on modulating the PI 3-K–Akt–mTOR axis that has been shown in preclinical studies to increase radiosensitivity against tumor cell growth and the vascular supply of tumors [109]. As we move forward, agents that can modulate the activity levels of the complete PI 3-K–Akt–mTOR axis as well as the individual components may provide the greatest treatment response for disorders of the nervous system. New clinical studies along this path are already underway to assess PI

3-K–Akt–mTOR axis modulators in early phase trials to treat hematological malignancies in children and adults [110, 111]. Furthermore, additional agents that can target different Akt classes with the alkyl-lysophospholipids and small molecule inhibitors of Akt, as well as combined targeting of mTORC1 and mTORC2 with or without PI 3-K inhibition, are under consideration for disorders that can involve the central nervous system. Treatments with rapamycin or its derivative compounds that block mTOR activity also have additional limitations. These agents can lead to a number of side effects that include oral and respiratory infections, stomatitis, hypertriglyceridemia, hypercholesterolemia, leukopenia, and immunosuppression [104, 105]. Therefore, the continued development of mimetics of mTOR signaling that can meet treatment objectives but eliminate the detrimental effects of current mTOR modulatory agents are highly warranted [93].

The treatment paradigms for mTOR signaling also require further investigation. Studies suggest that the degree and duration of mTOR signaling may be an essential factor for neuroprotection. Activation of mTOR can foster long-term potentiation and synaptic plasticity in models of AD [83] and block inflammatory cell death during toxic A β exposure [64]. However, sustained activation of mTOR can result in impaired neuronal stem cell maturation [38] and clinical disability with dyskinesia in PD patients [90], and prolonged inhibition of mTOR can lead to neuronal death [74]. In addition, the timing of treatments to alter mTOR activity may affect biological and clinical outcome. Early rather than late treatment with rapamycin can reduce plaques, tangles, and loss of cognition in murine models of AD [112]. To successfully move forward into the clinical realm with new avenues for treating neurodegenerative disorders by targeting mTOR, future work must continue to elucidate the fine modulatory role of mTOR and its intricate cellular pathways to achieve desired clinical outcomes without adverse effects.

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References

1. Chong ZZ, et al. Mammalian target of rapamycin: hitting the bull's-eye for neurological disorders. *Oxid Med Cell Longev*. 2010; 3:374–391. [PubMed: 21307646]
2. Brown EJ, et al. A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature*. 1994; 369:756–758. [PubMed: 8008069]
3. Heitman J, et al. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*. 1991; 253:905–909. [PubMed: 1715094]
4. Chong ZZ, et al. Shedding new light on neurodegenerative diseases through the mammalian target of rapamycin. *Prog Neurobiol*. 2012
5. Takahashi T, et al. Carboxyl-terminal region conserved among phosphoinositide-kinase-related kinases is indispensable for mTOR function in vivo and in vitro. *Genes Cells*. 2000; 5:765–775. [PubMed: 10971657]
6. Benjamin D, et al. Rapamycin passes the torch: a new generation of mTOR inhibitors. *Nat Rev Drug Discov*. 2011; 10:868–880. [PubMed: 22037041]
7. Acosta-Jaquez HA, et al. Site-specific mTOR phosphorylation promotes mTORC1-mediated signaling and cell growth. *Mol Cell Biol*. 2009; 29:4308–4324. [PubMed: 19487463]
8. Chiang GG, Abraham RT. Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. *J Biol Chem*. 2005; 280:25485–25490. [PubMed: 15899889]
9. Reynolds, THt, et al. Control of Ser2448 phosphorylation in the mammalian target of rapamycin by insulin and skeletal muscle load. *J Biol Chem*. 2002; 277:17657–17662. [PubMed: 11884412]

10. Soliman GA, et al. mTOR Ser-2481 autophosphorylation monitors mTORC-specific catalytic activity and clarifies rapamycin mechanism of action. *J Biol Chem.* 2010; 285:7866–7879. [PubMed: 20022946]
11. Wang L, et al. Mammalian target of rapamycin complex 1 (mTORC1) activity is associated with phosphorylation of raptor by mTOR. *J Biol Chem.* 2009; 284:14693–14697. [PubMed: 19346248]
12. Wang H, et al. Proline-rich Akt substrate of 40kDa (PRAS40): a novel downstream target of PI3k/Akt signaling pathway. *Cell Signal.* 2012; 24:17–24. [PubMed: 21906675]
13. Chen EJ, Kaiser CA. LST8 negatively regulates amino acid biosynthesis as a component of the TOR pathway. *J Cell Biol.* 2003; 161:333–347. [PubMed: 12719473]
14. Kim DH, et al. GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol Cell.* 2003; 11:895–904. [PubMed: 12718876]
15. Guertin DA, et al. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. *Dev Cell.* 2006; 11:859–871. [PubMed: 17141160]
16. Peterson TR, et al. DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell.* 2009; 137:873–886. [PubMed: 19446321]
17. Zhao Y, et al. DEPTOR, an mTOR inhibitor, is a physiological substrate of SCF(betaTrCP) E3 ubiquitin ligase and regulates survival and autophagy. *Mol Cell.* 2011; 44:304–316. [PubMed: 22017876]
18. Sarbassov DD, et al. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science.* 2005; 307:1098–1101. [PubMed: 15718470]
19. Chen CH, Sarbassov dos D. The mTOR (mammalian target of rapamycin) kinase maintains integrity of mTOR complex 2. *J Biol Chem.* 2011; 286:40386–40394. [PubMed: 21965657]
20. Pearce LR, et al. Protor-1 is required for efficient mTORC2-mediated activation of SGK1 in the kidney. *Biochem J.* 2011; 436:169–179. [PubMed: 21413931]
21. Gingras AC, et al. 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. *Genes Dev.* 1998; 12:502–513. [PubMed: 9472019]
22. Lenz G, Avruch J. Glutamatergic regulation of the p70S6 kinase in primary mouse neurons. *J Biol Chem.* 2005; 280:38121–38124. [PubMed: 16183639]
23. Cota D, et al. Hypothalamic mTOR signaling regulates food intake. *Science.* 2006; 312:927–930. [PubMed: 16690869]
24. Sarbassov DD, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol.* 2004; 14:1296–1302. [PubMed: 15268862]
25. Gulhati P, et al. mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. *Cancer Res.* 2011; 71:3246–3256. [PubMed: 21430067]
26. Hernandez-Negrete I, et al. P-Rex1 links mammalian target of rapamycin signaling to Rac activation and cell migration. *J Biol Chem.* 2007; 282:23708–23715. [PubMed: 17565979]
27. Cai SL, et al. Activity of TSC2 is inhibited by AKT-mediated phosphorylation and membrane partitioning. *J Cell Biol.* 2006; 173:279–289. [PubMed: 16636147]
28. Huang J, et al. The TSC1-TSC2 complex is required for proper activation of mTOR complex 2. *Mol Cell Biol.* 2008; 28:4104–4115. [PubMed: 18411301]
29. Inoki K, et al. TSC2 mediates cellular energy response to control cell growth and survival. *Cell.* 2003; 115:577–590. [PubMed: 14651849]
30. DeYoung MP, et al. Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. *Genes Dev.* 2008; 22:239–251. [PubMed: 18198340]
31. Murakami M, et al. mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. *Mol Cell Biol.* 2004; 24:6710–6718. [PubMed: 15254238]
32. Gangloff YG, et al. Disruption of the mouse mTOR gene leads to early postimplantation lethality and prohibits embryonic stem cell development. *Mol Cell Biol.* 2004; 24:9508–9516. [PubMed: 15485918]

33. Zhou J, et al. mTOR supports long-term self-renewal and suppresses mesoderm and endoderm activities of human embryonic stem cells. *Proc Natl Acad Sci U S A*. 2009; 106:7840–7845. [PubMed: 19416884]
34. Iriuchishima H, et al. Ex vivo maintenance of hematopoietic stem cells by quiescence induction through Fbxw7 α ; overexpression. *Blood*. 2011; 117:2373–2377. [PubMed: 21190997]
35. Easley CA, et al. mTOR-mediated activation of p70 S6K induces differentiation of pluripotent human embryonic stem cells. *Cellular reprogramming*. 2010; 12:263–273. [PubMed: 20698768]
36. Han J, et al. Mammalian target of rapamycin (mTOR) is involved in the neuronal differentiation of neural progenitors induced by insulin. *Mol Cell Neurosci*. 2008; 39:118–124. [PubMed: 18620060]
37. Malagelada C, et al. RTP801/REDD1 regulates the timing of cortical neurogenesis and neuron migration. *J Neurosci*. 2011; 31:3186–3196. [PubMed: 21368030]
38. Magri L, et al. Sustained activation of mTOR pathway in embryonic neural stem cells leads to development of tuberous sclerosis complex-associated lesions. *Cell Stem Cell*. 2011; 9:447–462. [PubMed: 22056141]
39. Maiese K, et al. New avenues of exploration for erythropoietin. *Jama*. 2005; 293:90–95. [PubMed: 15632341]
40. Shang YC, et al. Erythropoietin and Wnt1 Govern Pathways of mTOR, Apaf-1, and XIAP in Inflammatory Microglia. *Curr Neurovasc Res*. 2011; 8:270–285. [PubMed: 22023617]
41. Kim J, et al. Erythropoietin mediated bone formation is regulated by mTOR signaling. *J Cell Biochem*. 2012; 113:220–228. [PubMed: 21898543]
42. Miriuka SG, et al. mTOR inhibition induces endothelial progenitor cell death. *Am J Transplant*. 2006; 6:2069–2079. [PubMed: 16796720]
43. Sato A, et al. Regulation of neural stem/progenitor cell maintenance by PI3K and mTOR. *Neurosci Lett*. 2010; 470:115–120. [PubMed: 20045038]
44. Maiese K, et al. The Wnt signaling pathway: Aging gracefully as a protectionist? *Pharmacol Ther*. 2008; 118:58–81. [PubMed: 18313758]
45. Inoki K, et al. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell*. 2006; 126:955–968. [PubMed: 16959574]
46. Huang J, et al. Pivotal role for glycogen synthase kinase-3 in hematopoietic stem cell homeostasis in mice. *J Clin Invest*. 2009; 119:3519–3529. [PubMed: 19959876]
47. Chong ZZ, et al. Oxidative stress in the brain: Novel cellular targets that govern survival during neurodegenerative disease. *Prog Neurobiol*. 2005; 75:207–246. [PubMed: 15882775]
48. Zengi A, et al. Increased oxidative DNA damage in lean normoglycemic offspring of type 2 diabetic patients. *Exp Clin Endocrinol Diabetes*. 2011; 119:467–471. [PubMed: 21472659]
49. Vendelbo MH, Nair KS. Mitochondrial longevity pathways. *Biochim Biophys Acta*. 2011; 1813:634–644. [PubMed: 21295080]
50. Tupe RS, et al. Dietary nicotinic acid supplementation improves hepatic zinc uptake and offers hepatoprotection against oxidative damage. *Br J Nutr*. 2011 Jan 25; 1–9. [PubMed: 21262064]
51. Maiese K, et al. Oxidative stress: Biomarkers and novel therapeutic pathways. *Exp Gerontol*. 2010; 45:217–234. [PubMed: 20064603]
52. Maiese K, et al. Targeting disease through novel pathways of apoptosis and autophagy. Expert opinion on therapeutic targets. 2012 (in press).
53. Louneva N, et al. Caspase-3 is enriched in postsynaptic densities and increased in Alzheimer's disease. *Am J Pathol*. 2008; 173:1488–1495. [PubMed: 18818379]
54. Tatton NA. Increased caspase 3 and Bax immunoreactivity accompany nuclear GAPDH translocation and neuronal apoptosis in Parkinson's disease. *Exp Neurol*. 2000; 166:29–43. [PubMed: 11031081]
55. Canu N, et al. Role of the autophagic-lysosomal system on low potassium-induced apoptosis in cultured cerebellar granule cells. *J Neurochem*. 2005; 92:1228–1242. [PubMed: 15715672]
56. Qin AP, et al. Autophagy was activated in injured astrocytes and mildly decreased cell survival following glucose and oxygen deprivation and focal cerebral ischemia. *Autophagy*. 2010; 6:738–753. [PubMed: 20574158]

57. Wang JY, et al. Severe global cerebral ischemia-induced programmed necrosis of hippocampal CA1 neurons in rat is prevented by 3-methyladenine: a widely used inhibitor of autophagy. *J Neuropathol Exp Neurol.* 2011; 70:314–322. [PubMed: 21412169]
58. Baba H, et al. Autophagy-mediated stress response in motor neuron after transient ischemia in rabbits. *J Vasc Surg.* 2009; 50:381–387. [PubMed: 19631873]
59. Spencer B, et al. Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in alpha-synuclein models of Parkinson's and Lewy body diseases. *J Neurosci.* 2009; 29:13578–13588. [PubMed: 19864570]
60. Spilman P, et al. Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease. *PLoS One.* 2010; 5:e9979. [PubMed: 20376313]
61. Jeong JK, et al. Autophagy induced by resveratrol prevents human prion protein-mediated neurotoxicity. *Neurosci Res.* 2012; 73:99–105. [PubMed: 22465415]
62. Chen L, et al. Hydrogen peroxide inhibits mTOR signaling by activation of AMPKalpha leading to apoptosis of neuronal cells. *Lab Invest.* 2010; 90:762–773. [PubMed: 20142804]
63. Wu X, et al. Insulin promotes rat retinal neuronal cell survival in a p70S6K-dependent manner. *J Biol Chem.* 2004; 279:9167–9175. [PubMed: 14660591]
64. Shang YC, et al. Prevention of beta-amyloid degeneration of microglia by erythropoietin depends on Wnt1, the PI 3-K/mTOR pathway, Bad, and Bcl-xL. *Aging (Albany NY).* 2012; 4:187–201. [PubMed: 22388478]
65. Chong ZZ, et al. The pro-survival pathways of mTOR and protein kinase B target glycogen synthase kinase-3beta and nuclear factor-kappaB to foster endogenous microglial cell protection. *Int J Mol Med.* 2007; 19:263–272. [PubMed: 17203200]
66. Pastor MD, et al. mTOR/S6 kinase pathway contributes to astrocyte survival during ischemia. *J Biol Chem.* 2009; 284:22067–22078. [PubMed: 19535330]
67. Choi KC, et al. A novel mTOR activating protein protects dopamine neurons against oxidative stress by repressing autophagy related cell death. *J Neurochem.* 2010; 112:366–376. [PubMed: 19878437]
68. Chong ZZ, et al. PRAS40 Is an Integral Regulatory Component of Erythropoietin mTOR Signaling and Cytoprotection. *PLoS ONE.* 2012; 7:e45456. [PubMed: 23029019]
69. Nascimento EB, et al. Phosphorylation of PRAS40 on Thr246 by PKB/AKT facilitates efficient phosphorylation of Ser183 by mTORC1. *Cell Signal.* 2010; 22:961–967. [PubMed: 20138985]
70. Carayol N, et al. Critical roles for mTORC2- and rapamycin-insensitive mTORC1-complexes in growth and survival of BCR-ABL-expressing leukemic cells. *Proc Natl Acad Sci U S A.* 2010; 107:12469–12474. [PubMed: 20616057]
71. Luo S, Rubinsztein DC. Apoptosis blocks Beclin 1-dependent autophagosome synthesis: an effect rescued by Bcl-xL. *Cell Death Differ.* 2010; 17:268–277. [PubMed: 19713971]
72. Wang S, et al. WISP1 (CCN4) autoregulates its expression and nuclear trafficking of beta-catenin during oxidant stress with limited effects upon neuronal autophagy. *Curr Neurovasc Res.* 2012; 9:89–99.
73. Le XF, et al. Dasatinib induces autophagic cell death in human ovarian cancer. *Cancer.* 2010
74. Salminen A, et al. AMP-activated protein kinase: a potential player in Alzheimer's disease. *J Neurochem.* 2011; 118:460–474. [PubMed: 21623793]
75. Yu L, et al. Termination of autophagy and reformation of lysosomes regulated by mTOR. *Nature.* 2010; 465:942–946. [PubMed: 20526321]
76. Rong Y, et al. Spinster is required for autophagic lysosome reformation and mTOR reactivation following starvation. *Proc Natl Acad Sci U S A.* 2011; 108:7826–7831. [PubMed: 21518918]
77. Jung CH, et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell.* 2009; 20:1992–2003. [PubMed: 19225151]
78. Hosokawa N, et al. Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy.* 2009; 5:973–979. [PubMed: 19597335]
79. Slipczuk L, et al. BDNF activates mTOR to regulate GluR1 expression required for memory formation. *PLoS One.* 2009; 4:e6007. [PubMed: 19547753]

80. Griffin RJ, et al. Activation of Akt/PKB, increased phosphorylation of Akt substrates and loss and altered distribution of Akt and PTEN are features of Alzheimer's disease pathology. *J Neurochem.* 2005; 93:105–117. [PubMed: 15773910]
81. An WL, et al. Up-regulation of phosphorylated/activated p70 S6 kinase and its relationship to neurofibrillary pathology in Alzheimer's disease. *Am J Pathol.* 2003; 163:591–607. [PubMed: 12875979]
82. Paccalin M, et al. Activated mTOR and PKR kinases in lymphocytes correlate with memory and cognitive decline in Alzheimer's disease. *Dement Geriatr Cogn Disord.* 2006; 22:320–326. [PubMed: 16954686]
83. Ma T, et al. Dysregulation of the mTOR pathway mediates impairment of synaptic plasticity in a mouse model of Alzheimer's disease. *PLoS One.* 2010; 5
84. Lafay-Chebassier C, et al. mTOR/p70S6k signalling alteration by Abeta exposure as well as in APP-PS1 transgenic models and in patients with Alzheimer's disease. *J Neurochem.* 2005; 94:215–225. [PubMed: 15953364]
85. Chano T, et al. RB1CC1 insufficiency causes neuronal atrophy through mTOR signaling alteration and involved in the pathology of Alzheimer's diseases. *Brain Res.* 2007; 1168:97–105. [PubMed: 17706618]
86. Imai Y, et al. Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in *Drosophila*. *Embo J.* 2008; 27:2432–2443. [PubMed: 18701920]
87. Tain LS, et al. Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss. *Nat Neurosci.* 2009; 12:1129–1135. [PubMed: 19684592]
88. Malagelada C, et al. RTP801 is elevated in Parkinson brain substantia nigral neurons and mediates death in cellular models of Parkinson's disease by a mechanism involving mammalian target of rapamycin inactivation. *J Neurosci.* 2006; 26:9996–10005. [PubMed: 17005863]
89. Crews L, et al. Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of alpha-synucleinopathy. *PLoS One.* 2010; 5:e9313. [PubMed: 20174468]
90. Santini E, et al. Inhibition of mTOR signaling in Parkinson's disease prevents L-DOPA-induced dyskinesia. *Science signaling.* 2009; 2:ra36. [PubMed: 19622833]
91. Berger Z, et al. Rapamycin alleviates toxicity of different aggregate-prone proteins. *Hum Mol Genet.* 2006; 15:433–442. [PubMed: 16368705]
92. Ravikumar B, et al. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat Genet.* 2004; 36:585–595. [PubMed: 15146184]
93. Floto RA, et al. Small molecule enhancers of rapamycin-induced TOR inhibition promote autophagy, reduce toxicity in Huntington's disease models and enhance killing of mycobacteria by macrophages. *Autophagy.* 2007; 3:620–622. [PubMed: 17786022]
94. Roscic A, et al. Induction of autophagy with catalytic mTOR inhibitors reduces huntingtin aggregates in a neuronal cell model. *J Neurochem.* 2011; 119:398–407. [PubMed: 21854390]
95. Fox JH, et al. The mTOR kinase inhibitor Everolimus decreases S6 kinase phosphorylation but fails to reduce mutant huntingtin levels in brain and is not neuroprotective in the R6/2 mouse model of Huntington's disease. *Molecular neurodegeneration.* 2010; 5:26. [PubMed: 20569486]
96. Hyrskyluoto A, et al. GADD34 mediates cytoprotective autophagy in mutant huntingtin expressing cells via the mTOR pathway. *Exp Cell Res.* 2012; 318:33–42. [PubMed: 21925170]
97. Holmes GL, Stafstrom CE. Tuberous sclerosis complex and epilepsy: recent developments and future challenges. *Epilepsia.* 2007; 48:617–630. [PubMed: 17386056]
98. Jozwiak J, et al. Fibroblasts from normal skin of a tuberous sclerosis patient show upregulation of mTOR pathway. *The American Journal of dermatopathology.* 2009; 31:68–70. [PubMed: 19155728]
99. Waltereit R, et al. Enhanced episodic-like memory and kindling epilepsy in a rat model of tuberous sclerosis. *J Neurochem.* 2006; 96:407–413. [PubMed: 16300636]
100. Zeng LH, et al. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol.* 2008; 63:444–453. [PubMed: 18389497]

101. Buckmaster PS, et al. Inhibition of the mammalian target of rapamycin signaling pathway suppresses dentate granule cell axon sprouting in a rodent model of temporal lobe epilepsy. *J Neurosci.* 2009; 29:8259–8269. [PubMed: 19553465]
102. Zeng LH, et al. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J Neurosci.* 2009; 29:6964–6972. [PubMed: 19474323]
103. Curran MP. Everolimus: in patients with subependymal giant cell astrocytoma associated with tuberous sclerosis complex. *Paediatr Drugs.* 2012; 14:51–60. [PubMed: 22136276]
104. Krueger DA, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med.* 2010; 363:1801–1811. [PubMed: 21047224]
105. Pavel ME, et al. Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. *Lancet.* 2011; 378:2005–2012. [PubMed: 22119496]
106. Jin N, et al. Dual inhibition of mitogen-activated protein kinase kinase and mammalian target of rapamycin in differentiated and anaplastic thyroid cancer. *J Clin Endocrinol Metab.* 2009; 94:4107–4112. [PubMed: 19723757]
107. Gulhati P, et al. Sorafenib enhances the therapeutic efficacy of rapamycin in colorectal cancers harboring oncogenic KRAS and PIK3CA. *Carcinogenesis.* 2012
108. Mi R, et al. Efficacy of combined inhibition of mTOR and ERK/MAPK pathways in treating a tuberous sclerosis complex cell model. *Journal of genetics and genomics = Yi chuan xue bao.* 2009; 36:355–361. [PubMed: 19539245]
109. Fokas E, et al. NVP-BEZ235 and NVP-BGT226, dual phosphatidylinositol 3-kinase/Mammalian target of rapamycin inhibitors, enhance tumor and endothelial cell radiosensitivity. *Radiat Oncol.* 2012; 7:48. [PubMed: 22452803]
110. Barrett D, et al. Targeting the PI3K/AKT/mTOR Signaling Axis in Children with Hematologic Malignancies. *Paediatr Drugs.* 2012
111. Grzybowska-Izydorzyc O, Smolewski P. mTOR kinase inhibitors as a treatment strategy in hematological malignancies. *Future Med Chem.* 2012; 4:487–504. [PubMed: 22416776]
112. Majumder S, et al. Inducing autophagy by rapamycin before, but not after, the formation of plaques and tangles ameliorates cognitive deficits. *PLoS ONE.* 2011; 6:e25416. [PubMed: 21980451]

Box 1. Nomenclature

- !! Rapamycin: A metabolite isolated from the bacterial strain *Streptomyces hygroscopicus* found in a soil sample from Rapa Nui Island (Easter Island) in the South Pacific in 1970s. The metabolite was found to have the structure of a macrocyclic lactone and identified as macrolide antibiotic, which then was designated as rapamycin in honor of the location of the original discovery. The alternate name of rapamycin is sirolimus.
- !! mTOR (mammalian target of rapamycin): The investigation of the function and mechanism of action of rapamycin led to the discovery of the yeast target protein TOR. TOR was isolated in *Saccharomyces cerevisiae* with the generation of rapamycin-resistant TOR mutants that resulted in the identification of proteins participating in rapamycin toxicity. In yeast, the genes *TOR1* and *TOR2* encode two protein isoforms Tor1 and Tor2. In mammals, a single gene *FRAP1* encodes mTOR and is known as the mammalian target of rapamycin and the mechanistic target of rapamycin. mTOR also is termed FRAP (FK506-binding protein 12 –rapamycin complex-associated protein) since rapamycin inhibits mTOR by binding to immunophilin FK-506-binding protein 12 (FKBP12) that attaches to the C-terminal FKBP12 -rapamycin-binding domain (FRB) of mTOR.

Box 2. Outstanding questions

- !! What are the signal transduction pathways linked to mTOR that determine the stem cell fate and lineage commitment? mTOR signaling is necessary for stem cell development, proliferation, quiescence, and homeostasis that is intimately tied to growth factors, PI 3-K, forkhead transcription factors, and Wnt proteins. However, chronic or long-term activity of mTOR can lead to premature neuronal stem cell differentiation and impaired maturation.
- !! What are the underlying cellular conditions that determine whether mTOR signaling will be clinically beneficial or lead to disability for specific neurodegenerative disorders? Activating mTOR signaling pathways and inhibiting autophagy may be critical for the treating neurodegenerative disorders, such as Parkinson's disease and the protection of dopaminergic neurons. However, some studies of Parkinson's disease and Huntington's disease suggest that inhibiting mTOR with the activation of autophagy is required to clear toxic cellular aggregate-prone proteins.
- !! How can mTOR signaling be targeted to maximize efficacy but eliminate biological dysfunction? mTOR activation may protect against the loss of dopaminergic neurons during Parkinson's disease, but cross reactivity of mTOR with dopamine D1 receptors may lead to dyskinesias. How can mTOR signaling be targeted to maximize efficacy but eliminate biological dysfunction?
- !! What are the signal transduction pathways that determine resistance to drugs that inhibit mTOR signaling? Clinical trials demonstrate either a limited response or the ability to treat only a subpopulation of patients for agents that inhibit mTOR signaling.
- !! What components of the PI 3-K, Akt, mTOR axis should be targeted with new therapies to potentiate high clinical efficacy and limit feedback pathways that activate PI 3-K, Akt, and RAS-MAPK signaling, which can limit clinical efficacy?
- !! Can mTOR signaling mimetics be developed to specifically target desired mTOR signaling pathways and exclude the development of clinical toxicity? Current agents approved for clinical treatment that block mTOR signaling have multiple side effects that include immunosuppression and secondary infection onset.

Figure 1. Signaling pathways of the mammalian target of rapamycin (mTOR)

mTOR signaling relies upon the formation of protein complexes, either mTOR Complex 1 (mTORC1) or mTOR Complex 2 (mTORC2). mTORC1 is composed of the regulatory-associated protein of mTOR (Raptor) protein, the proline rich Akt substrate 40 kDa (PRAS40), the DEP domain-containing mTOR interacting protein (Deptor), and the mammalian lethal with Sec13 protein 8 (mLST8). mTORC2 is similar to mTORC1 because it is also contains mTOR, mLST8, and Deptor, but contains the protein Rictor as a component rather than Raptor. mTORC2 also associates with the mammalian stress-activated protein kinase interacting protein (mSIN1) and protein observed with Rictor-1 (Protor-1). The signaling pathways of mTOR have many regulators and stimuli. Growth

factors or cytokines, such as erythropoietin (EPO) can stimulate phosphoinositide-3-kinase (PI 3-K) and activate Akt. Once active, Akt can phosphorylate tuberous sclerosis complex-2 (TSC2), resulting in the disruption of its interaction with TSC1, the loss of its ability to convert the Ras homologue enriched in brain active form (Rheb-GTP) to the inactive GDP-bound form (Rheb-GDP), and result in the subsequent activation of mTORC1. Akt also can phosphorylate proline rich Akt substrate 40 kDa (PRAS40) leading to its dissociation from the regulatory associated protein of mTOR (Raptor). Nutrient deficiency stimulates AMP activated protein kinase (AMPK) that can phosphorylate TSC2. AMPK also leads to the expression of transcriptional regulation of DNA damage response 1 (REDD1/RTP801), which releases TSC2 from binding to protein 14-3-3 and inhibits mTORC1 activity. The glycoprotein Wnt phosphorylates and inhibits glycogen synthase-3 β (GSK-3 β). Without this inhibition, GSK-3 β can phosphorylate TSC2 to inhibit mTORC1. During activation, mTORC1 phosphorylates its two major downstream targets, p70 ribosome S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding protein 1 (4EBP1), to promote protein synthesis and cell survival. In contrast to the inhibition of mTORC1, TSC1/TSC2 can positively regulate mTORC2 by associating with rapamycin insensitive companion of mTOR (Rictor). Activation of mTORC2 promotes protein kinase C alpha (PKC α) activation to regulate cytoskeleton organization, activates Rho-GTPase to control cell to cell contact, and phosphorylates Akt to promote cell survival.

Table 1

Selected phosphorylation sites on mTOR and Raptor

Target	Phosphorylating Kinase or Pathway
mTOR	
Serine ¹²⁶¹	PI3K pathway
Serine ²¹⁵⁹	ND ^a
Serine ²⁴⁴⁸	AKT; p70S6K
Serine ²⁴⁸¹	mTOR
Threonine ²¹⁶⁴	ND ^a
Threonine ²⁴⁴⁶	AMPK, p70S6K
Raptor	
Serine ⁶⁹⁶	Regulated by Rheb
Serine ⁸⁵⁵	
Serine ⁸⁵⁹	
Serine ⁸⁶³	
Serine ⁸⁷⁷	
Threonine ⁷⁰⁶	

^aNot Determined

Table 2

The components of mTORC1 and mTORC2

mTORC1	mTORC2	Functions
mTOR	mTOR	Contains the kinase activity of mTORC1 and mTORC2
Raptor		Promotes the binding of 4EBP1 and p70S6K to mTORC1 for phosphorylation, and likely interacts with other proteins
	Rictor	Promotes the assembly and the activity of mTORC2; associates with and stabilizes mSIN1
PRAS40		Associates with Raptor and negatively regulates the activity of mTORC1
	mSIN1	Promotes the assembly and the activity of mTORC2 to allow for Akt activation
mSLT8	mSLT8	Promotes the activity of mTORC1 to phosphorylate p70S6K and 4EBP1, stabilizes the Rictor-mTOR association, controls insulin signaling through FoxO3, and is necessary for the phosphorylation of Akt and PKC α .
Deptor	Deptor	Negative regulator of both mTORC1 and mTORC2
	Protor-1	Functions as a Rictor binding subunit and is necessary for phosphorylation of specific proteins such as SGK1

Akt: protein kinase B; 4EBP1: eukaryotic initiation factor 4E-binding protein 1; Deptor: DEP domain-containing mTOR-interacting protein; mSLT8: mammalian lethal with Sec13 protein 8; mSIN1: mammalian stress-activated protein kinase interacting protein; mTOR: mammalian target of rapamycin; p70S6K: p70 ribosomal S6 kinase; PRAS40: proline-rich Akt substrate 40 kDa; Protor-1: protein observed with Rictor-1; Raptor: regulatory-associated protein of mTOR; Rictor: rapamycin-insensitive companion of mTOR; SGK1: serum and glucocorticoid induced protein kinase 1.

Table 3**mTOR signaling in neurodegenerative disease**

Diseases or targets	Functions
Stem cells	Activated mTOR promotes the development, proliferation, and quiescence of embryonic stem cells and neuronal stem cells. Stem cell proliferation and homeostasis with mTOR also tied to growth factors, PI 3-K, and Wnt signaling
Apoptosis and Autophagy	mTOR controls p70S6K, BAD, Akt, PRAS40, FoxO3a, Atg13, ULK1, and FIP200 to prevent apoptotic and autophagic cell death.
Alzheimer's disease	mTOR may have dual roles. In some experimental models, mTOR and p70S6K promote tau phosphorylation in neurons with inhibition of mTOR leading to improved memory. Other work supports a role for mTOR activation that can prevent β -amyloid toxicity and block neuronal atrophy.
Parkinson's disease	Activation of mTOR and inhibition of autophagy protects dopaminergic neurons, but may also potentiate dyskinesia.
Huntington's disease	Pathways that inhibit mTOR signaling and promote autophagy can attenuate huntingtin accumulation and cell death in models of Huntington's disease.
Tuberous sclerosis and Epilepsy	Blockade of mTOR signaling in clinical studies can limit giant-cell astrocytoma cell growth and decrease seizure frequency in the majority of patients examined, but treatment related immunosuppression and infections can occur.