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Therapeutic role of mammalian target of rapamycin (mTOR) inhibition in preventing epileptogenesis

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Abstract

Traditionally, medical therapy for epilepsy has aimed to suppress seizure activity, but has been unable to alter the progression of the underlying disease. Recent advances in our understanding of mechanisms of epileptogenesis open the door for the development of new therapies which prevent the pathogenic changes in the brain that predispose to spontaneous seizures. In particular, the mammalian target of rapamycin (mTOR) signaling pathway has recently garnered interest as an important regulator of cellular changes involved in epileptogenesis, and mTOR inhibitors have generated excitement as potential antiepileptogenic agents. mTOR hyperactivation occurs in tuberous sclerosis complex (TSC), a common genetic cause of epilepsy, as a result of genetic mutations in upstream regulatory molecules. mTOR inhibition prevents epilepsy and brain pathology in animal models of TSC. mTOR dysregulation has also been demonstrated in a variety of other genetic and acquired epilepsies, including brain tumors, focal cortical dysplasias, and animal models of brain injury due to status epilepticus or trauma. Indeed, mTOR inhibitors appear to possess antiepileptogenic properties in animal models of acquired epilepsy as well. Thus, mTOR dysregulation may represent a final common pathway in epilepsies of various causes. Therefore, mTOR inhibition is an exciting potential antiepileptogenic strategy with broad applications for epilepsy and could be involved in a number of treatment modalities, including the ketogenic diet. Further research is necessary to determine the clinical utility of rapamycin and other mTOR inhibitors for antiepileptogenesis, and to devise new therapeutic targets by further elucidating the signaling molecules involved in epileptogenesis.

Keywords

epilepsy; seizure; rapamycin; kainate; tuberous sclerosis complex; ketogenic diet

Introduction

Epilepsy is a common neurologic condition, affecting 1% of the world's population. Epilepsy is not a single disease, but rather represents the common symptomatic manifestation of numerous brain abnormalities, including genetic syndromes, traumatic brain injury, central nervous system infection, and structural brain lesions such as strokes or brain tumors. Despite the increasing number of anticonvulsant medications developed in

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recent years, approximately one third of epilepsy patients are refractory to available medications [59]. Medications used to treat epilepsy act via a variety of mechanisms which suppress the abnormal synchronous firing of neuronal populations. However, none of the currently-available "antiepileptic" medications have been shown to alter the progression or prevent the development of the disease [105]. Future treatments with "antiepileptogenic" or disease-modifying properties aimed at preventing the development and progression of epilepsy would be extremely beneficial, not only in improving seizure control and reducing refractory epilepsy, but in preventing epilepsy in patients at high risk.

Progress has been made in recent years in identifying potential mechanisms of epileptogenesis, namely the pathophysiologic changes that occur in nervous system tissue that predispose to spontaneous seizures. These changes include molecular, cellular, or neuronal network alterations that occur in response to a variety of genetic or environmental insults, and that predispose to seizures via either increased excitation or decreased inhibition. After an insult, there is typically a latent period in which these changes take place, before the onset of spontaneous seizures. This latent period provides an opportunity to study the alterations that occur, and potentially a window to prevent epileptogenesis. In this review, we will analyze the recent evidence implicating altered mTOR signaling in epileptogenesis and the potential antiepileptogenic role of mTOR inhibitors. We will discuss the classic mTOR inhibitor rapamycin, as well as alternative strategies for mTOR inhibition.

mTOR: a master switch in the maintenance of cellular homeostasis

mTOR is a highly-conserved serine-threonine protein kinase that integrates various energy, nutrient, and growth factor signals to regulate cell growth, proliferation, and survival. Through upstream signaling molecules, mTOR senses the presence of growth factors, including insulin, and nutrients, including amino acids and glucose, to promote protein translation and consequent cell growth and differentiation (Fig. 1) [89, 91, 110]. The best-characterized upstream signaling pathway involves the activation of PI3K/Akt by growth factors and nutrients. These phosphorylate and inhibit the hamartin/tuberin complex, an important inhibitor of mTOR via the small GTPase Rheb, thus leading to increased mTOR activity [47, 71, 92, 98, 104, 120]. Conversely, under conditions of energy deprivation, AMPK phosphorylates and activates tuberin to inhibit mTOR activity [7, 46, 48, 55]. This puts the brakes on protein synthesis and cell growth when energy and nutrients are not available, and protects cells from undergoing apoptosis in the setting of energy starvation. In addition to responding to energy and nutrient signals, mTOR is also involved in cellular responses to environmental stressors. For example, hypoxia activates REDD1 and REDD2, which serve as upstream regulators to inhibit mTOR activity. Animals with mutations in *TSC2*, the gene encoding tuberin, are more vulnerable to oxidative and endoplasmic reticulum stress, presumably due to an inability to inhibit mTOR in these settings [10, 29].

mTOR exerts its control over protein synthesis and cell growth via numerous downstream effectors. The first component of the signaling pathway identified was p70 ribosomal S6 kinase1 (S6K1), which is phosphorylated and activated by mTOR [19, 36]. Activated S6K1 phosphorylates ribosomal protein S6, which promotes production of ribosomal proteins and elongation factors to increase translation [13, 19, 36]. Additionally, mTOR phosphorylates the eukaryotic initiation factor 4E binding protein-1 (4E-BP1), a protein that binds to and inhibits activity of the eukaryotic initiation factor eIF4E. When 4E-BP1 is phosphorylated by mTOR, it dissociates from eIF4E, allowing eIF4E to complex with other initiation factors to initiate mRNA translation. Thus, activation of mTOR and phosphorylation of its targets S6K1 and 4E-BP1 stimulates translation of mRNA for ribosomal proteins and elongation factors to contribute to cell growth [13, 36]. mTOR has also been demonstrated to stimulate protein synthesis by inducing nucleophosmin-mediated export of ribosomal subunits from

the nucleus to the cytoplasm [83, 90]. In addition, there is some evidence that mTOR may exert actions on cell cycle progression and proliferation via effects on the cyclin-dependent kinase inhibitor p27 and the cyclin D1 regulator beta-catenin [26, 52, 54, 69, 96]. Finally, mTOR increases binding of microtubules to a protein called CLIP-170, an interaction that promotes microtubule growth and thus contributes to the role of mTOR in cell growth [101].

In addition to playing an essential role in cell growth and proliferation, mTOR is also involved in other cellular processes related to cell survival and death. Specifically, mTOR inhibits macroautophagy, which in different situations can either promote cell survival or cell death [3, 20, 95]. Macroautophagy, hereafter referred to as autophagy, is the process by which intracellular proteins and organelles are degraded and recycled, typically to promote survival in the setting of energy deprivation. mTOR, acting as a sensor of intracellular amino acids and of cellular energy status, inhibits autophagy when nutrients and energy are available. Starvation results in decreased levels of intracellular amino acids, which decreases the activity of mTOR, and consequently relieves the inhibition of autophagy [20]. Autophagy in the absence of apoptotic machinery prevents cell death, and thus serves as a survival mechanism. However, in the presence of apoptotic inducers, autophagy may assist in cell death. mTOR also appears to have differential effects on apoptosis independent of autophagy. S6K can phosphorylate and inactivate the pro-apototic molecule BAD to inhibit apoptosis [16]. On the other hand, mTOR can induce apoptosis via activation of p53 and inhibition of the antiapoptotic protein Bcl-2 in response to cellular damage [2, 16]. Thus, mTOR acts as a critical switch that, depending on the context, can promote either cell survival or cell death.

While the most extensively studied roles of mTOR relate to cell growth, proliferation, and survival or death, mTOR also appears to be important in numerous other cellular functions that may play a role in epileptogenesis as well. For example, mTOR induces IL-2 mediated T cell proliferation, which may play a role in inflammatory reactions thought to contribute to injury and epileptogenesis [109]. In neuronal development, mTOR is critical for axon guidance in response to extracellular cues, and in dendrite development and dendritic spine morphogenesis [14, 51, 58, 80, 101, 103]. In the adult brain, this translates to a role of mTOR in synaptic plasticity, as mTOR has been demonstrated to regulate ion channel and neurotransmitter receptor expression [84, 112]. Since putative learning mechanisms, such as long term potentiation and long term depression, rely on protein synthesis for sustained changes in synaptic strength, it is not surprising that they require mTOR activity [40, 50, 102]. Given the role of mTOR in neuronal development and plasticity, it seems plausible that mTOR may be responsible for the aberrant axonal sprouting and neurogenesis implicated in epileptogenesis.

mTOR dysregulation in disease states

Given the critical roles of mTOR in regulating cell growth, proliferation, survival, and death, it is not surprising that dysregulation of mTOR has been found in a variety of disease states, particularly in cancers, where tumorigenesis is related to loss of control of these functions. Amplification or overexpression of protooncogenes upstream of mTOR, including PI3K and Akt, have indeed been demonstrated in a variety of human cancers. Loss of function of tumor suppressors involved in the pathway, including phosphatase and tensin homolog on chromosome 10 (PTEN) and TSC1/2, have been linked to tumor predisposition syndromes [reviewed in 25, 41]. Alterations in mTOR activity have also been identified in patients with neurodegenerative diseases including Alzheimer’s disease, Parkinson’s disease, and Huntington’s disease [1, 70, 85, 101, 110]. These findings have led to the investigation of mTOR inhibition as a therapeutic strategy for both cancer treatment and neuroprotection.

mTOR inhibition

Rapamycin is a macrolide discovered in 1970 on Easter Island, a.k.a. *Rapa Nui*. It was initially developed as an antifungal agent, but fell out of favor when it was found to have potent immunosuppressive activity. In subsequent decades, the discovery of mTOR led to the elucidation of rapamycin's mechanism of action as an mTOR inhibitor (Fig. 2C), thereby initiating a resurgence of interest in rapamycin. It was first approved by the US FDA in 1997 as an anti-rejection agent for kidney transplant patients, and since that time rapamycin and its analogs have been used for a number of other clinical applications. The immunosuppressive effects have led to a potential role of rapamycin in treatment of autoimmune diseases, and its antiproliferative properties led to approval for preventing restenosis in patients with coronary artery stents [109, 110]. Oncologists have sought to take advantage of rapamycin's antiproliferative and growth-limiting properties to treat cancer, and clinical trials have demonstrated efficacy in renal cell carcinoma and other human cancers [25]. In the central nervous system, mTOR inhibitors appear to have neuroprotective effects, in models of Huntington's disease, Alzheimer's disease, Parkinson's disease, and hypoxic-ischemic injury [15, 81, 110]. Although the mechanism of rapamycin's neuroprotective effects are unclear, it is tempting to speculate that induction of autophagy helps prevent the accumulation of abnormal proteins seen in neurodegenerative disorders, and aids in cell survival in the setting of hypoxic injury. With regard to epilepsy, accumulating evidence suggests a role of mTOR in epileptogenesis. The remainder of this review will analyze the evidence for mTOR dysregulation in epilepsy and the therapeutic potential of mTOR inhibitors as antiepileptogenic agents.

Tuberous Sclerosis Complex as a model of mTOR dysregulation in epilepsy

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder affecting 1 in 6000 people worldwide that results in hamartoma formation in the brain, heart, kidneys, eyes, and skin [23]. The pathognomonic brain lesion of TSC is the tuber, a cortical lesion consisting of dysplastic neurons, astrocytes, and large, poorly-differentiated cells, called giant cells [113]. Other central nervous system lesions include subependymal nodules, which are hamartomas protruding from the walls of the ventricles, and subependymal giant cell astrocytomas (SEGAs), which are benign tumors arising from subependymal nodules that can cause obstructive hydrocephalus and death. Patients with TSC frequently come to the attention of neurologists because of a high incidence of epilepsy, mental retardation, and autism. Upwards of 90% of patients develop epilepsy, in some series, and 20–30% develop infantile spasms, a particularly devastating childhood epilepsy syndrome [18, 24, 33, 43]. Epilepsy due to TSC is especially severe, with a tendency to progress over time, and has a high rate of medical intractability [18, 43, 97].

TSC is perhaps the best model for studying mTOR deregulation, particularly with regard to its role in epileptogenesis. The last decade has seen significant progress in our understanding of the pathophysiology of TSC due to the discoveries in the 1990's. These demonstrated that TSC is caused by mutations on either chromosome 9q34 (*TSC1* gene) or 16p13.3 (*TSC2* gene), and that their respective gene products hamartin and tuberin are upstream regulators of mTOR activity [60]. As described above, hamartin and tuberin form a complex containing a GTPase activate protein (GAP) domain that inactivates the small GTPase Rheb, thereby turning off mTOR activity [32, 39, 46, 71, 92, 98, 104, 120]. Mutations in the *TSC1* or *TSC2* genes result in loss of this inhibitory mechanism, and therefore constitutive activation of mTOR (Fig. 2A). Overactive mTOR can explain many of the pathologic findings of TSC, including giant cells and tumors or hamartomas, due to loss of control over cell size, proliferation, and survival or death. Consequently, one might predict that mTOR

inhibition could reverse or prevent the pathophysiologic manifestations of TSC. Indeed, mTOR inhibitors have been demonstrated to slow tumor growth in murine models of TSC [62]. mTOR inhibitors have also shown efficacy in treating kidney and lung tumors as well as SEGAs in patients with TSC [6, 22, 38]. Based on results of these trials, the rapamycin analog everolimus has recently been granted FDA approval for treatment of SEGAs [57].

While the contribution of abnormal mTOR activity to tumorigenesis in TSC has been clearly demonstrated, mechanisms of epileptogenesis in this disease are less certain. It is well-established that seizures are associated with cortical tubers, although it is not clear whether tubers themselves are epileptogenic, whether tubers are irritative to surrounding tissue, or whether cellular and structural abnormalities in the perilesional area contribute [43, 68, 113]. In general, seizures can result from either increased excitation or decreased inhibition, and many of the histopathologic features of tubers may confer hyperexcitability to the tissue, including abnormalities in axonal projections, dendritic arbors, and glial proliferation [43]. Additionally, tubers show abnormalities in expression of neurotransmitter receptor subunits, including both decreases in inhibitory GABA receptors and increases in excitatory NMDA glutamate receptors [43]. All of these features that have been implicated in epileptogenesis in TSC may result from mTOR hyperactivation, given the evidence described above that mTOR plays a role in dendrite morphogenesis, axon outgrowth, cell proliferation, and even neurotransmitter receptor expression.

If mTOR is in fact involved in epileptogenesis in TSC, the next logical step is to assess whether mTOR inhibition is antiepileptogenic in TSC, and several studies have recently addressed this question [75, 118, 119]. Mice with conditional inactivation of the *Tsc1* gene in astrocytes ($Tsc1^{GFAP}$CKO) have abnormal glial proliferation, seizures beginning at about 4 weeks of age that increase in frequency over time, and premature death at around 3 months of age [34, 111]. The $Tsc1^{GFAP}$CKO mice have diffuse glial proliferation rather than discrete tubers, but they do have abnormal glial glutamate transport, similar to the astrocyte dysfunction and impaired glutamate transport seen in human TSC, which may result in increased extracellular glutamate levels [114, 115]. The mice also have decreased expression of inward-rectifier potassium channels which may contribute to hyperexcitability and epileptogenesis [49]. In this model, treatment with rapamycin prior to onset of seizures prevented the development of seizures, histopathologic abnormalities, and premature death [119]. Treatment with rapamycin after onset of seizures reduced seizure frequency and prolonged survival, but did not reverse the histopathologic abnormalities. Of note, if rapamycin treatment was stopped, the abnormal phenotype would then emerge. In this study, rapamycin also restored normal glutamate transport. In another study, using conditional *Tsc1* inactivation in post-mitotic neurons, early treatment with rapamycin dramatically improved the neurologic phenotype, including seizures, tremor, and premature death, observed in untreated mice [75]. These studies suggest that rapamycin is antiepileptogenic, and actually alters the underlying pathological process to prevent epilepsy in TSC models. This suggestion is supported by evidence that rapamycin itself does not alter neuronal excitability, so it is unlikely to suppress seizures via traditional anticonvulsant mechanisms [28, 86]. Another recent study demonstrated that mice with a heterozygous *Tsc2* mutation have impairment in hippocampal-dependent learning and memory, although they do not develop seizures or obvious neuropathology [31]. Rapamycin treatment in these mice reversed the learning deficits and abnormalities in long-term potentiation. This suggests that mTOR inhibition may not only be antiepileptogenic, but may also impact cognitive function in TSC.

Clinically, TSC patients represent an ideal population in which to study antiepileptogenesis, since the diagnosis is often made before the onset of epilepsy, due to family history, characteristic skin lesions, or in-utero/neonatal diagnosis of cardiac rhabdomyomas. Also,

the extremely high prevalence of epilepsy in TSC would make an antiepileptogenic effect easier to detect than in acquired epilepsies such as after traumatic brain injury or febrile status epilepticus, where the risk of later epilepsy is lower. Finally, since epilepsy in TSC tends to be progressive and refractory to medication, and intractable epilepsy is associated with poorer cognitive outcomes [43], patients with TSC stand to benefit greatly from antiepileptogenic agents that could slow or halt epilepsy progression. Anecdotal reports suggest that rapamycin may be beneficial in reducing seizure burden in patients with TSC [37, 78]. Furthermore, in a prospective trial, everolimus treatment was associated with a significant, clinically relevant reduction in seizure frequency in TSC patients with intractable epilepsy [57]. Future trials will be necessary to determine whether mTOR inhibition can truly alter the clinical course rather than simply suppressing seizures in TSC patients with epilepsy. However, the laboratory evidence from animal models is promising that mTOR inhibitors may be antiepileptogenic in TSC.

Beyond TSC: the role of mTOR in other models of epilepsy

Malformations of cortical development

TSC shares many features with other malformations of cortical development, including type IIb focal cortical dysplasia with balloon cells (FCDIIb) and low-grade gangliоglioma (GG). Clinically, FCDIIb and GG, like TSC, are associated with particularly severe epilepsy and high rates of intractability [21, 113]. Pathologically, FCDIIb are virtually indistinguishable from tubers in TSC, and contain large dysplastic cells called balloon cells that are morphologically similar to the giant cells of TSC. Balloon cells, like giant cells, show increased levels of phosphorylated ribosomal protein S6 (pS6), consistent with mTOR hyperactivation, although differential expression of pS6K and other gene products suggest some mechanistic differences between TSC and FCDIIb [4, 66, 76, 93]. The finding that FCDIIb have increased expression of PDK1 suggests that PI3K/Akt signaling is increased; however, the mechanism of this activation is unknown (Fig. 2A) [93]. FCDIIb also contain cytomegalic neurons which, like the balloon cells, show mTOR hyperactivation as well [66]. Similarly, gangliogliomas exhibit activation of the mTOR pathway as evidenced by increased expression of pS6K and pS6 [87]. Furthermore, *TSC1* polymorphisms are present in ~50% of FCDIIb, and *TSC1* or *TSC2* polymorphisms are present in ~75% of GG resected for intractable epilepsy, although whether the polymorphisms represent loss of function mutations has not been established [21, 113]. Taken together, these findings suggest that mTOR dysregulation may be a common feature of TSC, FCDIIb, and GG.

Given the evidence that mTOR dysregulation may occur in several malformations of cortical development, results from studies using animals with *Tsc1* or *Tsc2* mutations may have broader applicability to brain malformations outside of TSC. Additionally, mutations in other regulators of mTOR may be useful for studying epileptogenesis in cortical malformations. PTEN is a tumor suppressor upstream of mTOR that inhibits PI3K activity, and *PTEN* mutation results in mTOR hyperactivity (Fig. 1). Mutations in human *PTEN* have been associated with tumor and hamartoma syndromes, as well as a variety of neurologic manifestations including ataxia, seizures, macrocephaly, mental retardation, and Lhermitte-Duclos disease [61, 72]. A mouse model of *Pten* mutation in neurons has molecular, cellular, and behavioral features of cortical dysplasia including mTOR hyperactivation, hypertrophic neurons, and spontaneous seizures (Fig. 2A) [67]. In this model, short-term treatment with rapamycin suppressed the abnormal mTOR activity and cellular hypertrophy, reduced epileptiform activity on EEG, and nearly abolished electrographic seizures. In another conditional knockout of *Pten* in post-mitotic neurons of the cortex and hippocampus, rapamycin ameliorated the phenotype of macrocephaly, seizures, cellular hypertrophy, and behavioral features of autism including abnormal social behavior and anxiety [121]. Although the use of rapamycin in patients with epilepsy due to non-TSC

cortical malformation has not been reported, overall both the clinical and animal studies suggest that mTOR inhibitors may have a therapeutic role in treatment of epilepsy associated with several types of cortical malformations.

Acquired epilepsies

Acquired epilepsy after a brain injury, such as after trauma, hypoxia-ischemia, or status epilepticus, is another interesting model for studying epileptogenesis as there is often a latent period after the insult before the onset of seizures which provides a potential window for antiepileptogenic intervention. To date, no treatment strategy has been shown to prevent the development or progression of epilepsy after a cerebral insult [105]. However, there is increasing evidence that mTOR dysregulation is also a feature of acquired epilepsy, which raises the possibility that mTOR inhibitors may have clinical utility in acquired epilepsy as well.

Much of our understanding of epileptogenesis comes from animal models of temporal lobe epilepsy. In some of these models, animals are treated with chemoconvulsant agents, such as kainic acid (KA) or pilocarpine, that induce status epilepticus and excitotoxicity. The animals recover from status epilepticus, but several days to weeks later develop spontaneous recurrent seizures [63]. In these models, cellular and molecular changes observed after status epilepticus but before the onset of epilepsy include neuronal death, neurogenesis, mossy fiber sprouting, altered ion channel expression, synaptic reorganization, and gliosis [30]. Mossy fiber sprouting in the dentate gyrus correlates with increased frequency and amplitude of spontaneous excitatory post-synaptic currents, implying that aberrant sprouting confers hyperexcitability. Similar abnormalities have been demonstrated in patients with mesial temporal sclerosis undergoing epilepsy surgery, suggesting that these models replicate the features of human mesial temporal lobe epilepsy [65, 108]. Given that these changes fall under the functions of mTOR described above, it is plausible that mTOR hyperactivation may be responsible for these changes that occur during the latent period of epileptogenesis. Several studies have recently begun to investigate this possibility and have demonstrated mTOR hyperactivation after status epilepticus induced by KA [94, 117] or pilocarpine [11, 45]. Interestingly, mTOR is activated in the neocortex and hippocampus during status epilepticus induced by KA, returns to normal levels after 24 hours, and has a second elevation in the hippocampus, but not neocortex, that peaks during the latent period 5–7 days after status epilepticus [117]. Rapamycin treatment for 3 days prior to status epilepticus blocked both the early and late elevations in mTOR activity, and reduced mossy fiber sprouting, neuronal death, neurogenesis, and spontaneous recurrent seizures. Treatment with rapamycin 1 day after status epilepticus did not affect neuronal death or neurogenesis, but did block the second phase of mTOR hyperactivation and decreased mossy fiber sprouting and subsequent seizures. This suggests that rapamycin is in fact altering epileptogenesis rather than simply reducing the severity of KA-induced status epilepticus. Similarly, rapamycin was able to prevent mossy fiber sprouting after pilocarpine-induced status epilepticus, but like the TSC animal studies, continued treatment with rapamycin was necessary to prevent later development of mossy fiber sprouting [11]. Furthermore, rapamycin treatment suppressed late, spontaneous seizures in the pilocarpine model [45], although it is not clear whether early treatment actually prevents epileptogenesis in this model [12].

mTOR activation after KA-induced status epilepticus is likely triggered by glutamate. Status epilepticus results in widespread glutamate release, and glutamate NMDA receptor activation in turn can stimulate mTOR through the PI3K/Akt signaling cascade (Fig. 2B) [40, 44, 64, 100, 122]. Follow-up experiments by Zeng et al. showed that phosphorylation of Akt accompanies both the early and late mTOR activation after KA-induced status epilepticus, implicating PI3K signaling in epileptogenesis in this model [116]. Neuronal

death in the KA model is presumably due to overactivation of NMDA receptors causing calcium influx, which activates calcium-dependent neuronal death pathways. By inhibiting mTOR, rapamycin induces autophagy, which may enhance cell survival in the setting of increased metabolic demand during status epilepticus, and thus may explain the prevention of neuronal death, as well as the antiepileptogenic effects, with rapamycin pretreatment before KA [117].

Traumatic brain injury (TBI), like kainate-induced status epilepticus, induces abnormal axonal sprouting and synaptic reorganization with decreased synaptic density [17]. The hippocampus is particularly vulnerable to trauma and often shows impaired synaptic plasticity and long-term potentiation after injury. mTOR may play a role in these changes, as mTOR phosphorylation has been shown to be significantly increased in the hippocampus and cortex in a rat model of TBI [17]. The concurrent discovery that rapamycin treatment improves functional recovery after TBI in a mouse model further supports the hypothesis that mTOR hyperactivation may be responsible for the post-traumatic changes, and that these changes likely contribute to epileptogenesis rather than being compensatory, desirable responses [35]. However, further investigations are needed to test the antiepileptogenic potential of rapamycin for posttraumatic epilepsy following TBI.

Alternatives to rapamycin: therapeutic strategies for mTOR inhibition in epilepsy

mTOR inhibitors currently approved or being tested in clinical trials include rapamycin and its analogs. Although mTOR inhibitors are fairly well-tolerated, they do have significant side effects including immunosuppression, mucositis, and increases in cholesterol and triglycerides [110]. Additionally, it appears from animal studies that sustained treatment with rapamycin is necessary to prevent epileptogenesis in both TSC and acquired epilepsy models [11, 119]; therefore, the long-term effects of continuous rapamycin treatment need to be investigated further. Additionally, alternative approaches to inhibiting mTOR activity that might be better tolerated are worth investigating. A few intriguing studies have recently suggested that curcumin, a natural polyphenol product of the turmeric plant that possesses antioxidant, anti-inflammatory, and anticancer properties, may represent a new class of mTOR inhibitor which can suppress epileptogenesis in animal models of epilepsy (Fig. 2C) [5, 53].

The role of mTOR in integrating nutritional and energy signals to regulate numerous cellular functions also raises the question of whether dietary treatments for epilepsy may work in part by inhibiting mTOR activity. The ketogenic diet (KD) is a high-fat, adequate-protein, low-carbohydrate diet that is an effective treatment for epilepsy [42, 73, 79, 82]. Although the KD has been used for nearly 100 years, the mechanisms of its effects on epilepsy remain poorly-understood. Recent studies have suggested that it is physiologic adaptations to the KD, rather than ketone bodies or constituents of the diet itself, that are responsible for its anticonvulsant effects [8]. In addition to being anticonvulsant, there is some evidence that the KD is also neuroprotective and antiepileptogenic. In animal models, beta-hydroxybutyrate, one of the ketone bodies elevated by the KD, protects organotypic hippocampal cultures from metabolic and excitotoxic insults [88]. Furthermore, KD-fed animals are more resistant to metabolic stress [9]. With regard to epilepsy, patients often have long-lasting improvement in seizure control that persists after discontinuation of the diet [56]. In animal models, Muller-Schwarze et al. illustrated that the KD, administered to rats after KA-induced status epilepticus, prevented mossy fiber sprouting and the development of spontaneous recurrent seizures [77]. In a subsequent study, initiation of the diet 2 days after KA-induced status epilepticus inhibited mossy fiber sprouting and spontaneous recurrent seizures, while initiation 14 days after status epilepticus suppressed

seizures but did not alter sprouting [99]. These findings suggest that the KD not only suppresses seizures, but is also antiepileptogenic in the KA model, if administered during the latent period of epileptogenesis. mTOR inhibition is an appealing explanation for these findings, taking into account the nutrient and energy-sensing ability of mTOR, the above-described increased mTOR activity after KA-induced status epilepticus and the role of mTOR in axonal sprouting and other pathophysiologic changes associated with epileptogenesis. The KD reduces insulin levels [106], which would be expected to decrease PI3K/Akt signaling and inhibit mTOR activity (Fig. 2C). Indeed, pAkt and pS6 levels are decreased in the hippocampus and liver of rats fed a ketogenic diet for two weeks [74]. Furthermore, like rapamycin, the KD blocks the increased hippocampal mTOR activity seen 7 days after KA-induced status epilepticus, correlating with the latent phase of epileptogenesis [74]. Thus, the KD appears to inhibit mTOR activity, which may explain its apparent neuroprotective and antiepileptogenic effects, although these associations will require further confirmation.

Because mTOR regulates numerous cellular functions, mTOR inhibition results in changes to many downstream functions, increasing the likelihood of adverse effects along with the desired antiepileptogenic effects. Since mTOR activity is important for neuronal development and synaptic plasticity, mTOR inhibition may have adverse effects on learning and memory and may be particularly disadvantageous in the developing brain. mTOR inhibition has been shown to prevent consolidation of long-term memory [107], and rapamycin infusion into rat hippocampus leads to long-term impairment in the Morris water maze, a test of spatial memory [27]. Because of these concerns, future research should aim to delineate the specific downstream effectors of mTOR involved in epileptogenesis, and to target potential therapies to these downstream molecules. Overall, the evidence for mTOR dysregulation as a common pathway in epileptogenesis of diverse etiologies is quite convincing. The potential for mTOR inhibition as a therapeutic strategy for preventing epileptogenesis is therefore promising and may be applicable not only to TSC, but also to epilepsy associated with other cortical malformations and even acquired epilepsies due to various brain injuries.

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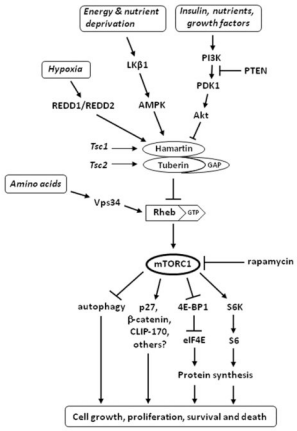

Fig. 1.

Schematic diagram of the mTOR signaling pathway. The rapamycin-sensitive mTOR complex (mTORC1) acts on numerous downstream effectors to inhibit macroautophagy and to promote protein translation, transcription, microtubule growth, and cell cycle progression. mTORC1 activity is largely regulated by extracellular nutrients and growth factors and intracellular energy and amino acid stores. Many of these upstream signaling pathways converge on the hamartin/tuberin complex, which are the gene products of *TSC1* and *TSC2*. Tuberin contains a GTP-ase activating protein (GAP) domain that, when complexed with hamartin, inactivates Rheb and thus inhibits mTOR. Insulin and other growth factors activate PI3K/Akt signaling, which relieves the hamartin/tuberin inhibition of mTOR activity, thereby promoting protein synthesis, cell growth, and proliferation. Amino acid-induced activation of mTOR occurs downstream of hamartin/tuberin, and also promotes mTOR-mediated anabolic processes. Conversely, energy or oxygen deprivation activates the hamartin/tuberin complex by stimulating AMPK or REDD1/2 signaling, respectively, to turn off mTOR activity and energy-consuming cellular processes when resources are scarce. 4E-BP1, elongation factor 4E binding protein 1; AMPK, AMP-activated protein kinase; eIF4E, elongation initiation factor 4E; PDK1, phosphoinositide-dependent kinase-1; PI3K, class I phosphoinositide-3 kinase; PTEN, phosphatase and tensin homolog on chromosome 10; RHEB, Ras homolog enriched in brain; S6K, ribosomal S6 kinase; VSP34, class III phosphoinositide-3 kinase vacuolar protein sorting 34.

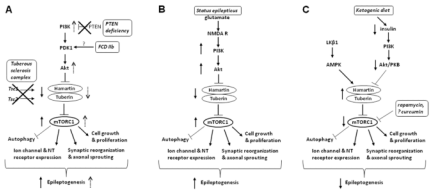

Fig. 2.

Mechanisms of mTOR dysregulation in epilepsy and mTOR inhibition in antiepileptogenesis. (A) Malformations of cortical development. In tuberous sclerosis complex, mutation in either the *TSC1* or *TSC2* gene results in overactivation of mTOR via loss of function of the hamartin/tuberin complex, leading to dysregulation of mTOR's downstream functions that contribute to tumor predisposition and epileptogenesis. *PTEN* mutations result in loss of inhibition of PI3K/Akt signaling, which may explain why mouse models with neuronal *Pten* mutations exhibit mTOR hyperactivation and seizures. FCDIIb have increased pS6 expression consistent with mTOR hyperactivation, as well as increased expression of PDK1 which is suggestive of increased PI3K/Akt signaling as a possible mechanism for mTOR dysregulation and epileptogenesis. Solid arrows denote expected direction of change with TSC; dotted arrows show expected direction of change with *PTEN* mutation. (B) Putative mechanism of mTOR hyperactivation in models of acquired epilepsy after status epilepticus or traumatic brain injury. Excessive glutamate release during status epilepticus or after trauma may result in NMDA receptor-mediated activation of PI3K/Akt signaling, which would be expected to relieve the hamartin/tuberin inhibition of mTOR, causing a cascade of cellular events that likely contribute to epileptogenesis. (C) Proposed mechanisms of antiepileptogenic effect of mTOR inhibition. Rapamycin directly inhibits mTORC1, thereby preventing the downstream effects implicated in epileptogenesis caused by mTOR dysregulation of any etiology. Curcumin has also been shown to inhibit mTOR, which may explain its apparent antiepileptogenic effects. The ketogenic diet decreases insulin levels, and thus would be expected to inhibit mTOR activity indirectly by decreasing PI3K/Akt signaling.