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Mammalian Target of Rapamycin (mTOR) Inhibition: Potential for Antiseizure, Antiepileptogenic, and Epileptostatic Therapy

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Abstract

New epilepsy treatments are needed that not only inhibit seizures symptomatically (antiseizure) but also prevent the development of epilepsy (antiepileptogenic). The mammalian target of rapamycin (mTOR) pathway may mediate mechanisms of epileptogenesis and serve as a rational therapeutic target. mTOR inhibitors have antiepileptogenic and antiseizure effects in animal models of the genetic disease, tuberous sclerosis complex. The mTOR pathway is also implicated in epileptogenesis in animal models of acquired epilepsy and infantile spasms, although the effects of mTOR inhibitors are variable depending on the specific conditions and model. Furthermore, beneficial effects on seizures are lost when treatment is withdrawn, suggesting that mTOR inhibitors are “epileptostatic” in only stalling epilepsy progression during treatment. Clinical studies of rapamycin in human epilepsy are limited, but suggest that mTOR inhibitors at least have antiseizure effects in tuberous sclerosis patients. Further studies are needed to assess the full potential of mTOR inhibitors for epilepsy treatment.

Keywords

epilepsy; seizure; epileptogenesis; rapamycin; everolimus; tuberous sclerosis complex; traumatic brain injury; status epilepticus; mice; rat

Introduction

Epilepsy is a common, neurological disorder affecting approximately 1% of the population. To date, the treatment of epilepsy has generally been unsatisfactory with approximately one-third of individuals refractory to medication. In addition, with the possible exception of epilepsy surgery, current treatments ameliorate seizures symptomatically (i.e., are antiseizure), with no effect on the underlying pathophysiology or course of the disease itself. Therefore, the ability to develop new epilepsy treatments that alter or prevent the development of epilepsy (i.e., are antiepileptogenic) represents a holy grail in epilepsy research.

Epileptogenesis is the process leading to the development of epilepsy following a brain injury or defect from either environmental or genetic causes. After the initial insult, epileptogenesis results from a complex interplay of multiple factors during a latent period typically lasting months to years, ultimately culminating in neuronal hyperexcitability and the expression of seizures. Multiple model systems have been developed to study

epileptogenesis. Perhaps the most relevant models are those that mimic common causes of acquired epilepsy in humans, such as following traumatic brain injury or status epilepticus. Amongst genetic causes of epilepsy, tuberous sclerosis complex (TSC) is an attractive model given its high propensity for seizures and the possibility of early detection via genetic testing and non-neurological findings. TSC, an autosomal dominant disorder due to mutations in either the *TSC1* (hamartin) or *TSC2* (tuberin) genes, affects multiple organ systems, but neurological involvement, especially epilepsy, usually accounts for the most disabling symptoms [1]. The exciting discovery that the hamartin and tuberin proteins limit activation of the mammalian target of rapamycin (mTOR) signaling pathway sparked a series of discoveries that are relevant not only to epileptogenesis in TSC, but potentially also to more common, acquired epilepsies.

This review aims to discuss the role that the mTOR pathway plays in epileptogenesis and how inhibition of this pathway has potential for epilepsy treatment. While other works have also covered this subject [2–4], this review will analyze the most recent, up-to-date animal and human studies, including some published in preliminary form, related to mTOR inhibitors and epilepsy.

The mTOR pathway

mTOR is a critical protein kinase that functions to integrate multiple intra- and extracellular signals to regulate cell growth, metabolism, proliferation, and survival via alterations in gene expression and protein translation (Fig. 1). The complex details of mTOR biology have recently been reviewed elsewhere and are outside the scope of this review [4,5]. In brief, mTOR forms two complexes, mTORC1, which can be inhibited by rapamycin, and mTORC2, which is largely rapamycin-insensitive. mTORC1 activates a number of downstream pathways including stimulation of mRNA translation via activation of the p70 ribosomal S6 kinase 1 (S6K1) and the eukaryotic initiation factor 4E binding protein-1 (4E-BP1). These actions mediate many of the functional effects of the mTOR pathway via modulation of protein synthesis.

Hamartin and tuberin act as a complex upstream of mTOR and inhibit the mTOR pathway via inhibition of Rheb (Ras homolog enriched in brain) (Fig. 1). Multiple upstream pathways stimulate or inhibit the mTOR pathway by interacting with the hamartin and tuberin complex to control important physiological functions. For example, regulation of energy metabolism in response to conditions promoting growth or starvation is achieved through opposite effects on the hamartin-tuberin complex via either growth factor stimulation of the PI3K pathway or energy deprivation leading to LK 1/AMPK pathway activation. Several interesting findings further support the role of this pathway in regulation of energy and growth that may relate to epilepsy. The high-fat, low carbohydrate, ketogenic diet is a well established treatment for epilepsy [6], but the mechanism of action is unknown. Interestingly, rats fed a ketogenic diet demonstrated reduction of both upstream and downstream mTOR pathway markers, suggesting that the mTOR pathway may be involved in effects of the ketogenic diet on growth and seizures [7]. The link between mTOR and the role of nutrient signaling is further elucidated in patients with a rare disorder known as polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome (PMSE), in which deletions in the *STRADA* gene cause dysregulation of mTOR signaling via a reduction in the LKB1/AMPK pathway [8].

Probably the strongest link between mTOR and epilepsy occurs in TSC, one of the most common genetic causes of epilepsy. In TSC, the result of inactivating mutations in *TSC1* or *TSC2* is to reduce inhibition of the mTOR pathway and therefore hyperactivate downstream targets of mTOR [1]. Abnormal mTOR signaling was initially implicated in causing

abnormal cell growth and proliferation contributing to cortical malformations (e.g., tubers) and brain tumors (e.g., astrocytomas) in TSC [9]. This finding has led to the establishment of mTOR inhibitors for treating astrocytoma growth in TSC patients [10•]. Intriguingly, some forms of focal cortical dysplasias (FCD), specifically type IIB, which resemble cortical tubers histologically and represent a common cause of intractable epilepsy, also exhibit abnormalities in TSC gene polymorphisms, hamartin and tuber expression, and other mTOR markers [11–19]. Thus, a strong association between the mTOR pathway and epilepsy has been found in several types of human epilepsy. However, evidence supporting a causal role of mTOR in epileptogenesis is less clear and primarily derives from animal models of epilepsy.

mTOR and Epileptogenesis in Animal Models

Genetic Epilepsies

i. TSC—Multiple mouse models of TSC have been generated by specific disruption of the *Tsc1* [20–24] or *Tsc2* genes [25–27] in neurons, glia, or brain progenitor cells (Table 1). These models recapitulate many aspects of the central nervous system phenotype found in TSC, including epilepsy and deficits in learning and memory. At the cellular level, these animal models also exhibit abnormalities found in human TSC brain specimens, such as enlarged and dysplastic neurons or vacuolated giant cells, abnormal neuronal organization, reduced myelin, and astrogliosis. Although the precise molecular mechanisms causing epileptogenesis in TSC are not known, specific molecular defects, such as in astrocyte glutamate transporters, have been identified and may contribute to epileptogenesis in these mouse models.

A critical role of the mTOR pathway in epileptogenesis has been firmly established in many of these TSC models (Table 1). The brain abnormalities are associated with hyperactivation of the mTOR pathway and postnatal treatment with mTOR inhibitors decreased megalencephaly, neuronal hypertrophy, and astrogliosis. Postnatal treatment with mTOR inhibitors decreased seizures when started after the onset of seizures (i.e. antiseizure) [28] or prevented the development of epilepsy when treatment was initiated prior to the onset of seizures (i.e. antiepileptogenic) [22–23,27–29]. Rapamycin also improved the cognitive deficits seen in *Tsc2* mutant mice [26]. Unfortunately, neither the antiseizure nor antiepileptogenic benefits persisted in any of the TSC mutant mouse models once rapamycin was withdrawn, indicating that chronic, indefinite treatment was necessary to maintain efficacy.

ii. Pten Models—An additional window into the role that abnormal activation of the mTOR pathway plays in genetic epilepsies arises from study of Pten (phosphatase and tensin homolog deleted on chromosome ten) mutant animal models. Pten is a tumor suppressor gene that exerts its effects on growth via modulation of the phosphatidylinositol-3 kinase (PI3K) / AKT pathway which is an upstream regulator of *TSC1* and *TSC2* (see Figure 1). Various human diseases with Pten mutations have been identified involving tumor or hamartoma growth in various organs. In addition, a common theme of some of these diseases is the presence of seizures [30].

A couple of Pten knockout mice have been developed targeting the central nervous system. In one mouse model, Pten inactivation in postnatal granule neurons of the dentate gyrus and cerebellum led to megalencephaly, neuronal hypertrophy, seizures and premature death [31,32]. Treatment with mTOR inhibitors decreased seizures and associated pathological abnormalities and prolonged life [33, 34]. Similar to the TSC models, seizures returned within weeks of cessation of rapamycin therapy, although subsequent intermittent rapamycin treatment was able to maintain a long-term antiseizure effect [35•]. In another, similar Pten

mouse model, rapamycin not only decreased seizures and neuronal hypertrophy, but also reversed deficits in social behavior suggestive of autism [36].

Acquired Epilepsies

While the link between certain genetic diseases, especially TSC, and the mTOR pathway is well-established, these genetic diseases are relatively rare causes of epilepsy. Environmental insults or other acquired brain disorders represent more common etiologies of epilepsy. Thus, a question of potentially broader clinical relevance and impact is whether abnormal mTOR signaling may contribute to various epilepsies due to acquired brain injury. In a general scheme of epileptogenesis in acquired epilepsy, the initial precipitating injury triggers epileptogenic mechanisms during a latent period, which eventually leads to the development of spontaneous seizures. In theory, activation of the mTOR pathway could represent one of the initial signals during the latent period, which trigger a number of downstream cellular and molecular changes in the brain that ultimately cause increased neuronal excitability and seizure generation. There is now evidence that the mTOR pathway is indeed activated following various types of brain injuries in animal models. Furthermore, in some models of acquired epilepsy, mTOR appears to mediate cellular abnormalities implicated in epileptogenesis, such as mossy fiber sprouting in the hippocampus, as well as possibly contributing to seizure generation itself. Experimental evidence for the involvement of the mTOR pathway in animal models of different types of acquired epilepsy will be briefly reviewed in sequence, as also summarized in Table 2.

i. Status Epilepticus Injury Models—Some of the most popular animal models of epilepsy involve an initial episode of status epilepticus, triggered by drugs or electrical stimulation, which then causes brain injury and subsequent development of spontaneous seizures. The mechanisms of epileptogenesis in these status epilepticus models are still debated, but may involve neuronal death, synaptic reorganization (e.g. mossy fiber sprouting), neurogenesis, and molecular changes in ion channels. The mTOR pathway has been clearly shown to be activated during and following status epilepticus in both the kainate and pilocarpine models [37–39]. Furthermore, mTOR almost certainly mediates some of the cellular changes that have been implicated in epileptogenesis, particularly mossy fiber sprouting, as mTOR inhibitors consistently reduce the degree of mossy fiber sprouting using different models and paradigms [37–39]. Interestingly, however, the effects of mTOR inhibition on mossy fiber sprouting requires continued treatment, as withdrawal of rapamycin leads to reemergence of mossy fiber sprouting [38]. The mTOR pathway may also be involved in neuronal death and neurogenesis following status epilepticus, although this appears to be dependent on the specific timing and model [37•].

The effect of mTOR inhibitors on epilepsy itself in status epilepticus models is more complicated and variable. In the kainate model in rats, rapamycin treatment shortly before or after status epilepticus decreases the subsequent development of epilepsy [37•], suggesting a possible antiepileptogenic effect. However, in the pilocarpine model, late rapamycin treatment in rats that have already developed spontaneous epilepsy can significantly reduce seizure frequency, more consistent with a direct antiseizure effect [39]. In contrast, in another study involving the pilocarpine model in mice, early rapamycin did not appear to have an antiepileptogenic effect for preventing epilepsy; however, the concurrent late use of rapamycin in the control group during the monitoring period to control for anticonvulsant effects does not preclude the possibility of an undetected short-term antiseizure effect [40•]. Furthermore, another electrical stimulation model of the amygdala did not find an effect of rapamycin on subsequent development of epilepsy, but since rapamycin was stopped before the end of the monitoring period, the possibility of an undetected transient short-term effect also exists [41]. Other explanations for variation in these studies may involve differences in

species, dose, and timing of the effects, as well as EEG monitoring methods. At a minimum, the status epilepticus models demonstrate the complexity of mTOR's involvement in epileptogenesis and ictogenesis and raise the question as to whether the effects of mTOR inhibitors are more consistent with antiepileptogenic or antiseizure mechanisms [42].

ii. Traumatic Brain Injury Models—Traumatic brain injury (TBI) and posttraumatic epilepsy represent one of the most common and important types of human epilepsy. Furthermore, antiepileptogenic drug trials, attempting to prevent posttraumatic epilepsy, have been successfully designed and conducted, although the results have been negative [43]. Similar to status epilepticus models, abnormal activation of the mTOR pathway has been demonstrated in several animal models of TBI [44–47]. Rapamycin decreases neuronal death and functional deficits, including spatial memory impairment, following traumatic brain injury [45,47]. While effects of mTOR inhibitors on posttraumatic epilepsy have not yet been rigorously tested, preliminary data suggest that rapamycin does decrease the development of posttraumatic epilepsy and associated neuronal death and mossy fiber sprouting in the controlled cortical impact model in mice [46], as well as in an in vitro organotypic culture model of posttraumatic epileptogenesis [48]. If these preliminary results are confirmed, this would have direct clinical implications for patients with TBI.

iii. Infantile spasms models—Infantile spasms represents a characteristic epilepsy syndrome of infancy and early childhood, characterized by epileptic spasms, developmental delay, and hypsarrhythmia on EEG and is usually associated with a poor prognosis. Infantile spasms can be caused by a variety of different brain insults during early development or can be of unknown cause (“cryptogenic”). Recently a number of animal models of infantile spasms have been developed and characterized and the potential involvement of the mTOR pathway has been tested in a couple of them. In a “multiple-hit” model involving several pharmacologically-induced “injuries” to model symptomatic infantile spasms, rapamycin can decrease the frequency of preexisting spasms in neonatal rats [49], suggesting a direct antiseizure effect. In addition, rapamycin treatment decreased the subsequent development of cognitive deficits observed in this model, indicating a disease-modifying effect. Interestingly, however, rapamycin did not decrease the development of other non-spasm seizure types, indicating that mTOR may only be involved in mediating specific types of seizures in this animal model. Furthermore, in another model of “cryptogenic” spasms, rapamycin appeared to have no direct effect on the spasms [50]. Again differences in effects of rapamycin in different models suggest that the role of the mTOR pathway in epileptogenesis may vary depending on the type and cause of epilepsy and the timing of rapamycin administration.

iv. Other models—Preliminary studies have also investigated the potential role of mTOR in other types of acquired epilepsy models. In a model of neonatal hypoxic brain injury, acute hypoxia induces immediate seizures in neonatal rats, which later develop a decreased seizure threshold and spontaneous seizures in adulthood. Preliminary studies indicate that the mTOR pathway is hyperactivated following neonatal hypoxia and rapamycin inhibits the subsequent change in seizure threshold, suggesting an antiepileptogenic effect [51]. In a model of rapid electrical kindling, repetitive electrical stimulation leads to increasing epileptiform discharges. In a limited number of experiments, rapamycin was reported to decrease the development of epileptiform activity in this model [52].

Data from Human Studies

Given the reports from animal models, there is burgeoning excitement to further assess the role that inhibition of the mTOR pathway might play in the treatment of clinical epilepsy in humans. Although this would ideally be assessed for its antiepileptogenic potential for

preventing epilepsy in high-risk patients, there are clear barriers to designing and conducting antiepileptogenesis trials in people. The limited data available to date have only assessed the role of mTOR inhibition as an antiseizure medication in patients with established, intractable epilepsy.

The human data that exists comes almost exclusively from TSC patients. A single case report initially suggested a beneficial effect of rapamycin in reducing seizure frequency in a child with TSC and intractable epilepsy [53]. The largest available human data derives from a Phase II prospective, open-label trial of everolimus (a rapamycin analog) that was designed primarily to assess the efficacy of everolimus in inhibiting growth of subependymal giant-cell astrocytomas (SEGAs) in patients with TSC [10•]. Seizure frequency was a secondary endpoint of the study, as assessed by self-reports of seizure frequency, as well as twenty-four hour video EEG at baseline and after six months of treatment. Twenty-eight patients with TSC were enrolled ranging in age from 3 to 34 years and received oral everolimus (3 mg/m²/d, serum levels 5–15 ng/ml). Treatment with everolimus resulted in a significant decrease in self-reported seizure frequency. Sixteen patients underwent video EEG evaluation with nine of ten patients with active seizures demonstrating a decrease in seizure frequency and the tenth patient demonstrating an increase in seizure frequency. The remaining six patients had no change in seizure frequency but five of these were seizure free both at baseline and in follow-up.

These intriguing results have sparked a subsequent clinical trial of everolimus with seizures as the primary endpoint. Preliminary data from this study was recently presented in abstract form [54]. In this open-label, multi-center combined phase I/II clinical trial, patients greater than two years old with medically refractory epilepsy secondary to TSC were treated with everolimus (5 mg/m²/day, 5–15 ng/ml). Seizures were assessed by seizure diary and 24 hour video EEG during the baseline and at the end of an eight week treatment period. Fifteen of 17 total patients reported at least a 25% reduction in seizure frequency with six patients reporting greater than 50% reduction. One patient reported an increase in seizure frequency.

Conclusions

There is now a myriad of data, mostly from animal models, but also from limited human studies, supporting a role of the mTOR pathway in epilepsy and suggesting that mTOR inhibitors may represent rational therapeutic agents. However, the specific contexts and mechanisms involving the mTOR pathway and the type of therapeutic action of mTOR inhibitors is uncertain and may vary depending on a number of factors, such as timing of treatment, seizure type, and epilepsy syndrome. The main issue is whether the mTOR pathway is involved in epileptogenesis (i.e., the development of epilepsy) or the maintenance of epilepsy/ictogenesis (i.e., seizure generation) and correspondingly whether mTOR inhibitors primarily have antiepileptogenic (i.e., preventing the development or progression of epilepsy) or antiseizure actions (i.e., suppressing the symptoms of seizures in patients that already have epilepsy).

At this point, there is evidence that mTOR inhibitors may have both antiepileptogenic and antiseizure actions, again depending on the context and type/model of epilepsy. In theory, antiepileptogenic and antiseizure effects may have completely separate and unique mechanisms of action. However, a distinct separation between antiepileptogenic and antiseizure mechanisms may be artificial. Given the diversity of cellular and molecular processes that may be regulated by the mTOR pathway, it is possible that mTOR inhibitors could have a range of overlapping actions spanning both antiepileptogenic and antiseizure effects. Plausible antiepileptogenic actions of mTOR inhibition include regulation of synaptic plasticity, neuronal death, neurogenesis, and long-term actions on metabolism and

protein synthesis. In addition, while there is minimal evidence that mTOR inhibitors have immediate, direct effects on neuronal excitability akin to traditional anticonvulsant drugs [55,56], mTOR inhibition could regulate protein synthesis of ion channels, neurotransmitter receptors, or other proteins involved in neuronal signaling, resulting in antiseizure effects in patients with established epilepsy.

Regardless of mechanism, one key principle that has emerged from the animal studies is the importance of the timing and duration of treatment with mTOR inhibitors. In the TSC models, mTOR inhibitors consistently have apparent antiepileptogenic actions in preventing epilepsy when treatment is initiated early; however, when treatment is then discontinued, epilepsy subsequently emerges [22,28,29]. Thus, mTOR inhibitors are not a cure for the epilepsy found in TSC and persistent, life-long treatment may be required to maintain effectiveness. The requirement for long-term treatment is not surprising for a genetic disease like TSC, as the underlying genetic defect persists and becomes unmasked upon rapamycin discontinuation, allowing reactivation of the mTOR pathway. In theory, acquired epilepsies may not have a requirement for long-term treatment, as some epileptogenic mechanisms may only occur for a limited, critical period after the initial brain injury. Interestingly, however, there is some evidence that discontinuation of rapamycin treatment also leads to loss of effectiveness at least for suppression of late seizures and mossy fiber sprouting in the pilocarpine model of acquired epilepsy [38,39]. Although not specifically examined, a requirement for persistent mTOR inhibition might also explain variability in effects on seizures among various models of acquired seizures, including status epilepticus and infantile spasms models [37,39–41,49,50]. In summarizing rapamycin's actions, Galanopoulou proposed that mTOR inhibitors may have “epileptostatic” properties [57] that are intermediate between antiepileptogenic and antiseizure actions, with the potential to prevent epilepsy but the requirement for chronic treatment to maintain efficacy.

The clinical data in people are much more limited, only involving ongoing antiseizure trials in TSC patients with intractable epilepsy. If the current clinical trials definitively demonstrate an antiseizure effect of mTOR inhibitors, a logical next step would involve a true antiepileptogenic trial. Antiepileptogenic drug trials have been attempted in the past, primarily involving conventional antiseizure medications in patients with traumatic brain injury, but the results have been negative [43]. Patients with TSC may be an appropriate initial target population for an antiepileptogenic trial with mTOR inhibitors for several reasons including the high rate of epilepsy, the medical refractory nature of epilepsy in TSC patients, and the ability to diagnosis TSC prior to epilepsy onset and in many cases prenatally. However, there are many potential barriers to an antiepileptogenic trial. Although the rate of epilepsy is quite high, it is not 100% even in this population and to date there are no clearly identifiable predictors of epilepsy in TSC. As such, treatment of all patients with TSC would needlessly expose some patients to side effects without clear benefit. Additionally, the timing of treatment initiation is not clear. One could argue that prenatal treatment of women carrying fetuses with TSC identified by genetic testing or prenatal ultrasound may be necessary to attain the full benefit of rapamycin treatment since cortical tubers are present at birth. However, prenatal treatment would obviously increase the risk of serious side effects, including impairment of critical developmental processes that may be mTOR-dependent. Prenatal treatment trials in mice have demonstrated potential side effects and incomplete rescue of the neuronal phenotype. In one mouse model, a single prenatal dose of rapamycin followed by postnatal continuation of rapamycin rescued the lethal phenotype of the mutant mice but they ultimately still developed an abnormal neurological phenotype including enlarged brains and cognitive deficits [58]. Further, in comparison with controls, reduced neonatal weight gain was seen. Difficulty with weight gain during prolonged postnatal treatment with rapamycin has also been seen in other mouse models [22,23,28,29,36]. Limited data on safety of prenatal exposure to rapamycin in humans

largely stems from transplant patients on rapamycin for immune suppression [59–61]. In these handful of case reports and seven women reported to the National Transplantation Pregnancy Registry, rapamycin does not appear to be associated with any specific birth defects although 3 of seven pregnancies resulted in first trimester spontaneous abortions. Clearly more preclinical and clinical data are needed before considering prenatal treatment of rapamycin in TSC patients.

Besides prenatal treatment, an intermediate, less risky approach might involve identifying high-risk infants/children with TSC. Biomarkers for epilepsy have not been definitively established, but there is the possibility that specific EEG or brain MRI abnormalities may predict TSC patients with a worse neurologic prognosis and thus identify those patients most appropriate for antiepileptogenic therapy. For example, an abnormal EEG has been used to identify TSC patients prior to the onset of clinical seizures for starting vigabatrin treatment, which may decrease epilepsy severity and developmental delay [62]. If EEG is validated as an early biomarker for intractable epilepsy, a similar approach could be used with mTOR inhibitors. Additionally, although it seems likely that prolonged treatment with rapamycin may be necessary to sustain its effects, the optimal course of this treatment is yet to be determined. There is hope from mouse studies that intermittent treatment may both maintain an antiepileptic effect and reduce side effects [35]. Beyond TSC, other high-risk populations, such as patients with traumatic brain injury, could be considered for antiepileptogenic or neuroprotective trials, but should await more definitive data from animal models. Clearly the safety and efficacy of rapamycin as an antiepileptogenic treatment remain to be seen but in certain high risk populations the time to consider formal trials is near.

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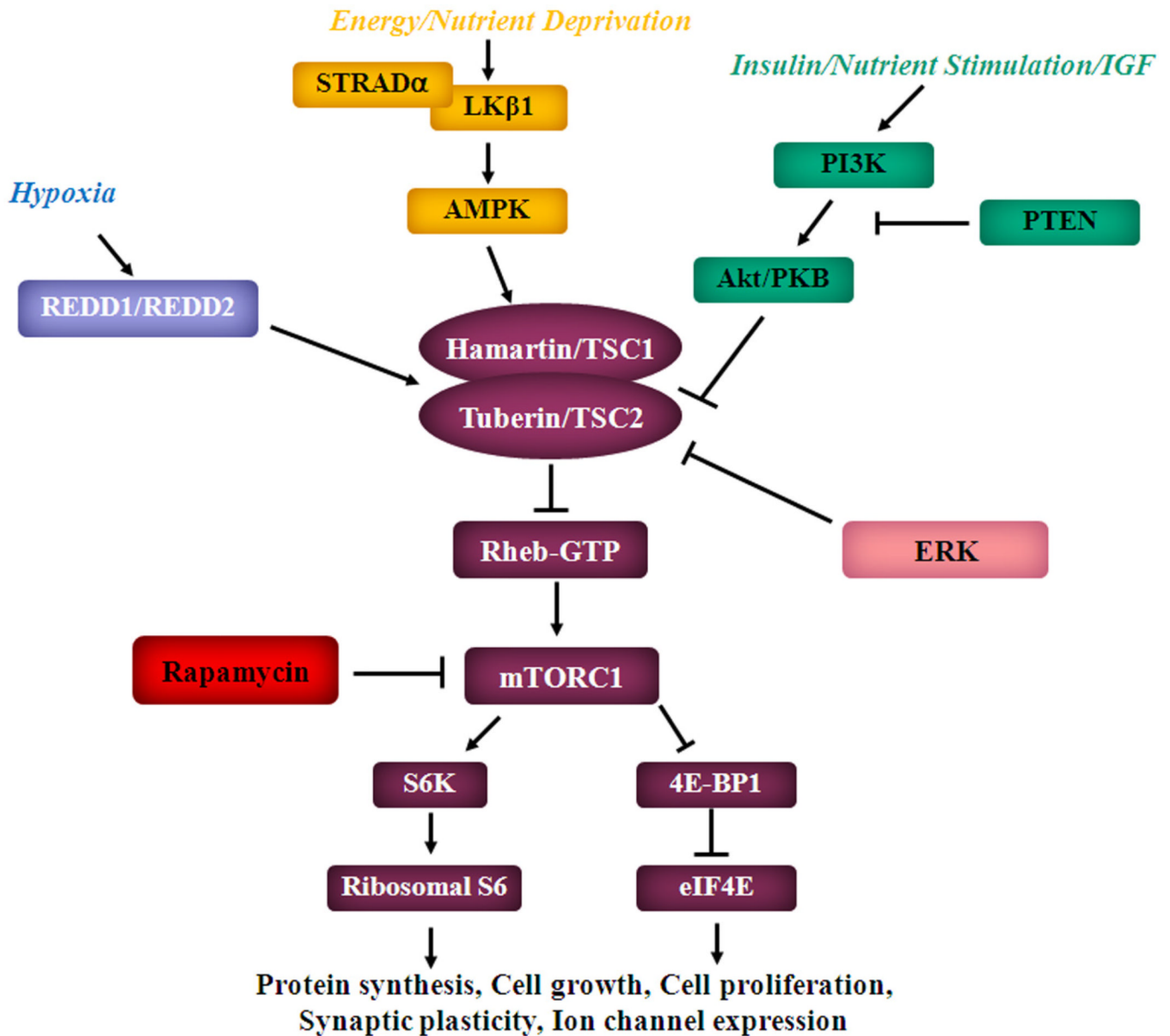


Figure 1. Regulation of the mTOR signaling pathway. The serine-threonine protein kinase, mTOR, forms two complexes, mTORC1, which is rapamycin sensitive, and mTORC2 (not shown). The mTOR pathway activates downstream signaling mechanisms primarily involved in regulating protein synthesis related to multiple functions, such as cell growth and proliferation, as well as other processes that may relate directly to epileptogenesis. In turn, the mTOR pathway may be activated or inhibited by various physiological or pathological stimuli via various upstream signaling pathways and intermediary proteins (TSC1, TSC2, Rheb). AMPK - 5' adenosine monophosphate-activated protein kinase; eIF4E, elongation initiation factor 4E; ERK – extracellular-signal-regulated kinases; IGF - insulin growth factor; mTOR - mammalian target of rapamycin; PI3K - phosphoinositide-3 kinase; PTEN, phosphatase and tensin homolog on chromosome 10; Rheb - Ras homolog enriched in brain; STRADα – STE20-related kinase adapter alpha; S6 - ribosomal protein S6; S6K - ribosomal

S6 kinase; TSC1 - tuberous sclerosis complex 1 protein; TSC2 - tuberous sclerosis complex 2 protein; 4E-BP1 - elongation factor 4E binding protein 1.

Table 1

Effects of mTOR Inhibitors in Animal Models of Genetic Epilepsy

Animal model [references]	Timing of treatment (pre/post-epilepsy onset)	Inhibition of Pathological Abnormalities	Inhibition of Epilepsy Phenotype	Maintenance of inhibitory effect after drug withdrawal
		Cell size/number	Existing seizures	
		Megal-encephaly	Epilepsy development	
		Other	Cognitive deficits	
			Survival	
<i>TSC models</i>				
Gfap-Tsc1 CKO mice [28]	P14(pre)	+	n/a	-
	P42 (post)	+	n/a	n/a
Gfap-Tsc2 CKO mice [27]	P14(pre)	+	n/a	n/a
Synapsin-Tsc1 CKO mice [29]	P7-9 (pre?)	+	+(?) ^a	-
Emx-Tsc1 CKO mice [23]	P13 (pre?)	+	+(?) ^a	-
Inducible nestin-Tsc1 CKO mice [22]	P8 (pre)	+	n/a	-
Tsc2+/-mice [26]	Adult (n/a)	n/a	n/a	n/a
<i>Pten models</i>				
NS-Gfap-Pten CKO mice [33,34,35]	~P28 (post)	+	n/a	-
Nse-Pten CKO mice [36]	~P35 (pre)	+	n/a	n/a
	~P70 (post)	+	n/a	n/a

^a effect on seizures noted subjectively, not quantitatively or by EEG.

n/a – not applicable or not done

Table 2

Effects of mTOR Inhibitors in Animal Models of Acquired Epilepsy

Animal model [reference]	Timing of treatment (pre/post-epilepsy onset)	Inhibition of Pathological Abnormalities			Inhibition of Epilepsy Phenotype		Maintenance of inhibitory effect after drug withdrawal	
		Mossy fiber sprouting	Neuronal death	Other	Existing seizures	Epilepsy development		Cognitive deficits
<i>Status Epilepticus (SE) Injury Models</i>								
Kaimate rats [37]	1–3d before SE (pre)	+	+	neurogenesis	n/a	+	n/a	n/a
	1 d after SE (pre)	+	-		n/a	+	n/a	n/a
Pilocarpine rats [39]	10 wk after SE (post)	+	n/a		+	n/a	n/a	-
Pilocarpine mice [40]	1 d after SE (pre)	+	-	dentate gyrus hypertrophy	n/a	-	n/a	-
Amygdala stimulation [41]	1 d after SE (pre)	-	n/a		n/a	-	n/a	n/a
<i>Traumatic Brain Injury Models</i>								
Weight drop mice [45]	4 h after injury (n/a)	n/a	+		n/a	n/a	+	n/a
CCI mice [46]*	1 h after injury (pre)	+	+		n/a	+	n/a	n/a
Organotypic culture [48]*	3 d after injury (pre)	n/a	+		n/a	+	n/a	n/a
<i>Infantile Spasms Models</i>								
Multiple-hit rat [49]	P4 (post)	n/a	n/a		n/a	+ ^a	- ^a	+
Betamethasone-NMDA rat [50]	1 or 8 d before NMDA (pre)	n/a	n/a		n/a	n/a	-	n/a
<i>Neonatal Hypoxia Model</i>								
Neonatal hypoxia rat [51]*	1 d before hypoxia (pre)	n/a	n/a		n/a	n/a	+	n/a
<i>Kindling</i>								
Rapid electrical kindling [52]*	During kindling	n/a	n/a		n/a	+	n/a	n/a

* published/presented in preliminary form

^a reduction in spasms but not other seizure types

n/a – not applicable or not done