Dysregulation of the mTOR signaling pathway is associated with highly epileptogenic conditions such as tuberous sclerosis, focal cortical dysplasia, hemimegalencephaly and ganglioglioma, grouped under the term of ‘mTORopathies’. Brain abnormalities associated with mTOR overactivation include enlarged and dysplastic neurons, abnormal cortical organization and astrogliosis. mTOR signaling intervenes in several molecular/biochemical processes leading to epileptogenesis. Animal models demonstrated that mTOR inhibitors could exert both an anticonvulsant action and an antiepileptogenic effect in models of genetic and acquired epilepsy. Preliminary studies in patients affected by tuberous sclerosis and treated with rapamycin or everolimus demonstrated potential benefits in seizure frequency reduction, suggesting that mTOR inhibition could be a promising treatment option for mTORopathies-related epilepsy. The authors reviewed the current knowledge of mTOR overactivation in different forms of epilepsy, and discuss the potential clinical use of mTOR inhibitors.

**KEYWORDS:** epilepsy • epileptogenesis • everolimus • focal cortical dysplasia • infantile spasms • mTOR inhibitors • phosphatase and tensin homolog • rapamycin • tuberous sclerosis

The mTOR pathway is the central controller of cell growth and proliferation that has been observed to play a crucial role during the development of the cerebral cortex [1]. Its activation is associated with abnormal cellular differentiation, proliferation and growth [3]. Dysregulation of the mTOR is a common molecular substrate of a number of conditions grouped under the term ‘mTORopathies’ [2]. These disorders include focal cortical dysplasia (FCD), tuberous sclerosis complex (TSC), ganglioglioma and hemimegalencephaly. Brain abnormalities associated with the activation of the mTOR pathway include disordered cortical lamination, enlarged and dysplastic neurons and astrogliosis, providing a likely histopathological substrate for epileptogenesis [2]. The neurological phenotype associated with these highly epileptogenic malformations is characterized by early onset and intractable seizures, often associated with cognitive deficit [2]. Hyperactivated mTOR signaling pathway has been also observed in animal models of acquired epilepsy, such as infantile spasms, temporal lobe epilepsy (TLE), status epilepticus (SE), traumatic brain injury (TBI) and neonatal hypoxia–ischemia [3-5].

In this article, the authors reviewed the possible role of mTOR signaling pathway in epileptogenesis, the preclinical studies of mTOR inhibitors treatment in different models of epilepsy, and preliminary clinical experience of rapamycin or everolimus treatment in human epilepsy.

**mTOR & epileptogenesis**

The mTOR is a serine/threonine protein kinase that plays a role in a wide spectrum of important cellular processes, such as the regulation of cell growth, development and proliferation to adaptive immune function [6]. mTOR signaling pathway integrates key information from nutrients, growth factors, cytokines and hormones through tyrosine kinase receptors [6]. mTOR forms two distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The mTORC1 regulates the activity of downstream targets that eventually lead to enhanced translation, cell growth, angiogenesis, transcription, ribosomal biogenesis and inhibit autophagy [4]. mTOR signaling is crucially involved in CNS development, intervening in axon guidance, dendritic development and spine morphogenesis [7,8], synaptic plasticity, memory function and neuronal repair mechanisms following an injury [9]. In brain cells, the mTOR signaling pathway is also modulated by

**mTOR inhibitors as a new therapeutic option for epilepsy**

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glutamate and dopamine receptors [10]. The molecular upstream and downstream cascade revealing the central role of the mTORC1 in cellular processes is summarized in Figure 1.

The mTOR pathway is negatively regulated by different genes, among which there are tumor suppressor genes TSC1 and TSC2 [11], but also upstream regulators including PTEN [12], the STRADα [13] and NF1 [1]. Overactivation of the mTOR pathway is associated in both humans and mouse models with several hamartoma and genetic syndromes, such as TSC, NF1, PTEN-related hamartoma syndromes (including Cowden syndrome), which are characterized by epilepsy and cognitive impairment [14]. As a result of its role in cell growth, both physiologic and pathologic tissue hypertrophy are associated with mTOR activation [15]. One of the most striking features of some epileptogenic malformations, such as FCD and TSC, is therefore the presence of enlarged cells in the context of an abnormal laminated cortex. This led to the assumption that cytomegaly can be a significant source of hyperexcitability, thus leading to epileptogenesis [4].

**Figure 1.** Molecular upstream and downstream cascade revealing the central role of mTORC1 in cellular processes, and the regulating action exerted by TSC1/2 complex. mTOR forms two complexes, mTORC1, which can be inhibited by mTOR inhibitors and mTORC2. mTORC1 activates a number of downstream pathways including stimulation of mRNA translation via activation of S6K1 and 4E-BP1. These actions mediate many of the functional effects of the mTOR pathway via modulation of protein synthesis.

In Table 1, the authors have listed the principal syndromes caused by these genes. Mutations of these genes lead to overactivation of the mTOR pathway with subsequent abnormal cell differentiation, proliferation and growth and a high comorbidity with epilepsy. Furthermore, mTOR dysregulation appears to play a key role in the pathogenesis of other malformative and neurocutaneous syndromes characterized by early onset and refractory epilepsy, such as hemimegalencephaly and Sturge–Weber syndrome. Hemimegalencephaly is a hamartomatous overgrowth limited to one hemisphere, which can often be associated with neurocutaneous syndromes. It has been postulated that it is a genetic mosaic determined by increased function of mTOR and some of its upstream regulator in the PI3K–Akt pathway [16]. Pathological examination in hemimegalencephaly revealed mTOR hyperactivation in the dysplastic neurons of the enlarged cerebral hemisphere [17]. Furthermore, the mTOR pathway was activated on specimen of vascular malformations taken from patients affected by Sturge–Weber syndrome, a condition characterized by early onset seizures and SE [18].

Owing to its role in neuronal development and plasticity, the mTOR signaling pathway could be responsible for the abnormal axonal sprouting and neurogenesis involved in epileptogenesis. The mTOR pathway has been implicated in contributing to progressive epileptogenesis; but at the same time, seizures themselves may directly cause acute activation of the mTOR pathway. In different animal models of induced seizures, mTOR appears to be overactivated both in the acute and chronic phase; while in some others, it only presents an immediate acute overactivation [19]. This hypothesis is supported by the evidence of an antiepileptogenic and neuroprotective effect of mTOR inhibitors in different animal models of epilepsy [20]. Weston et al. showed that hyperactivated mTOR signaling by loss of Pten leads to an increase in evoked synaptic responses in both glutamatergic and GABAergic neurons, and it also impairs presynaptic function [21]. They demonstrated that decreasing mTOR signaling with prolonged rapamycin treatment normalized glutamatergic but not GABAergic synapses [21].

**mTOR inhibition & seizure protection**

mTOR inhibitors are a small class of drugs that act by blocking the activity of the mTOR signaling pathway. The most established mTOR inhibitors are the so-called rapalogs, which are rapamycin and...
Rapamycin is a macrolide antibiotic produced as a fermentation product of *Streptomyces hygroscopicus*. The drug was first developed as an antifungal agent, but was later found to have potent antiproliferative and immunosuppressive properties. Everolimus is an orally bioavailable, structurally similar derivative of rapamycin that also exhibits antiproliferative and immunosuppressive effects. Everolimus was developed in an attempt to improve upon the pharmacokinetics of rapamycin, and has been shown to provide greater stability, solubility and more favorable pharmacokinetics.

Rapamycin and the other mTOR inhibitors share similar mechanisms of action, exerting their inhibitory effects on mTOR-regulated processes by reducing the phosphorylation of downstream mTOR effectors, including the translational repressor 4E-BP1 and S6K1. They form a complex with intracellular FKBP12, which subsequently binds to mTOR at the FKBP12-rapamycin binding domain, thus inhibiting downstream signaling events.

**Table 1. Summary of the main syndromes in which the mTOR activation has been demonstrated.**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>CNS pathological manifestations</th>
<th>Rapamycin effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberous Sclerosis</td>
<td>TSC1/TSC2</td>
<td>Tubers, subependymal nodules, white matter bands, tumors [57]</td>
<td>Presymptomatic treatment: prevention of seizures, prevention of premature death [64]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Symptomatic treatment: reduction of seizure frequency and severity, prolonged survival [64]</td>
</tr>
<tr>
<td>Cowden syndrome</td>
<td>PTEN</td>
<td>Macrocephaly (38%), Lhermitte–Duclos disease [87]</td>
<td>NA</td>
</tr>
<tr>
<td>Proteus syndrome</td>
<td>PTEN</td>
<td>Macrocephaly, hemimegalencephaly (20%), hamartomas</td>
<td>NA</td>
</tr>
<tr>
<td>Other PTEN-related disorders</td>
<td>PTEN</td>
<td>Neuronal hypertrophy, macrocephaly</td>
<td>Presymptomatic treatment: normal brain/body ratio, prevention of neuronal hypertrophy and loss of neuronal polarity. Reduced anxiety and increased social interactions [56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Symptomatic treatment: decreased brain/body ratio, reversion of neuronal hypertrophy, reversion of dendritic hypertrophy but not of the loss of neuronal polarity. Decreased duration and frequency of seizures [56]</td>
</tr>
<tr>
<td>PMSE syndrome</td>
<td>STRADα</td>
<td>Macrocephaly, subependymal dysplasias, ventriculomegaly [13]</td>
<td>Cytomegaly reversion [86]</td>
</tr>
<tr>
<td>Focal cortical dysplasia type IIB</td>
<td>Different genetic polymorphisms (TSC?)</td>
<td>Focal cortical dysplasias, abnormal lamination [46]</td>
<td>Suppression of seizures and neuronal hypertrophy [50]</td>
</tr>
<tr>
<td>Hemimegalencephaly</td>
<td></td>
<td>Asymmetric macrocephaly, abnormal gyration [16]</td>
<td>NA</td>
</tr>
<tr>
<td>Sturge–Weber syndrome</td>
<td></td>
<td>Cerebral calcifications, leptomeningeal angiomatosis [18]</td>
<td>NA</td>
</tr>
<tr>
<td>Neurofibromatosis I</td>
<td>Neurofibromin I</td>
<td>Possible glial tumors, white matter abnormalities [88]</td>
<td>NA</td>
</tr>
</tbody>
</table>

In some studies, efficacy of rapamycin treatment has been observed in animal studies. NA: Not available; PMSE: Polyhydramnios, megalencephaly, symptomatic epilepsy; TSC: Tuberous sclerosis complex. Modified from [4].

In its analogs, such as temsirolimus and everolimus. These drugs specifically act on mTORC1, while mTORC2 activity is resistant to short-term treatments.

Rapamycin’s mechanism of action involves the inhibition of mTOR, a serine/threonine kinase that regulates the translation of downstream protein synthesis. By inhibiting mTOR, rapamycin can block the proliferation of cancer cells, making it a valuable therapeutic option for cancer treatment. Its use in epilepsy, however, has been more recently explored.

**FIGURE 2** summarizes the role of the mTOR pathway in cellular and molecular processes underlying epileptogenesis. mTOR inhibition can have an antiepileptic effect due to its multiple actions on neuronal excitability by acting on voltage- and ligand-gated channels, neurotransmitter receptors and signaling pathways [4]. However, acute administration of rapamycin demonstrated a limited anticonvulsant effect [22], and recent data have shown that it presents a weak short-term anticonvulsant effect, with loss of efficacy over time, associated with downregulation of neuropeptide Y [23]. Chronic administration of rapamycin can reduce cortical excitability by prolonging mean open channel time of calcium and sodium channels and by increasing Kv1.1 expression in cortical and hippocampal neurons [24,25]. Furthermore, chronic administration of rapamycin reduced overall excitability by increasing γ-aminobutyric acid (GABA)A receptor-mediated synaptic activity and hyperpolarized resting membrane potentials [4] and by reducing AMPA-receptor surface expression usually observed after blockade of synaptic activity [26].

Recent data show that mTOR inhibition not only has an antiseizure action, but also presents an antiepileptogenic potential, with the possibility of preventing or delaying the onset of epilepsy [27]. In a rodent model of pilocarpine-induced SE, the
prolonged hippocampal infusion of rapamycin reduced aberrant mossy fiber sprouting [28]. Furthermore, rapamycin acts on axonal sprouting, and is able to revert abnormal cell growth [29]. Rapamycin plays also a role on inflammation pathway, controlling microglial activation in response to proinflammatory cytokines, therefore having the potential of controlling neuroinflammation [5,30]. However, these data are still controversial since not all studies succeeded in replicating these findings [31]. The link between its anticonvulsant and anti-inflammatory action has been reinforced by the observation that resveratrol pre-treatment reduces early inflammatory response induced by SE via mTOR signaling [32]. McMahon et al. demonstrated that autophagy is suppressed in mouse models of TSC and PTEN mutation and that it is negatively regulated by the mTOR pathway; these findings suggest that impaired autophagy contributes to epileptogenesis, thus representing a further potential target for epilepsy treatment and/or prevention [33]. The final antiepileptic or antiepileptogenic effect of mTOR inhibitors could depend on different animal models, timing of treatment and administration protocols and types of seizure [28,34,35].

**Animal models**

The mTOR signaling pathway is implicated in the epileptogenesis of animal models of genetic diseases such as TSC [4]. However, recent data showed that the mTOR dysregulation also plays a role in the epileptogenesis of some acquired forms of epilepsy, such as infantile spasms (IS) [35], SE, TLE, TBI [36] and neonatal hypoxia–ischemia [37]. Recently, overactivation of the mTOR signaling pathway has also been proved in the animal model of absence epilepsy [38], suggesting that this mechanism could be a common pathological pathway for epileptogenesis in models of different epilepsy syndromes.

**Acquired epilepsies**

Infantile spasms

Recent evidence from animal models supports a pathogenetic role of the mTOR pathway in IS of genetic and nongenetic etiology. The therapeutic potential of rapamycin was tested in a multiple-hit rat model of symptomatic ACTH refractory IS that recreates a combination of cortical and subcortical lesions, suggesting that pathologic overactivation of mTORC1 may be implicated in the pathogenesis of IS per se [35]. Raffo et al. showed that low dosage of rapamycin has no anticonvulsant effect on the acute stage of spasms, but very high dosages can suppress them [35]. They suggested that this dose-dependent effect might be due to an excessive early activation of mTORC1 in IS because of an increased activity of upstream regulators, which therefore requires an increased rapamycin dose [4]. In the same model, other types of emerging seizures were not affected by rapamycin, despite the suppression of spasms. These data suggest that the neuronal networks generating and controlling spasms are distinct from those implicated in other types of seizures [35]. The same study also demonstrated that a 3-day pulse protocol was able to stop spasms permanently, with no recurrence after its discontinuation [35]. Therefore, these findings suggest that in acquired IS, rapamycin treatment may not need to be continuous, but could be given only during the acute phase of spasms, reducing the potential side effects of chronic exposure to the drug [4]. Interestingly, in treated mutant mice, a significant improvement of memory, cognitive and exploratory ability was observed more than 10 days after the last dose of rapamycin suggesting a disease-modifying effect [35]. Whereas mutant mice had a clear learning benefit from rapamycin, naïve pups treated with rapamycin worsened their performances in some of the early cognitive trials [35]. These findings might suggest that rapamycin exerts a disease-modifying effect, not determining a cognitive improvement per se, but acting on the molecular pathways that may cause learning disabilities associated with IS.

**Status epilepticus**

The mTOR pathway has been demonstrated to play a key role both during and after SE in different animal models reducing mossy fiber sprouting [28,39,40]. However, treatment administration must be continued to exert this effect, as withdrawal of rapamycin leads to re-emergence of mossy fiber sprouting [28]. In a Pten knockout rodent model, Sunnen et al. demonstrated that epilepsy recurred a few weeks after rapamycin withdrawal, but both mossy fiber sprouting and epilepsy were inhibited with repetitive pulses of rapamycin [41]. The effect of mTOR inhibition on epilepsy itself...
in the SE models is more complicated and variable [3]. In some models, rapamycin showed a potential but short-term antiseizure effect, significantly reducing existing seizures. However, these results failed to be replicated in all experiments [34,40,42]. Similarly, animal models of TLE indicate that rapamycin treatment during or soon after SE might reduce the risk of subsequent epilepsy, thus exerting an antiepileptogenic effect [39]. However, this observation was not confirmed by other studies [42]. Since results from different studies diverge, no definite conclusion can be achieved concerning the efficacy of mTOR inhibition in SE.

Temporal lobe epilepsy
Different studies on animal models observed that TLE following a SE might be responsive to rapamycin [39,40]. In these studies, rapamycin administered before the SE decreased neuronal cell death, neurogenesis, mossy fiber sprouting and the development of spontaneous epilepsy. Late rapamycin treatment after SE blocked the chronic phase of mTOR activation and reduced mossy fiber sprouting, but had no effect on neurogenesis or cell death [39,40]. In TLE models, contradictory findings exist as to whether rapamycin may produce antiepileptogenic effects. It is possible that different findings may be obtained by modifying model- or species-related factors, or the timing and protocols of rapamycin administration. However, all the available data indicate that continuous treatment seems to be necessary to maintain the obtained benefits, since a complete reversal of the epileptic phenotype has not been achieved [4,27].

Traumatic brain injury
Rapamycin was able to reduce the activation of microglia following a TBI [36], suggesting that mTOR inhibitors can exert at least part of their antiepileptogenic action following SE by reducing inflammation. However, recent data showed that mTOR inhibition could lead to seizure suppression without acting on microglia activation, thus suggesting that other mechanisms of action may be involved [31]. In rodent models of controlled cortical impact, mTOR inhibition reduced neuronal death and memory deficits following TBI, and also decreased mossy fiber sprouting and subsequent post-traumatic epilepsy [36,38]. Since up to now, no antiepileptic drugs (AEDs) have been found to be effective in epilogenesis following TBI, the possible protective action of mTOR inhibition in post-traumatic epilepsy is worthy of further investigation.

Neonatal hypoxia–ischemia
Neonatal insult determines a decrease of the seizure threshold with subsequent occurrence of spontaneous seizures; preliminary evidence indicates that the mTOR pathway is hyperactivated following neonatal hypoxic damage [44]. Interestingly, in a model of rapid electrical kindling, rapamycin treatment was able to exert an antiepileptogenic effect, modifying the seizure threshold and decreasing the development of epileptiform activity [45].

Other forms of epilepsy
Absence seizures
Recently in a genetic rat model of absence epilepsy, a chronic administration in a rat treated with rapamycin, Russo et al. were able to prevent seizure onset demonstrating an antiepileptogenic action [38]. However, while the chronic treatment determined an unexpected prodepressant action, the acute or subacute treatment exerted antidepressant action [38]. They concluded that these effects might be linked to mTOR inhibitors anti-inflammatory action.

Focal cortical dysplasia
FCD type IIB is a sporadic developmental malformation of the cerebral cortex; FCDs are highly epileptogenic brain lesions and are a frequent cause of drug resistant epilepsy, usually with childhood onset [46]. Children with FCD may present both focal seizures and IS. Although the IS are easily controlled by AEDs, the prognosis for focal epilepsy is poor, with high refractoriness, depending on the intrinsic epileptogenicity of the lesion [47]. FCD belongs to the new category of mTORopathies, in which the mTOR dysregulation plays a key role in determining the epilepsy phenotype. Interestingly, balloon cells in FCD IIB exhibit activation of the mTORC1 signaling pathway, suggesting that FCD IIB is histologically similar to TSC [48]. Abnormalities in hamartin and tuberin expression as well as other mTOR markers have been found in FCD, indicating a pathogenic relationship to TSC [48,49]. In an animal model of FCD, Ljungberg et al. demonstrated that a short treatment with rapamycin was able to strongly suppress seizures severity and duration via mTOR pathway inhibition [50]. Recently, human papillomavirus type 16E6, a potent activator of mTOR signaling, was detected in human FCD IIB, suggesting that human papillomavirus expression during fetal brain development may have intrinsic and increased epileptogenicity in FCD and may represent a potential antiepileptogenic target [51].

Genetic epilepsies
Pten models
Pten is a tumor suppressor gene acting as an upstream regulator of TSC1/2 complex. Mutations of Pten are associated to a variety of human diseases such as Cowden syndrome and Lhermitte–Duclose, which are characterized by tumors and hamartomas associated with epilepsy [52]. Mouse models created by inactivation of Pten are usually characterized by megalencephaly, neuronal hypertrophy, seizures and premature death [53–55]. Ljungberg et al. demonstrated that histopathological abnormalities and neuronal hypertrophy can be reverted by treatment with mTOR inhibitors [50]. Furthermore, in these animal models, rapamycin administration was able to prolong survival, suppress seizures and improve neurodevelopmental outcome, also reversing autism-like features [41,50]. Antiseizure effects appeared to be strictly linked to the period of administration, with seizures appearing again after drug withdrawal [56].

Tuberous sclerosis
Seizures are the most common neurological symptom in TSC, affecting up to 90% of patients. Epilepsy in TSC often begins during the first years of life and, in most cases, in the very first months. Focal seizures may precede, coexist or evolve, into infantile spasms [57]. Early onset seizures are associated with a
high risk of later neurodevelopmental disabilities, such as mental retardation and autism spectrum disorders [57]. A prompt treatment with vigabatrin soon after seizure onset may be able to minimize these cognitive sequelae, improving the long-term outcome, but it is not able to totally revert the learning disability [58,59]. Interestingly, vigabatrin has been shown to exert part of its antiseizure action by reducing mTOR overactivation, and this may account for the unique efficacy of this drug for TSC-associated epilepsy [60].

Tavazoie et al. demonstrated that loss of Tsc1 or Tsc2 triggered enlargement of somas and dendritic spines, altering the properties of glutamatergic synapses [8]. Mutation of either the TSCI or TSC2 gene activates the mTOR pathway, inducing fundamental alterations in network properties as well as an imbalance in excitation/inhibition [8]. mTOR dysregulation in TSC is able to directly influence several downstream mechanisms, such as modification of neurotransmitter receptors and ion channel expression, as well as synaptic and neuronal organization, finally leading to epileptogenesis [24,61]. Cortical tubers are characterized by the presence of dysplastic neurons, giant cells and bizarrely shaped astrocytes [57]. Neuronal populations in the context of cortical tubers have intrinsic epileptogenicity, generating seizures by releasing neurotransmitters into the adjacent brain tissue [62]. New evidence suggests that a significant reduction in the potential for synaptic plasticity occurs in the hippocampus of mutant TSC2 rats, and that the deficit in synaptic plasticity could be even more pronounced following recurrent seizures [63].

**Preclinical studies**

Most of the actual knowledge on the relationship between mTOR and epilepsy comes from TSC, which widely serves as a model to better understand the key mechanisms linking together mTOR activation and epileptogenesis. The principal findings of animal models of TSC treated with mTOR inhibitor rapamycin are summarized in Table 2.

The first evidence of a potential antiepileptogenic effect of mTOR inhibitor rapamycin comes from a mouse model of TSC1 inactivation in glial cells [64]. In this TSCI<sup>Cre</sup> knockout mouse model, spontaneous seizures began at approximately 3 weeks of age and progressively worsened. Rapamycin was administered according to two different protocols, both before and after seizure onset. In the early-treated mice, rapamycin prevented seizure onset and premature death, reversing neuronal disorganization and astrogliosis [64]. On the other hand, late treatment with rapamycin at a symptomatic stage determined a reduction in seizure frequency and severity, and also a prolonged survival [64]. In a different study using a mouse neuronal TSC model, Meikle et al. have shown that mTOR inhibitors were highly effective on cell size and myelination restoration by acting on mTOR and Akt signaling. Interestingly, also in this study, early treatment proved effective in preventing development of epilepsy and prolonging survival, while late treatment suppressed existing seizures and prolonged survival [65]. These results have been subsequently replicated in other studies using different TSC mouse models [66,67], thus reinforcing the concept that mTOR inhibition in TSC has disease-modifying potential. Recent data also demonstrated that mTOR inhibition has the potential to reduce the severity of some of TSC-associated comorbidities, such as anxiety and mood disorders, in concordance with EEG power spectra changes [68].

The efficacy on histopathological and molecular abnormalities appear to be widely different between various experiments, thus partially explaining the antiepileptic versus antiepileptogenic effects observed in different treatment protocols. Rapamycin could act as an antiepileptogenic agent when it is able to alter the underlying pathological processes suggesting that timing of administration is crucial [66]. In the data provided by Zeng et al., rapamycin treatment after seizure onset exerted a clear antiepileptic effect without reverting the histopathological abnormalities [64].

Mutation of TSC genes, through downstream effect on neuronal and synaptic structures/neurotransmission, induces fundamental alterations in network properties, leading to epilepsy and cognitive and behavioral comorbidities [69]. Therefore, in this view, mTOR inhibition could potentially be useful to revert or minimize TSC-related cognitive impairment. Ehninger et al. showed that the poor spatial learning in adult Tsc2<sup>-/-</sup> mice was reversed following a brief treatment with rapamycin [70]. This preliminary evidence has been confirmed in a recent study on a non-TSC mouse model by Talos et al. that demonstrated that mTOR inhibition prevented autistic-like features following neonatal seizures, offering a potential treatment for neurodevelopmental comorbidities associated with epilepsy [71].

Different CNS lesions are already present during fetal life, as a consequence of early mTOR hyperactivation [72]. Tsai et al. demonstrated that TSC1 deletion results in cortical malformations, increased cell volume and focal cortical lamination defects of the cortical layers II–III [73]. They showed for the first time that mTORC1 and mTORC2 signaling is activated in fetal tubers. They also showed that migratory defects, focal cortical dyslamination and cytomegaly following TSC1 loss are prevented with rapamycin treatment in utero. These exciting results suggest that mTORC1 inhibition during fetal development could theoretically prevent or reduce the severity of the neurological phenotype associated with TSC [73]. Andrerl et al. administered rapamycin to pregnant dams, observing a reduced lethality of mutant Tsc1<sup>-/-</sup> Nes-cre<sup>+/-</sup> mice [74]. The extension of treatment after birth was able to extend survival, completely suppressing the mTOR overactivation, but treated mice presented very poor weight gain, developmental delay and severe neurological symptoms [74]. The differential effects of prenatal and/or postnatal administration of rapamycin on neurodevelopmental defects and cognition in a neoglial mouse model of TSC were studied by Way et al. [75]. While postnatal treatment had little effect on antenatal neuronal migration defects, they observed a significant benefit on histopathological abnormalities when rapamycin was administered both before and after birth. However, they also found that mice treated only after birth obtained better performances in learning and memory tasks [75]. These interesting results suggest a developmental window of opportunity for the perinatal rescue of TSC pathology with rapamycin [75].
Table 2. Effects of rapamycin in tuberous sclerosis complex-related neurologic manifestations and focal cortical dysplasia.

<table>
<thead>
<tr>
<th>Model</th>
<th>Timing</th>
<th>Cell size/number</th>
<th>Neuropathologic effects</th>
<th>Effects on Neurocognitive</th>
<th>Survival</th>
<th>Persistent efficacy after withdrawal</th>
<th>Neurocognitive</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsc1GFAPCKO mice</td>
<td>P14 pre-epilepsy</td>
<td>Yes</td>
<td>Neuronal dispersion, Glt-1 expression</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Tsc1Emx-Cre CKO mice</td>
<td>P13-P40</td>
<td>Yes</td>
<td>Increased cortical myelination</td>
<td>NA</td>
<td>NA</td>
<td>Severe developmental delay</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tsc1cc Nes-Cre+ mice</td>
<td>Pregnant dams</td>
<td>No</td>
<td>Increase in layer IV-V cell density</td>
<td>NA</td>
<td>NA</td>
<td>Improved social interaction</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>Tsc1+/- mice</td>
<td>Adult NA</td>
<td>Yes</td>
<td>Focal cortical dysplasia</td>
<td>Yes</td>
<td>Yes</td>
<td>For at least 3 weeks</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>Tsc2+/- mice</td>
<td>Prenatal and postnatal</td>
<td>No</td>
<td>Neuronal cell density in cerebral cortex</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tsc1+/- and Tsc2+/- mice</td>
<td>Focal cortical dysplasia</td>
<td>No</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Clinical studies

A number of studies have investigated the effect of sirolimus and everolimus on controlling the appearance and progression of TSC-related tumors. In a recent double-blind Phase III placebo-controlled study, everolimus proved to be an effective and safe drug for the treatment of TSC-associated subependymal giant cells astrocytomas (SEGAs) [76]. There are still limited data on the effect of mTOR inhibition in TSC-related epilepsy. A young child with clusters of intractable seizures despite previous tuberectomy was treated with rapamycin with a significant decrease in seizure frequency and severity [77]. In another child treated with everolimus for a regrowing SEG, a cessation of previously intractable seizures was reported at the follow-up of 12 months [78]. A prospective trial evaluating safety and efficacy of everolimus in decreasing the size of SEGAs, enrolled 28 patients followed-up for 6 months [79]. Sixteen of these patients also presented active epilepsy. The proportion of patients experiencing seizures on a daily basis decreased from 27% at baseline to 8% 6 months after everolimus administration; beneficial changes in 24-h video-EEG in terms of reduction of awake and asleep interictal activities were also observed [79]. Furthermore, quality of life in childhood epilepsy questionnaire showed an improvement from baseline at 3 and 6 months [79].

In general, mTOR inhibitors were well tolerated in all these studies. All patients reported at least one adverse event, such as aphthous ulcers, fatigue, rash, mucositis, anorexia, diarrhea, nausea, arthralgias, thrombocytopenia and effects on lipid metabolism [80]. However, these events were mostly grade 1 or 2 in severity and usually transient, never requiring everolimus withdrawal [80,81].

Further data from larger, prospective trials are now needed to establish the clinical efficacy, the optimal dosage regimens, and the safety profile of mTOR inhibitors in TSC. A new randomized, double-blind, placebo-controlled study of the efficacy and safety of two trough-levels of add-on everolimus in patients with tuberous sclerosis-associated refractory focal-onset seizures is in the pipeline [101]. In TSC and FCD mTOR inhibition could represent a rational therapy, and targeting the mTOR signaling pathway appears to be a promising treatment option for epilepsy associated with these disorders.

Expert commentary

Currently, despite the use of old and new AEDs, approximately a third of children with epilepsy still continue to present seizures. A major limitation for available AEDs is that they act primarily on the molecular mechanisms that mediate the endstage of epilepsy, which is the occurrence of seizures. Malformations of cortical development are a significant source of refractory epilepsy and the treatment of epilepsy associated with these malformations remains a major challenge. Although resective surgery may offer benefits in selected patients, no cure is currently available for neurocognitive dysfunction or behavioral symptoms associated with epilepsy, suggesting that there is an urgent need for new biologically targeted treatment options. mTOR inhibition has been tested in various mouse models with striking therapeutic effects, even on established symptoms. Different data confirm that mTOR
activation may play a pathogenetic role in the epileptogenesis of acquired forms of epilepsy such as IS, and that mTOR inhibition may act as a disease-modifying agent, and may also benefit cognitive and behavioral problems associated with epilepsy. Data from Galanopoulou's laboratory showed that mTOR inhibition may exert an 'epileptostatic' effect, being able to prevent epilepsy-related pathology and even the development of epilepsy itself. However, the withdrawal of treatment might determine the reappearance of symptoms, thus suggesting that chronic treatment would be necessary to maintain efficacy [3,4]. Successful anticonvulsant effects of both rapamycin and everolimus have been reported in individuals with focal-onset seizures in the context of TSC. Placebo-controlled studies are now needed to verify these preliminary findings. Several aspects can potentially limit the use of mTOR inhibitors in the clinical practice. Since the effect of long-term mTOR inhibitor pharmacotherapy on brain structure and function is not known, the potential side effects of chronic exposure to the drugs should be taken into account. Furthermore, mTOR inhibitors may have complex and clinically relevant interactions with some AEDs such as carbamazepine, oxcarbazepine and topiramate, frequently used alone or in combination in patients with TSC. Finally, there are still limited data of the effect of mTOR inhibitors in TSC patients with epilepsy not treated for SEGAs, and more rigorous clinical trials on epilepsy as the primary outcome are needed.

What is the current place for mTOR inhibition in seizures control and what the optimal timing is remains unknown. Further data from larger, prospective trials will help to establish the clinical efficacy, optimal dosage regimens and safety profile of mTOR inhibitors in epilepsy, and more clearly define their role in this setting.

Five-year view
Targeting the mTOR pathway with mTOR inhibitors can be a promising treatment option for patients with epilepsy. Animal models have shown that mTOR hyperactivation is not always associated with epileptogenesis in animal models, and in some cases, mTOR inhibition has no effects. More definite data from animal models are now needed to clarify when mTOR activation leads to structural and functional changes that predispose to abnormal synaptic excitability that favor seizure onset and when the optimal timing is for the mTOR inhibition option. Further studies will clarify whether rapamycin is directly anticonvulsant, leading to a decrease of cortical excitability, or whether its effect is due to circuitry reorganization. The search for more selective agonists targeting specific effectors of the mTOR pathway can lead to the discovery of better drugs to treat epilepsy. mTOR inhibition is an exciting area of research, and hopefully, pharmacotherapy manipulation that targets the developing brain can potentially prevent or rescue the structural and functional changes resulting from loss of TSC genes with potential benefit on epilepsy and its comorbidities. The prenatal or perinatal diagnosis of TSC and hemimegalencephaly make an early postnatal treatment with Everolimus theoretically possible. In particular, the diagnosis of TSC is made before seizure onset in a growing number of patients, allowing close EEG monitoring during the first months of life, in order to have an early detection of seizures and start treatment as soon as possible. Presently, early postnatal treatment with vigabatrin increases the chances of successful seizure control and potentially reduces the risk of severe learning disabilities and autism-like symptoms, but it is not able to completely revert the negative neuropsychological impact. Of course it is still too early to consider a human prenatal treatment, since limited data on in utero exposure to rapamycin are available. Since mTOR is crucial for normal brain development, marked toxicity of mTORC1 inhibitors during the fetal period may prevent its usage. Currently there are no clinical data on the efficacy of mTOR inhibitors in infants with IS; in the next few years, clinical trials of mTOR inhibitors in infants with IS...
due to mTORopathies are needed. Starting the treatment at an early age, possibly even at infancy, might prevent the development of tumors, epilepsy and other disease manifestations associated with TSC. Furthermore, from a clinical point of view, we still lack significant data on the efficacy and long-term safety of mTOR inhibitors given during early development. Currently, one of the problems of treatment with mTOR inhibitors is that while mTORC1 is rapamycin sensitive, mTORC2 is refractory to presently available drugs. Other inhibitors of mTOR signaling pathway are now in development. In particular, ATP-competitive inhibitors that directly target the mTOR catalytic site display potent and comprehensive mTOR inhibition[82]. The identification of genetic biomarkers could help us identify patients which could benefit more from an early mTOR inhibition, thus allowing an optimized therapeutic regimen minimizing side effects. In the next years, further clinical trials should clarify whether low dosage or intermittent pharmacotherapy may maintain an antiepileptic effect avoiding unwarranted adverse events.

**Financial & competing interests disclosure**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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- Provided the first clear evidence that rapamycin may exert an antiepileptogenic effect in animal models of tuberous sclerosis complex (TSC).

- Demonstrated that combined pre- and postnatal rapamycin treatment resulted in almost complete histologic rescue.

- Provided clear evidence that neurobiological effects of chronic rapamycin treatment in a mouse model of Tuberous Sclerosis Complex.


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