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REVIEW



## DEPDC5 as a potential therapeutic target for epilepsy

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### ABSTRACT

**Introduction:** Dishevelled, Egl-10 and Pleckstrin (DEP) domain-containing protein 5 (DEPDC5) is a protein subunit of the GTPase-activating proteins towards Rags 1 (GATOR1) complex. GATOR1 is a recently identified modulator of mechanistic target of rapamycin (mTOR) activity. mTOR is a key regulator of cell proliferation and metabolism; disruption of the mTOR pathway is implicated in focal epilepsy, both acquired and genetic. Tuberous sclerosis is the prototypic mTOR genetic syndrome with epilepsy, however GATOR1 gene mutations have recently been shown to cause lesional and non-lesional focal epilepsy.

**Areas covered:** This review summarizes the mTOR pathway, including regulators and downstream effectors, emphasizing recent developments in the understanding of the complex role of the GATOR1 complex. We review the epilepsy types associated with mTOR overactivity, including tuberous sclerosis, polyhydramnios megalencephaly symptomatic epilepsy, cortical dysplasia, non-lesional focal epilepsy and post-traumatic epilepsy. Currently available mTOR inhibitors are discussed, primarily rapamycin analogs and ATP competitive mTOR inhibitors.

**Expert opinion:** DEPDC5 is an attractive therapeutic target in focal epilepsy, as effects of DEPDC5 agonists would likely be anti-epileptogenic and more selective than currently available mTOR inhibitors. Therapeutic effects might be synergistic with certain existing dietary therapies, including the ketogenic diet.

### ARTICLE HISTORY

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### KEYWORDS

DEPDC5; focal epilepsy; NPRL2; NPRL3; GATOR1; mechanistic target of rapamycin; tuberous sclerosis; cortical dysplasia; rapamycin

## 1. Introduction

Dishevelled, Egl-10, and Pleckstrin (DEP) domain-containing protein 5 (DEPDC5) is a subunit of the GTPase-activating proteins toward Rags 1 (GATOR1) complex. Loss of GATOR1 impairs inactivation of mechanistic (formerly mammalian) target of rapamycin (mTOR) complex 1 (mTORC1) [1]. The mTOR pathway plays a major role in the regulation of cell proliferation and cell metabolism. Disruption of mTOR pathway activity has been implicated in the pathogenesis of certain cancers as well as many genetic syndromes associated with tumors, including tuberous sclerosis, polyhydramnios megalencephaly symptomatic epilepsy syndrome (PMSE), neurofibromatosis 1 and 2, Peutz–Jeghers syndrome, Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome, Proteus syndrome, Lhermitte–Duclos syndrome, and von Hippel–Lindau syndrome [2–8].

The prototypic mTOR disease associated with epilepsy is tuberous sclerosis, with the majority of affected individuals having seizures [9–11]. This condition provided the initial motivation to consider the possible antiepileptic properties of medications targeting the mTOR pathway. Applicability at first appeared limited to individuals with tuberous sclerosis, a rare disease with an estimated incidence of 1 in 5800 live births [12]. However, the relatively recent observation of mutations in mTOR pathway genes in focal epilepsies raises the

possibility that mTOR inhibitors could have far wider therapeutic benefit for people with epilepsy.

Focal epilepsy syndromes with and without malformations have recently been associated with mutations in all three genes encoding proteins that make up the GATOR1 complex: *DEPDC5* (OMIM #614191), *NPRL2* (OMIM #607072), and *NPRL3* (OMIM #600928) [13–17]. The latter two genes encode nitrogen permeator regulator 2-like and nitrogen permeator 3-like proteins (NPRL2 and NPRL3).

In this paper, we review the current understanding of the role of the mTOR pathway in epilepsy, discuss current pharmaceutical options that elicit mTOR inhibition, and evaluate the potential of DEPDC5 as a therapeutic target in the treatment of seizures.

## 2. mTOR complexes and activation

The mTOR protein, encoded by *MTOR* (OMIM #601231) is a member of the phosphoinositide-3-kinase (PI3K)-related kinase family, having serine/threonine kinase activity [18–20]. This enzyme associates with other proteins to form one of two complexes, mTORC1 or mTORC2 [21]. mTORC2 will not be discussed in detail as it has not been clearly shown to be influenced by GATOR1 and is primarily involved in cytoskeletal organization, cell survival, and metabolism [21–23].

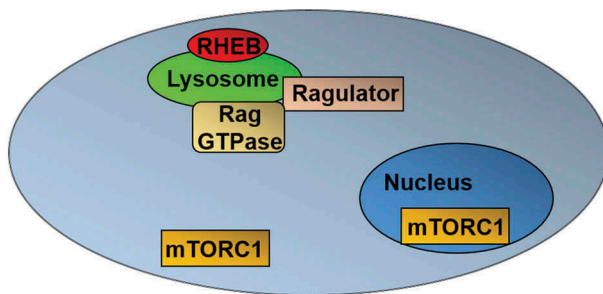
### Article highlights

- Dysfunction of the mTOR pathway occurs in tuberous sclerosis and other genetic syndromes, focal cortical dysplasia, non-lesional focal epilepsy, as well as post-traumatic epilepsy.
- DEPDC5 is a subunit of the GATOR1 complex, which selectively inhibits mTORC1 during periods of amino acid deprivation.
- Existing mTORC1 inhibitors such as rapamycin have shown promise in treatment of focal epilepsy associated with tuberous sclerosis, and might also be effective for other focal epilepsy syndromes.
- Agonists of DEPDC5 could theoretically have an anti-epileptogenic effect in certain types of focal epilepsy by inhibiting mTORC1 in an amino acid-dependent manner. This selective inhibition might allow for fewer side effects when compared to existing direct mTORC1 inhibitors (i.e. rapamycin).
- Though DEPDC5 agonism is an appealing therapeutic mechanism, considerable work in drug development is required, and such therapies would need to be superior to rapamycin analogs or other existing therapies in terms of efficacy and tolerability.

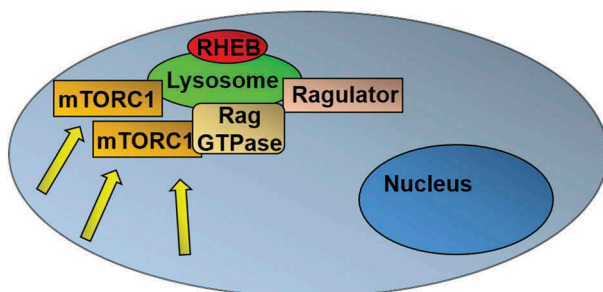
This box summarizes key points contained in the article.

mTORC1 is a large complex, containing six known protein components, mTOR, lethal with sec-13 protein 8 (LST-8) [24], DEP domain containing mTOR interacting protein (DEPTOR) [25], regulatory-associated protein of mTOR (RAPTOR) [26,27], protein-rich Akt substrate 40 (PRAS40) [28–31], and Tti1/Tel2 [21,32,33]. The mTORC1 multi-protein complex may be found in the cytoplasm or nucleus, but, in the presence of increased local amino acid concentrations, translocates to the lysosomal surface (Figure 1). This process involves amino acid sensing within the lysosomal lumen and a complex signaling cascade involving a vacuolar H<sup>+</sup>-adenosine triphosphatase (v-ATPase),

#### a) LOW AMINO ACIDS



#### b) HIGH AMINO ACIDS



**Figure 1.** mTORC1 Translocation to Lysosomal Membrane: When local amino acid concentrations are low, mTORC1 is distributed in the cytoplasm and within the nucleus. With increasing amino acid levels, mTORC1 translocates to the lysosomal membrane and is activated by RHEB.

Ragulator complex and Rag guanosine triphosphatases (GTPases) [34,35]. Once at the lysosomal surface, mTORC1 is activated by GTP-binding protein Rheb (RHEB) [33].

Once activated, mTORC1 acts primarily through phosphorylation of S6K1 and 4E-BP1 (Figure 2). These proteins are primarily involved in modulation of mRNA translation and protein synthesis [36–38], though S6K1 also has a role in regulation of lipid synthesis [39]. A third molecular target of mTORC1 is unc-51-like kinase 1/mammalian autophagy-related gene 13/focal adhesion kinase family-interacting protein of 200 kDa (ULK1/Atg13/FIP200), a kinase complex that plays a key role in autophagy [40–42].

Because mTORC1 activation is dependent on local amino acid concentrations, enzymatic activity is lower in times of cellular stress such as nutrient deprivation. Inhibition of mTORC1 activity triggers autophagy, and conversely autophagosome formation decreases with mTORC1 activation. Though the mechanisms by which these changes occur are not entirely clear, the interaction between ULK1/Atg13/FIP200 and mTOR appears to be crucial [33,40].

### 3. mTORC1 regulation

Control of mTORC1 activity is complex, and dependent on cellular energy and nutrient reserves, as illustrated in Figure 3. There are multiple actors, both direct and indirect, including the tuberous sclerosis-related proteins, TSC1 and TSC2, as well as other proteins known to be disrupted in the other mTOR-related genetic syndromes. The main elements that have direct activity on mTORC1 are AKT, RHEB, and GATOR1 (Figure 3), with the latter being the focus of this review.

The GATOR complex is composed of GATOR1 (DEPDC5, NPRL2, NPRL3) and GATOR2 (Mios, WDR24, WDR59, SEH1L, SEC13) sub-complexes (Figure 4) [1]. When local amino acid concentrations are low, GATOR1 blocks mTORC1 activation. However, with increasing local amino acid levels, GATOR2 inhibits GATOR1, resulting in mTORC1 disinhibition. Consequently, disruption of GATOR1 results in mTORC1 activation becoming insensitive to amino acid deprivation [1,33,43].

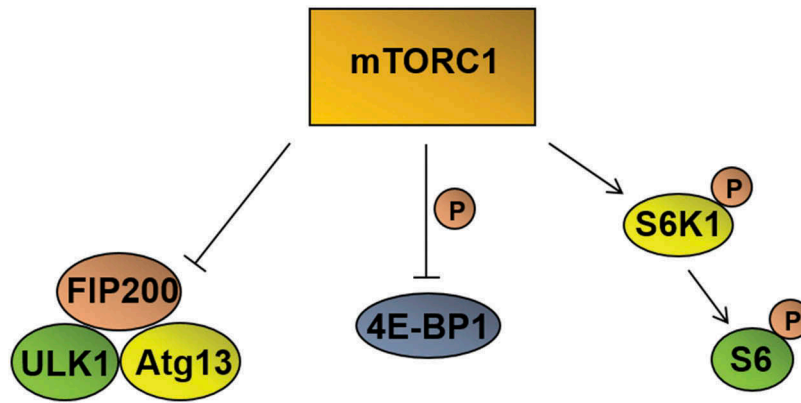
In a rat DEPDC5-knockout model, homozygotes died in utero, and displayed dramatically increased mTORC1 activity [44]. Heterozygotes survived and had increased cortical neuron excitability and brain pathology resembling focal cortical dysplasia (FCD).

### 4. mTORC1 and brain pathology

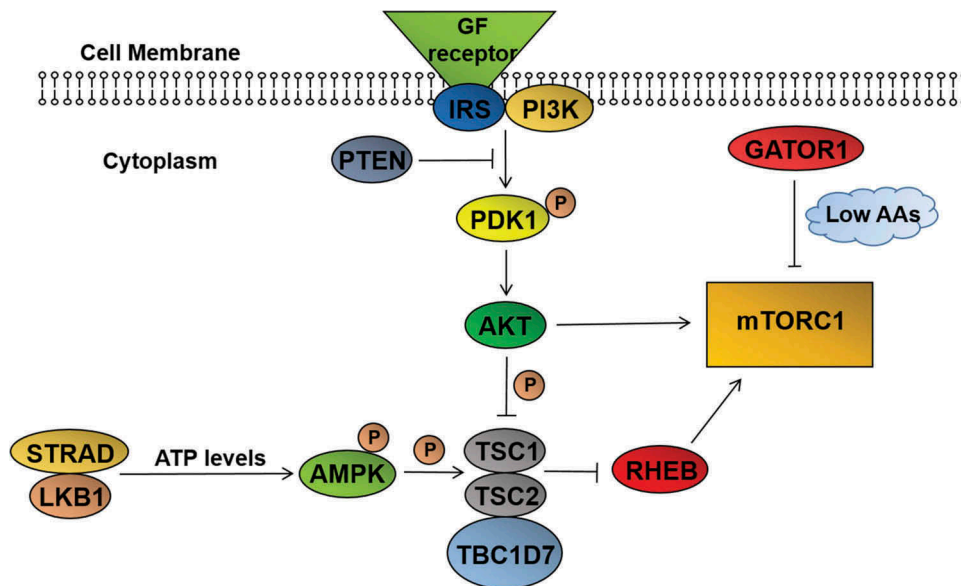
Disruption of the mTOR pathways can result in epilepsy with or without structural abnormalities. Three important conditions to consider are tuberous sclerosis, FCD, and focal epilepsy with normal neuroimaging. We will also briefly discuss the very rare clinical entity of PMSE, as well as posttraumatic seizures and epilepsy.

#### 4.1. Tuberous sclerosis

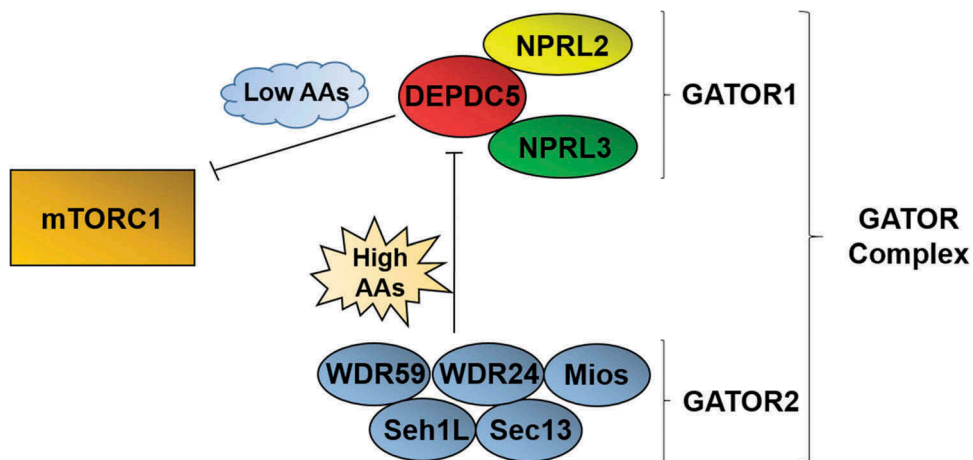
Tuberous sclerosis is an autosomal dominant multisystem disorder caused by mutations in one of two genes, *TSC1* or *TSC2*



**Figure 2.** mTORC1 Downstream Effects: The three primary molecular targets of mTORC1. Phosphorylation (denoted by P in circle) activates S6K1 which in turn phosphorylates S6, a ribosomal protein. Eukaryotic translation initiation protein 4E binding protein 1 (4E-BP1) is inhibited by phosphorylation. ULK1/Atg13/FIP200 is also inhibited, resulting in reduced autophagosome formation.



**Figure 3.** mTORC1 Regulation: Direct and indirect modulators of mTORC1 activity. Decreased cellular ATP results in decreased mTORC1 activation via STRAD-LKB1 complex activity. Figure adapted from Crino 2016 [33].



**Figure 4.** GATOR Modulation of mTORC1: GATOR1 inhibits mTORC1 activation when local amino acid levels are low. GATOR2 inhibits GATOR1 with elevated amino acid concentrations.

[45,46]. The multisystem clinical presentation may involve any or all of skin abnormalities (i.e. angiofibromas, hypopigmented macules, shagreen patches, confetti lesions, ungual fibromas), cardiac rhabdomyomas, renal cysts or angiomyolipomas, lymphangioleiomyomatosis, eye abnormalities (i.e. retinal nodular hamartomas, achromic patches), and oral abnormalities (i.e. enamel pitting, intraoral fibromas) [47]. Central nervous system pathology involves formation of nonmalignant tumors, including cortical tubers, subependymal nodules and subependymal giant cell astrocytomas (SEGA) [47].

Epilepsy occurs in more than 80% of individuals with tuberous sclerosis, typically beginning in infancy, and most frequently involving focal seizures and epileptic spasms [9–11]. Spasms are usually accompanied by hypsarrhythmia and developmental plateau or regression, the classic triad of West syndrome. Treatment of epilepsy in tuberous sclerosis has until recently involved traditional antiepileptic medications and occasional surgical removal of tubers [48].

Seizures in patients with tuberous sclerosis are presumed to originate primarily from cortical tubers, hamartomatous lesions located at the surface of the brain, and the perituberal tissue. Histopathologic analysis reveals that these lesions exhibit cortical disorganization and are composed of dysmorphic neurons, giant cells, and abnormal white matter [49–51]. These were originally considered to be static lesions representing disordered neuroblast migration and organization; however, there is evidence that cortical tubers are dynamic entities that evolve over time. Cyst-like cortical tubers progress on serial magnetic resonance imaging (MRI), and display reactive gliosis on pathologic analysis consistent with a chronic process [52]. Furthermore, molecular analysis reveals that tuberous tissue has elevated mTOR activity, when compared to perituberal tissue [53].

#### 4.2. Cortical dysplasia

FCD are discrete malformations of cortical development from which seizures frequently originate. Lesions are classified as type I–III based on pathologic characteristics including dysmorphic neurons, cortical dyslamination, presence/absence of balloon cells, and association with other abnormalities [54]. The appearance on MRI varies based on type, and can be very subtle and often initially not detected. Common features include T2 hyperintensity, abnormally deep sulcus, transmantle sign, cortical thickening, and blurring of the gray-white matter border [55].

The underlying cause of FCD is usually unknown; however, genetic causes have recently been identified. Somatic and germ line mutations in mTOR pathway genes were the first identified genetic etiology. Somatic mutations in *DEPDC5* [56] and *MTOR* [57,58] have been identified in sporadic FCD, as well as a 1q21.1-q44 duplication affecting *AKT3* [59].

Where more extensive dysplasia is found such as hemimegalencephaly (also known as hemispheric cortical dysplasia), somatic 1q duplication, and missense mutations in *MTOR*, *AKT3*, and *PIK3CA*, have also been identified [60–63]. In this condition, an entire hemisphere is abnormally large and malformed and affected individuals typically have severe refractory epilepsy. Extending the spectrum further, the rare

megalencephaly syndromes, megalencephaly-capillary malformation and megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndromes, have also been associated with both germ line and somatic mutations in mTOR genes including *AKT3*, *PIK3CA*, and *PIK3R2* [64,65]. As with cortical tubers in tuberous sclerosis, mTOR activity is elevated in resected dysplastic tissue [60,66–68].

Germ line mutations in mTOR pathway genes have also been identified in familial FCD. Although familial reports of FCD [69] were thought by some to be coincidental, mutations in all three GATOR1 genes, *DEPDC5*, *NPRL2*, and *NPRL3*, have recently been implicated in individual families [14,56,70–72]. A recent study found that mutations in these three genes account for 3/14 (21%) cases of familial focal epilepsy with cortical dysplasia [14]. Individuals with *de novo* germ line *DEPDC5* mutations have also been found with FCD and epilepsy [73,74].

These exciting findings nevertheless raise the intriguing question of how focal brain malformations result from a germ line mutation present in all cells in the body. One hypothesis is that individuals carrying a mutation in a GATOR1 gene are susceptible to a ‘second hit,’ be that genetic or environmental. Brothers, one with quadrantic and one with hemispheric FCD, shared a germ line *DEPDC5* mutation with their father and paternal uncle who had non-lesional focal epilepsy [70]. The brothers also inherited *DEPTOR* and *NF1* mutations, suggesting that multiple mutations interacted to produce FCD. Alternatively, the second genetic hit may be somatic, as illustrated by a case in which a patient with FCD had an inherited germ line *DEPDC5* mutation and a second, somatic, *DEPDC5* mutation in the dysplastic tissue [56].

Recent studies demonstrate that somatic brain mutations are much more common than previously thought. Using single cell genetic analysis tools, researchers have demonstrated that normal human brains contain a surprising degree of genetic heterogeneity with 1500 single nucleotide variants present in each neuron. Somatic copy number variants and mutations are found in brain tissue of normal people [43,75–77]. Each human brain has clonal mosaicism where somatic mutations may be limited to individual neurons, a specific gyrus or an entire hemisphere [76,77]. It is possible that this genetic variation may be protective against deleterious mutations in some instances, and may actually prevent more widespread disease.

These findings provide further support for the ‘second hit’ explanation of FCD occurring with germ line mutations in GATOR1 genes. Whether the second hit must occur in a GATOR1 gene, another mTOR pathway gene, or via other genetic or environmental mechanisms has yet to be determined.

#### 4.3. Non-lesional focal epilepsy

Non-lesional focal epilepsy is a common clinical entity that can be sporadic or familial. The first epilepsy gene, *CHRNA4*, was discovered 21 years ago and is associated with a focal epilepsy syndrome, autosomal dominant sleep-related hypermotor epilepsy [78,79]. Since then a number of other genes related to function of neurotransmitter receptors or ion channels have

been implicated in non-lesional focal epilepsy, including *GRIN2A* [80–83], *GRIN2B* [84], *CHRNA2* [87], *KCNT1* [88,89], *LGI1* [90], and *SCN1A* [91].

Mutations in *DEPDC5* were first identified in non-lesional familial autosomal dominant focal epilepsy [13,92]. Familial focal epilepsy with variable foci differs considerably from other genetic focal epilepsies in that seizure type varies considerably between individuals, rather than showing phenotypic homogeneity [13]. *DEPDC5* mutations are estimated to account for 12% of cases of familial focal epilepsy [13]. Sporadic cases of non-lesional focal epilepsy may also occur due to *de novo* germ line *DEPDC5* mutations [73].

Similarly, mutations of the other two GATOR1 genes, *NPRL2* and *NPRL3*, have been associated with familial FCD. These two genes are each estimated to account for 1–2% of focal epilepsy cases [15,93]. Given the finding of mutations in all three GATOR1 genes in both lesional and non-lesional FCD, it is tempting to speculate that the underlying substrate may be very subtle FCD not appreciable on current neuroimaging.

#### 4.4. Polyhydramnios megalencephaly symptomatic epilepsy (PMSE)

PMSE (OMIM #611087) is a rare autosomal recessive neurodevelopmental syndrome described in Old Order Mennonite individuals in the northeastern USA [8]. Clinical features include refractory focal epilepsy, macrocephaly, and severe cognitive impairment. Brain tissue is abnormal, with neuronal heterotopia, enlarged neurons, and increased mTORC1 activity [94]. Affected individuals are homozygous for a truncating intragenic deletion of *LYK5/STRADA* [8]. This gene encodes STRADA, and mutation affects association with serine threonine kinase-11 (STK-11, also known as LKB5), impairing formation of the STRAD-LKB5 complex, an upstream regulator of mTORC1 (Figure 1) [95].

#### 4.5. Posttraumatic seizures and epilepsy

Following traumatic brain injury (TBI), seizures may occur immediately or after an interval of time. The latter is posttraumatic epilepsy, the underlying pathophysiology of which is an ongoing area of study [96]. Among the many identified biological changes that occur in the brain following injury, some involve mTORC1 activation. Axon sprouting, increased synthesis and phosphorylation of proteins, cell migration, and neurogenesis may all contribute to epileptogenesis, and have been identified following traumatic injury [97]. Studies using a mouse model of TBI have demonstrated that rapamycin, a direct mTORC1 inhibitor, can modulate post-injury changes in GABAergic inhibition, reduce mossy fiber sprouting and excitation of dentate granule cells, while also reducing seizure frequency [97,98]. The significance of the GATOR1 complex signaling in posttraumatic epilepsy has not yet been specifically investigated, however, so the importance of *DEPDC5* remains unclear.

### 5. mTORC1 modulation as a therapeutic target for epilepsy

There are a number of existing pharmaceutical agents that modulate mTORC1 activity. This is an exciting drug class as the

effects are disease modifying (antiepileptogenic) versus the antiseizure medications which currently dominate pharmaceutical treatment of epilepsy. In this section, we will discuss the mechanism of action of selected mTORC1 modulators, and their potential utility in seizure treatment.

#### 5.1. Rapamycin and analogs

Recognition of the molecular pathology of tuberous sclerosis led to the use of drugs that directly inhibit mTORC1. Rapamycin (also known as sirolimus) is produced by a streptomycete, *Streptomyces hygroscopicus*, originally isolated from soil from Easter Island (Rapa Nui) [99]. Initially developed for its antifungal actions [100], rapamycin was subsequently discovered to have immunosuppressive properties as well [101].

Rapamycin and structural analogs (rapalogs), everolimus (Afinitor, Novartis), temsirolimus (Torisel, Wyeth), deforolimus (ARIAD/Merck) have different physiochemical properties but all directly inhibit mTOR (Figure 5) [102]. Rapalogs form a complex with FK 506-binding protein of 12 kDa (FKB12), and bind to a C terminus mTOR region known as FKB12-rapamycin binding (FRB) [103,104]. Binding of rapamycin-FKB12 to FRB primarily inhibits mTORC1; mTORC2 activity is largely unaffected though impairment of complex assembly leads to lower levels of mTORC2 with long-term treatment [105,106].

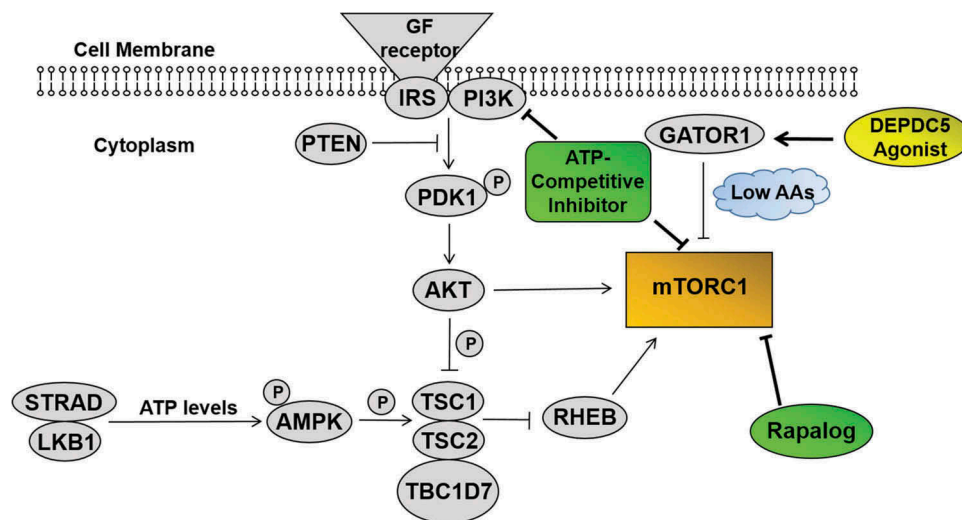
The antiseizure effects likely occur via inhibition of formation and growth of cortical tubers that are presumed to be epileptogenic. The underlying molecular basis for this is sound as mTORC1 overactivity has been demonstrated in resected cortical tubers from tuberous sclerosis patients, with mTORC1 activity higher in tuber versus perituberal tissue [53]. Antitumor effects of rapamycin have been demonstrated in tuberous sclerosis with studies showing shrinkage of SEGA and renal angiomyolipomas [107,108].

An alternate hypothesis is that the antiepileptic properties of rapamycin and analogs relate to immunosuppressive effects. Inflammation appears to play a role in the epileptogenesis in tuberous sclerosis, with microglial activation, increased inflammatory markers including IL-1 $\beta$ , and down-regulation of CD47 and CD200 observed in animal models [109–111]. The increases in IL-1 $\beta$  and other inflammatory cytokines and chemokines in the neocortex and hippocampus can be reversed with rapamycin [111].

Though clinical trials of rapamycin analogs in treatment of epilepsy have thus far focused on tuberous sclerosis, there is also evidence of benefit from one open-label study of a small cohort of patients with PMSE. Patients in that study had fewer seizures and improved receptive language after treatment with rapamycin [112]. Additionally, there is a mouse model of FCD involving knock out of neuron subset-specific Pten, in which rapamycin inhibits epilepsy progression, reduces seizure frequency, improves EEG, and prolongs survival [113].

#### 5.2. ATP competitive mTOR inhibitors

A number of agents have been developed that act by competitively binding to an ATP-binding cleft with the resultant inhibition of the kinase activity of both mTORC1 and mTORC2, as well as PI3K in some cases [114]. The



**Figure 5.** Pharmaceutical Inhibition of mTORC1: Rapalogs directly block mTORC1 activation via allosteric inhibition. ATP competitive mTOR inhibitors also non-specifically bind to an ATP-binding cleft, inhibiting both mTORC1 and PI3K in some cases. A DEPDC5 agonist would indirectly block mTORC1 activation by increasing mTORC1 sensitivity to decreased local amino acid concentrations.

resultant effects include cell cycle arrest in G1, thus these agents have been primarily used in oncology for their antitumor properties. *In vitro* studies demonstrated that one agent, INK128, decreased lipopolysaccharide-stimulated upregulation of inflammatory markers, IL-1 $\beta$  and IL-6, suggesting this class of drugs also modulates inflammatory mechanisms [115]. Given the broad mTOR inhibition, observation of anti-inflammatory properties is not surprising, as these are described with rapamycin as well. Though some of these agents have been studied in tuberous sclerosis for their tumor suppressive effects, there are no data regarding possible efficacy in treating seizures.

## 6. Expert opinion

Direct targeting of DEPDC5 represents an as yet unexplored avenue to modulate mTORC1 activity; however, whether such drugs would be superior to existing mTORC1 inhibitors remains unclear. In comparison to the allosteric inhibition of rapalogs and the broad kinase blockade of ATP competitive inhibitors, DEPDC5 modulation would be expected to *selectively* influence mTORC1 activity. This is an important distinction from the currently available medications which produce broader inhibition of mTORC1, independent of the local nutritional milieu. Drugs enhancing DEPDC5 activity, either directly or via GATOR2 antagonism, would be expected to most significantly enhance mTORC1 inactivation in times of amino acid deprivation.

This novel mechanism would likely result in clinical effects that differed from rapalogs, possibly with less immune suppression and increased autophagy. The antitumor effects are difficult to predict as nutrient availability is likely to be different within a neoplastic lesion when compared to normal tissue. DEPDC5 agonists would be expected to exert their greatest effects in the CNS, as protein levels have been shown to be highest in the brain, when compared to other organs [13]. Consequently, such agents could be effective in

treating epilepsy associated with tuberous sclerosis or PMSE, potentially with better side effect profiles than existing agents.

Nevertheless, the wider applicability of mTORC1 inhibitors in epilepsy treatment remains unclear. At present, mTORC1 inhibitors have only demonstrated success in treating epilepsy in the context of specific genetic syndromes, primarily tuberous sclerosis. While it is true that mutations in genes related to the mTOR pathway, including *DEPDC5* and other GATOR1 genes, have a causative role in both lesional and non-lesional focal epilepsies, the mechanism of epilepsy in these conditions may be similar to, or differ significantly from, tuberous sclerosis.

Cortical tubers are recognized to be dynamic entities that evolve over time; however, similar properties are not known to be present in cortical dysplasia. In these congenital brain malformations, mTORC1 likely contributes to disruption of neuroblast migration during brain development; its ongoing contribution to the epileptogenicity of these lesions needs to be further elucidated. Nevertheless, the observation of intralésional increased mTORC1 activity in FCD suggests mTORC1 modulation has therapeutic potential. More research is needed to investigate what role, if any, the GATOR complex plays during the complex biochemical and cellular changes that occur following TBI, and the therapeutic potential of mTORC1 modulators.

The relationship of DEPDC5 mTORC1 inhibition to amino acid levels raises the question of whether DEPDC5 agonists might have synergistic effects with dietary therapy. The ketogenic diet is often used in refractory epilepsy and has also shown potential in slowing tumor growth [116,117]. There are likely multiple mechanisms by which antiseizure effects occur, some of which may relate to mTORC1 inhibition. In rats, the ketogenic diet has been demonstrated to reduce mTORC1 activity [118], an outcome which may relate to simulating a cellular starvation milieu, and thus enhancing DEPDC5 inhibition of mTORC1. Use of DEPDC5 agonists could effectively lower the amino acid threshold at which mTORC1 activity is blocked.

If DEPDC5 agonism is pursued as a therapeutic avenue, determining the best pharmacologic approach will be crucial. An important consideration in drug development would be whether to develop agents that mimic the structure of DEPDC5 and thus inhibit mTORC1 independent of the native protein, versus agents that simply enhance the activity of native DEPDC5. The latter approach might be less successful in individuals carrying mutations of *DEPDC5* or the other *GATOR1* genes, *NPRL2* and *NPRL3*, as the genetic differences might have produced a structurally altered molecular target (*GATOR1*). Given that relatively little is currently known about DEPDC5 and *GATOR1*, development of a clinically useful DEPDC5 agonist will almost certainly be challenging.

In summary, DEPDC5 agonists have an exciting potential in focal epilepsy treatment as the effects are expected to be anti-epileptogenic, directly addressing the underlying etiology of seizure generation. There is currently good reason to believe that pharmaceutical agents that enhance DEPDC5 activity could be used in the treatment of tuberous sclerosis-associated epilepsy, given that direct mTORC1 inhibitors (rapalogs) have already shown effectiveness in human trials. DEPDC5 agonists also have the potential for effectiveness in treatment of focal epilepsy due to cortical dysplasia; however, the current evidence of effectiveness with other mTORC1 inhibitors is much more limited. Our evolving understanding of the contribution of mutations in mTOR-related genes to non-lesional focal epilepsy suggests that DEPDC5 agonists should be investigated in the focal epilepsies more broadly, which account for 60% of all epilepsies.

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