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Mechanistic Target of Rapamycin Pathway in Epileptic Disorders

Jang Keun Kim, B.S.,[1] Jeong Ho Lee, M.D., Ph.D.[1,2]

Biomedical Science and Engineering Interdisciplinary Program,[1] Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea
Graduate School of Medical Science and Engineering,[2] Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea

The mechanistic target of rapamycin (mTOR) pathway coordinates the metabolic activity of eukaryotic cells through environmental signals, including nutrients, energy, growth factors, and oxygen. In the nervous system, the mTOR pathway regulates fundamental biological processes associated with neural development and neurodegeneration. Intriguingly, genes that constitute the mTOR pathway have been found to be germline and somatic mutation from patients with various epileptic disorders. Hyperactivation of the mTOR pathway due to said mutations has garnered increasing attention as culprits of these conditions : somatic mutations, in particular, in epileptic foci have recently been identified as a major genetic cause of intractable focal epilepsy, such as focal cortical dysplasia. Meanwhile, epilepsy models with aberrant activation of the mTOR pathway have helped elucidate the role of the mTOR pathway in epileptogenesis, and evidence from epilepsy models of human mutations recapitulating the features of epileptic patients has indicated that mTOR inhibitors may be of use in treating epilepsy associated with mutations in mTOR pathway genes. Here, we review recent advances in the molecular and genetic understanding of mTOR signaling in epileptic disorders. In particular, we focus on the development of and limitations to therapies targeting the mTOR pathway to treat epileptic seizures. We also discuss future perspectives on mTOR inhibition therapies and special diagnostic methods for intractable epilepsies caused by brain somatic mutations.

Key Words : mTORC1 · mTORC2 · Epilepsy · Malformation of cortical development.

INTRODUCTION

The discovery of the mechanistic target of rapamycin (mTOR) began with the identification of new antimicrobial agents in soil samples from Rapa Nui (also known as Easter Island)[93]. This new antimicrobial agent, called rapamycin (clinically called sirolimus), was found to exhibit immunosuppressive and anti-cancer potential and to be of use as an antiepileptic drug[43,47,144]. For two decades, endeavors to define the function of mTOR revealed that mTOR coordinates environmental signals and metabolic activity by forming two distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2)[130]. mTOR was first found in brain lysates, suggesting the importance of the brain-specific function of mTOR[125]. Indeed, the mTOR pathway has been found to regulate a variety of functions in the brain from brain develop-

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• Address for reprints : **Jeong Ho Lee, M.D., Ph.D.**
Biomedical Science and Engineering Interdisciplinary Program, Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon 34141, Korea
Tel : +82-42-350-4246, Fax : +82-42-350-4240, E-mail : jhlee4246@kaist.ac.kr

ment to degeneration[85].

Human genetic studies of epileptic patients and epilepsy models of aberrant activation of the mTOR pathway have demonstrated the importance of activating mutations in the mTOR pathway in neurodevelopmental disorders with epilepsy[66]. With the discovery of mutations activating the mTOR pathway as genetic causes of medically intractable epilepsy[4], mTOR inhibitors, such as sirolimus and everolimus, have emerged as being of potential medical use in treating intractable epilepsy patients[33]. However, with the limited efficacy and significant drawbacks of clinically available mTOR inhibitors, new drugs that are more effective and tolerable based on the understanding of pathogenic mechanisms are required. Key discoveries in research on the role of the mTOR pathway in epilepsy are summarized in Supplementary Box 1.

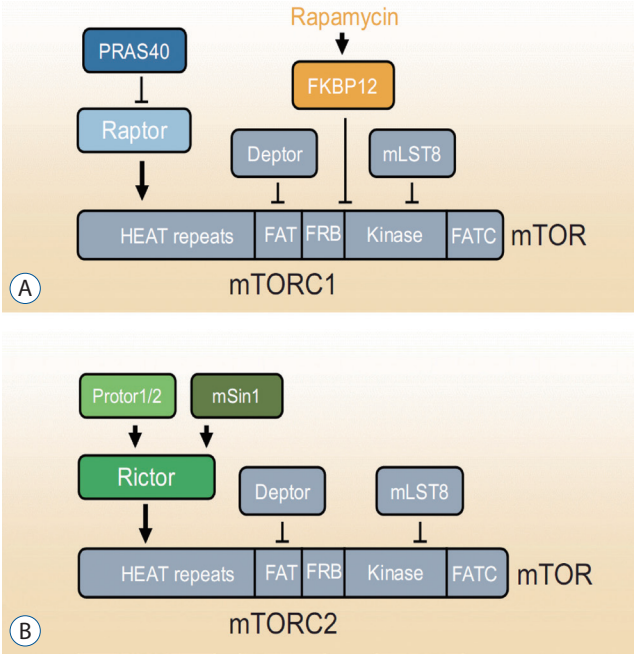

Fig. 1. mTORC1 and mTORC2. A : The components of mTORC1 and respective binding site on mTOR. B : The components of mTORC2 and respective binding site on mTOR. PRAS40 : proline-rich Akt substrate of 40 kDa, Raptor : regulatory protein associated with mTOR, FKBP12 : FK506 binding protein 12, Deptor : DEP domain-containing mTOR-interacting protein, mLST8 : mammalian lethal with Sec13 protein 8, mTOR : mechanistic target of rapamycin, mTORC1 : mTOR complex 1, mSin1 : mammalian stress-activated protein kinase-interacting protein, Rictor : rapamycin-insensitive companion of mammalian target of rapamycin, mTORC2 : mTOR complex 2.

THE MTOR PATHWAY

mTOR is a serine/threonine protein kinase that forms the core subunit of two functionally distinct protein complexes : mTORC1 and mTORC2[130] (Fig. 1). mTORC1 comprises three core components : mTOR, Raptor (regulatory protein associated with mTOR), and mLST8 (mammalian lethal with Sec13 protein 8, also known as GßL)[130]. In response to environmental signals, mTORC1 acts to control the balance between anabolism and catabolism[130]. mTORC2 controls proliferation, survival, and cytoskeleton organization[130]. In a nutrient-rich environment, cells convert energy sources into macromolecules, such as proteins, lipids, and nucleotides. In nutrient-starved environments, however, cells downregulate the production of macromolecules and rely on catabolic pathways, such as autophagy, for energy.

The mTOR pathway receives various inputs from upstream signaling pathways in response to growth factors, amino acids, energy, oxygen, and stress[130], and the upstream pathways of mTOR can be divided as energy-sensing *PI3K-PTEN-AKT-TSC* and amino acid-sensing *GATOR2-GATOR1-Rag* GTPase pathways (Fig. 2) : phosphatidylinositol-3-kinase (PI3K) is critical to integrating insulin signaling for growth and survival[130]. PTEN antagonizes the action of PI3K. Akt is activated by PI3K and is a positive regulator of mTORC1 via inhibition of Tuberous Sclerosis Complex (TSC). TSC is a heterotrimeric complex comprising TSC1, TSC2, and TBC1D7[38]. TSC inhibits mTORC1 by acting as a GTPase activating protein for Ras homolog enriched in brain (Rheb)[67]. Rheb is a small GTPase that activates mTORC1 by directly binding to mTORC1 on the surface of lysosomes[88]. Meanwhile, Rag GTPase, a component of the amino acid sensing pathway[127], activates mTORC1 by promoting translocation of mTORC1 to the lysosomal surface. Upstream regulators of Rag GTPase in amino acid signaling are the GATOR1 and GATOR2 complexes[10]. The GATOR1 complex, consisting of DEPDC5, Nprl2, and Nprl3, inhibits the mTORC1 pathway by acting as a guanine exchange factor for Rag GTPase. The GATOR2 complex, consisting of Mios, WDR24, WDR59, Seh1L, and Sec13, is a positive regulator of the mTORC1 pathway by inhibiting GATOR1. KICSTOR, which is composed of four proteins, KPTN, ITFG2, C12orf66, and SZT2, recruits GATOR1 to the lysosome to inhibit Rag GTPase[150]. Leucyl-tRNA synthetase, which is another amino acid sensor, functions as a GTPase ac-

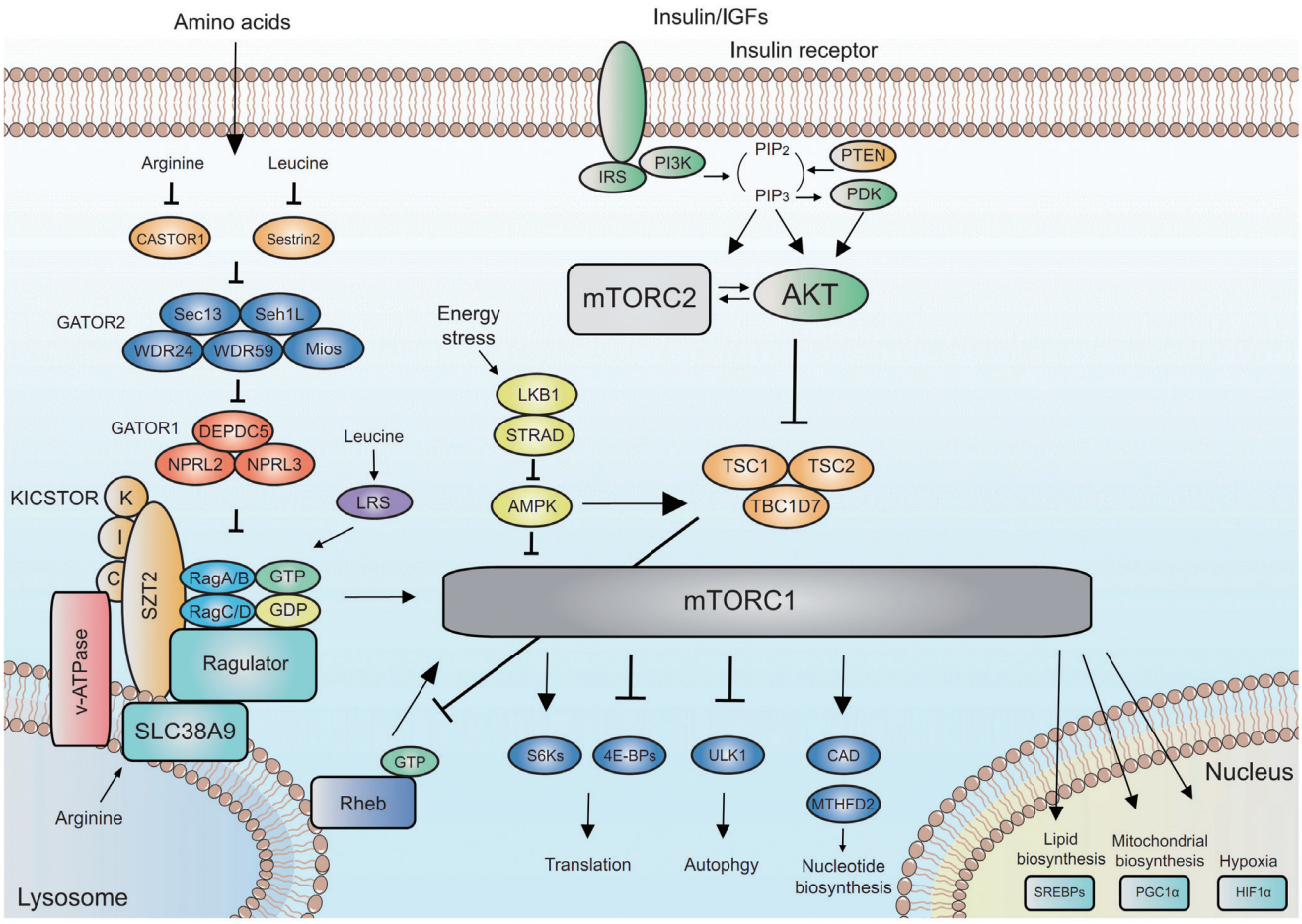

Fig. 2. Upstream and downstream of mTORC1 and mTORC2. The signaling network of mTORC1 and mTORC2. Positive regulators of mTORC1 signaling are shown in blue to green. Negative regulators of mTORC1 signaling are shown in red to yellow.

tivating protein for Rag GTPase[57]. mTORC1 senses amino acids in an intra-lysosome fashion. Lysosomal amino acid regulates Rag GTPase via v-ATPase, which increases the guanine exchange factor activity of Ragulator towards Rag GTPase[159]. SLC38A9 is a sensor of lysosomal arginine and activates mTORC1[69]. Additional novel mTOR upstream regulators, including a methionine sensor, have recently been discovered[1,55].

For macromolecule metabolism, mTORC1 regulates translation through inhibitory eukaryotic initiation factor 4E (eIF4E)-binding protein 1/2/3 (4E-BPs) and the S6 kinases (S6Ks)[21,50]. Translational control occurs predominantly at the initiation step, which commences with the binding of the eukaryotic translation initiation factor 4F (eIF4F) complex to the 5'cap[52,135]. As the limiting component of the eIF4F complex, eIF4E is considered to be a critical determinant in translation of mRNA[37]. Facilitating eIF4F formation and the progression of translation, mTORC1 phosphorylates (inactivates) the 4E-BPs, leading to their dissociation from eIF4E[51,60]. The S6Ks activate the eukaryotic translation initiation factor 4B (eIF4B), which is an activator of the eukaryotic translation initiation factor 4A, leading to an increase in the helicase activity of eIF4A and the initiation of translation[39,61].

Stimulating lipid synthesis, mTORC1 interacts with the sterol responsive element binding proteins transcription factors[116]. For sufficient supply of nucleotides during growth, mTORC1 promotes purine and pyrimidine nucleotide biosynthesis through MTHFD2 and the carbamoyl-phosphate synthetase[17]. Through increased translation of the HIF1α transcription factor that drives the expression of glycolytic enzymes, mTORC1 further promotes growth by changing glucose metabolism from oxidative phosphorylation to glycolysis[130]. The mTORC1 pathway also activates the transcriptional coactivator PGC1α for increased mitochondrial biosynthesis[34]. Meanwhile, mTORC1 inhibits autophagy, which plays

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an important role in scavenging damaged and harmful cellular structures and sustains energy homeostasis, through ULK1[118]. Recently, it was also demonstrated that mTORC1 regulates ribosomal protein degradation through NUFIP1[151].

mTORC2 largely functions as a primary effector of the insulin/PI3K signaling[130] (Fig. 2). Generally, the role of the mTORC2 pathway is thought to overlap with the insulin signaling pathway, considering that phenotypes caused by perturbation of the mTORC2 pathway are very similar to those elicited by perturbation of insulin signaling[105]. Upon phosphorylation by mTORC2[128], Akt promotes cell survival and proliferation. mTORC2 further regulates cellular proliferation and survival through the AGC (PKA/PKG/PKC) family of protein kinases.

PHYSIOLOGICAL ROLES OF THE MTOR PATHWAY IN THE BRAIN

The mTOR pathway plays important roles in development and aging of the central nervous system (CNS) : the mTORC1 pathway is a core regulator of neural development, cortical architecture, neuronal morphology, circuit formation, synaptic plasticity, and neurodegeneration[85]. Perturbation of the mTOR pathway has been found to disrupt several developmental processes. Defective telencephalic development was reported in mTOR mutant rats, called "flat-top" mutants[59]. The loss of function of tuberous sclerosis complex 2 (*Tsc2*), which is an upstream inhibitor of mTOR, was found to disrupt neuroepithelial growth[119]. Altogether, these studies demonstrated that the mTOR pathway is important for brain development by regulating neural stem cells. Meanwhile, activation of the mTOR pathway via conditional deletion of *Tsc2* was found to lead to the disruption of cortical layering[147]. Additionally, increased activity of the mTOR pathway due to mTOR mutations, loss of *Tsc1/2*, or *Pten*, was described as eliciting hypertrophy of soma, fewer dendritic spine, increased axon length, and increased dendritic complexity in cortical neurons[53,77,78,137,138].

Perturbation of the mTOR pathway has also been found to adversely affect neural circuit formation. The mTOR pathway regulates axon length[53] and axon guidance in response to environmental signals[156]. Among neurons, local protein synthesis in synapses distant from the soma is mediated by mTOR and is critical for the formation of the neural circuit. The expression levels of synaptic proteins, such as the Arc and Synapsin, are increased by activation of mTOR[81,117]. Hyperactivation of the mTOR pathway by loss of *Pten* has been shown to increase glutamatergic and GABAergic signals[148]. In addition to neurons, the mTOR pathway has been found to regulate glial cells during neural circuit formation. Deletion of the core component of the mTORC1 or the mTORC2, *Raptor* or *Rictor*, was reported to result in defective myelination and oligodendrocyte maturation[18,146]. Conditional *Tsc1* knockout in the astrocyte reportedly disrupted electrical signaling in the mouse brain by abrogating neural circuitry[141]. Interestingly, researchers have discovered crosstalk between neurons and glial cells as evidenced by a loss of myelination upon loss of TSC1 in neurons[96]. In TSC patients, the critical role of the mTOR pathway in neural circuit formation was echoed by reductions in white matter and cortical connectivity[112].

Neuronal synapses are crucial to storing memories. Observations of impaired memory formation and synaptic function in response to rapamycin treatment suggest the important role of the mTOR pathway in memory formation[25]. Indeed, the mTOR pathway has been found to play a crucial role in both long-term potentiation (LTP) and long-term depression (LTD) : activation of the mTOR pathway via *Tsc1*+/-, *Tsc2*+/-, or 4E-BP2 knockout (KO), results in a decrease in LTP threshold[8,41,145]. Meanwhile, reduced mTOR pathway activity disrupts LTD through metabotropic glutamate receptors (mGluRs) that disturb local protein synthesis[62,64].

ACTIVATION OF MTOR PATHWAY IN NEURODEVELOPMENTAL DISORDERS WITH EPILEPSY

Neurodevelopmental disorders, such as malformations of cortical development (MCD), Cowden syndrome (CS), PIK-3CA-related overgrowth spectrum (PROS), and hamartoma tumor syndromes, commonly present cortical abnormalities and are highly associated with epilepsy, developmental delay, and autism-spectrum disorders[4,12,70,80,97,106,133]. These neurodevelopmental disorders with epilepsy are a common cause of drug-resistant epilepsy in children requiring surgery for treatment[4,12]. However, about 50% of these individuals continue to experience seizures after surgical resection of the epileptic focus[94]. In addition to clinical intractability, refractory epileptic seizures also pose tremendous socioeconomic burden, based

on poor quality of life, to patients and their caregivers[28].

Aberrant activation of the mTOR pathway has been identified in brain lesions from patients with neurodevelopmental disorders with epilepsy, especially in MCD[15,86,100]. With these results, researchers have suspected genetic mutations as culprits of the observed mTOR pathway activation in neurodevelopmental disorders[32]. Indeed, a number of neurodevelopmental disorders with epilepsy has been shown to be caused by germline or somatic mutations in the mTOR pathway : these mutations can be categorized as those affecting the energy-sensing *PI3K-PTEN-AKT-TSC* pathway and those affecting the amino acid-sensing *GATOR2-GATOR1-Rag* GTPase pathway (Table 1).

MUTATIONS IN THE ENERGY-SENSING *PI3K-PTEN-AKT-TSC* PATHWAY

Germline mutations in the *PIK3CA* gene, which activates the mTOR pathway, have been reported in CS[106]. Character-

Table 1. Mutations in the mTOR pathway in the neurodevelopmental disorders with epilepsy

Gene	Mutation type	Effect of the mutation on the mTOR pathway	Disease
PIK3CA	Germline	Activating	CS, PROS
	Somatic	Activating	PROS, MEG, HME, MCAP, MPPH, FCD
PTEN	Germline	Inactivating	CS, BRRS, LDD, Proteus syndrome, Proteus-like syndrome
AKT3	Germline	Activating	MEG, HME, MEG with polymicrogyria, MEG with PNH
	Somatic	Activating	HME, FCD
TSC1/2	Germline	Inactivating	TSC
	Inherited – autosomal dominant	Inactivating	TSC
	Somatic	Inactivating	FCD
TBC1D7	Inherited – autosomal recessive	Inactivating	MEG
RHEB	Somatic	Activating	HME
STRADA	Inherited – autosomal recessive	Inactivating	PMSE
MTOR	Germline	Activating	SKS
	Somatic	Activating	SKS, FCD, HME
DEPDC5	Inherited – autosomal dominant	Inactivating	FFEVF, ADNFLE, FMTLE
	Germline	Inactivating	MCD, epileptic spasm, FCD
	Somatic	Inactivating	FCD, pachygyria, MCD
NPRL2	Germline	Inactivating	TLE, MCD
NPRL3	Germline	Inactivating	TLE, MCD, FCD
KPTN	Inherited – autosomal recessive	Inactivating	Familial intellectual disability-macrocephaly syndrome, Macrocephaly
SZT2	Inherited – autosomal recessive	Inactivating	Infantile encephalopathy with epilepsy
C12ORF66	Germline	Inactivating	Macrocephaly

mTOR : mechanistic target of rapamycin, CS : cowden syndrome, PROS : PIK3CA-related overgrowth spectrum, MEG : megalencephaly, HME : hemimegalencephaly, MCAP : megalencephaly-capillary malformation, MPPH : megalencephaly-polydactylyl-polymicrogyria-hydrocephalus, FCD : focal cortical dysplasia, PTEN : phosphatase and tensin homolog, BRRS : Bannayan-Riley-Ruvalcaba syndrome, LDD : Lhermitte-Duclos disease, PNH : periventricular nodular heterotopia, TSC : tuberous sclerosis, RHEB : Ras homolog enriched in brain, STRADA : STE20-related kinase adaptor α, PMSE : polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome, MTOR : mechanistic target of rapamycin, SKS : Smith-Kingsmore syndrome, FFEVF : familial focal epilepsy with variable foci, ADNFLE : autosomal dominant nocturnal epilepsy, FMTLE : familial mesial temporal lobe epilepsy, TLE : temporal lobe epilepsy

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ized by hamartomatous overgrowth of tissues, CS is also accompanied by epileptic seizure in a subset of patients[98,113]. There are also other brain and body overgrowth disorders with epilepsy that are caused by post-zygotic mutations in the *PIK3CA* pathway, which are termed PROS[70]. Additionally, mosaic or brain somatic mutations in *PIK3CA* have been reported in several other neurodevelopmental disorders with epilepsy, including the megalencephaly (MEG), hemimegalencephaly (HME), megalencephaly-capillary malformation (MCAP), megalencephaly-polydactyly-polymicrogyria-hydrocephalus syndromes, and focal cortical dysplasia (FCD) type IIa/IIb[36,68,79,99,122,132].

Classically, germline *PTEN* mutation has been reported in hamartoma tumor syndromes, including CS, Bannayan-Riley-Ruvalcaba syndrome, Lhermitte-Duclos disease, Proteus syndrome, and Proteus-like conditions that share the pathological phenotypes of macrocephaly and megalencephaly[97]. Epileptic seizures have been reported in patients with germline mutation in PTEN[29,30,90].

Brain somatic mutations in *AKT3* have primarily been detected in MCD, including the HME and FCD. Brain somatic gain of function mutations in *AKT3* were first demonstrated to cause HME[79], followed by identification of somatic *AKT3* mutations in FCD[68]. Germline *AKT3* mutations were also reported in MEG[104]. To date, germline *AKT3* mutations have been reported in HME, MEG, MEG with polymicrogyria, and MEG with periventricular nodular heterotopia[2].

TSC1 and *TSC2* germline mutations have been identified in TSC patients[142]. Brain somatic mutations in *TSC1* and *TSC2* have also been discovered in FCD type IIb[82]. Homozygous *TBC1D7* loss of function mutation have been identified in MEG patients[3,22]. More recently, brain somatic mutation in *RHEB* were identified in an HME patient[126].

STE20-related kinase adaptor α (STRADA) is a negative regulator of mTOR, by activating AMPK[107]. A loss-of-function mutation in STRADA was reported in autosomal recessive disease polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome, also called Pretzel syndrome (PMSE)[111].

Germline or mosaic mutation in *MTOR* has been described in the Smith-Kingsmore syndrome (SKS) OMIM #616638, which is characterized by epileptic seizure and neurodevelopmental defect[54]. SKS is a rare disease for which only 23 patients have been reported as of 2018[54,101,102]. Brain somatic mutations in *MTOR* have also been described in FCD and HME, with varying degrees of mutational burden[91]. All of these mutations have been found to be missense gain-of-function mutations that activate the mTOR pathway.

MUTATIONS IN THE AMINO ACID-SENSING *GATOR2-GATOR1-RAG* GTPASE PATHWAY

Amino acid-sensing pathway is mediated by the *GATOR2-GATOR1-Rag* GTPase pathway. Since GATOR1 is a negative regulator of the mTORC1 pathway, researchers proposed that loss-of-function of GATOR1 would lead to neurodevelopmental disorders with epilepsy via hyperactivation of the mTORC1 pathway. Germline mutations in *DEPDC5*, *NPRL2*, and *NPRL3* have been reported in familial focal epilepsy with variable foci, autosomal dominant nocturnal epilepsy, temporal lobe epilepsy, and familial mesial temporal lobe epilepsy[13,121]. In addition, germline mutations in *DEPDC5*, *NPRL2*, and *NPRL3* have also been discovered in MCD and epileptic spasms patients[24,131,134]. Additionally, mosaicism and brain somatic mutation in GATOR1 have been reported in MCD[36], pachygyria[26] and familial focal epilepsy with focal cortical dysplasia[14]. Interestingly, it was recently demonstrated that epilepsy patients with focal cortical dysplasia carry a germline mutation in one allele of *DEPDC5* and a brain somatic mutation in the other allele, suggesting a two-hit hypothesis as a disease mechanism[14,120]. Autosomal recessive mutations in the KICSTOR complex, including *KPTN*[9,108], *SZT2*[11,143], and the genomic locus that contains *C12orf66*[95], have been identified in neurodevelopmental disorders with epilepsy. Until now, mutations in *GATOR2* have not been identified in epilepsy patients.

EPILEPSY ANIMAL MODELS OF HYPERACTIVATION OF THE MTOR PATHWAY

For extensive research into epilepsy, genetic animal models of mutations activating the mTOR pathway in epilepsy patients have been developed (Table 2). The first genetic model of epilepsy was the *Pten* KO mouse reported in 2001[6,78]. Although Eker rats with germline mutations in the *Tsc2* gene were the first genetic animal model of mTOR hyperactivation, the model was not suitable for analyzing epileptic seizures due

Table 2. Epilepsy animal models with genetic mTOR pathway hyperactivation

Gene	Role and function	Genotype	Reference
PI3K	PI3K is a critical signaling pathway which integrates insulin signaling to the growth and survival	Hyperactive *Pik3ca* Nestin KI	22)
PTEN	PTEN is a phosphatase and a negative regulator of the PI3K-PTEN-AKT-TSC pathway	*Pten* KO	1,14)
		Pten GFAP KO	13,17,23)
		Pten NSE KO	33)
		Inducible *Pten* KO	145)
AKT	AKT is a positive regulator of the mTORC1 via inhibition of TSC and the mTORC2	Akt3 KI	25)
		Hyperactive *Akt3* KI via *in utero* electroporation at the SVZ	2)
TSC1/2	TSC1/2 is a negative regulator of the mTORC1 via inhibiting the action of RHEB	*Tsc1* GFAP KO	26)
		Tsc1 Synl KO	37)
		Tsc1 Nestin KO	8)
		Tsc1 Emx1 KO	4)
		Tsc1 KO via *in utero* electroporation at the SVZ	15)
		Tsc2 GFAP KO	27,44)
		Tsc2 KO via *in utero* electroporation at the SVZ	15)
mTOR	mTOR is a serine/threonine kinase regulating various downstream targets which coordinate metabolism	Hyperactive *MTOR* KI via *in utero* electroporation at the SVZ	16)
RHEB	RHEB activate the mTORC1 on the lysosomal membrane	Constitutively active *Rheb* KI via *in utero* electroporation at the SVZ	9)
DEPDC5	DEPDC5 comprise the amino acid-sensing pathway and is a negative regulator of the mTORC1	*Depdc5* KO via *in utero* electroporation at the SVZ in *Depec5* +/- mouse	21)

mTOR : mechanistic target of rapamycin, PI3K : phosphatidylinositol-3-kinase, KI : knock-in, PTEN : phosphatase and tensin homologon chromosome 10, GFAP : glial fibrillary acidic protein, KO : knock-out, NSE : neuron specific enonlase, SVZ : subventricular zone, TSC : tuberous sclerosis, Synl : synapsin I, RHEB : Ras homologue enriched in brain

to early phase lethality[155]. In 2002, the first TSC model with epilepsy, which was the astrocyte-specific *Tsc1* KO model, was reported[141]; neuron-specific KO of *Tsc1* was also found to lead to spontaneous seizure in the mouse model[96]. After these, the *Tsc2* GFAP KO mouse model was developed and shown to lead to epilepsy[157]. A mouse model expressing human *PI3KCA* mutation in developing neural progenitors was generated to recapitulate pathological features of PROS syndrome, including epilepsy[123]. A knock-in mouse model with gain-of-function mutation in *Akt3* has also been found to elicit spontaneous seizure[140].

Recently, epilepsy models of brain somatic mutations, which recapitulate focal malformations of cortical development (FMCD), have been reported. A mouse model of FMCD was first generated by *in utero* electroporation, thereby providing a small portion of neurons to express known mTOR mutations in human FMCD patients[7,83,110]; these models developed spontaneous seizures. Additionally, FMCD models of brain somatic mutation in mTOR pathway genes, such as *RHEB* or *TSC*, have consistently recapitulated spontaneous seizures[63,82]. Heterozygote GATOR1 gene (*Depdc5*, *Nprl2*, and *Nprl3*) KO animals have not been found to recapitulate epileptic seizures observed in patients with germline mutations in the GATOR1 genes[40,65,72,92]. Interestingly, FMCD mouse model caused by a biallelic two-hit mutation (brain somatic and germline) in *Depdc5* was shown to lead to spontaneous seizure[120]. Homozygous KO of *Szt2*, a component of the KICSTOR complex, has been reported as leading to low seizure threshold in mice[46].

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MTOR PATHWAY ACTIVATION IN EPILEPTOGENESIS

Epileptogenesis refers to the process leading to the first spontaneous seizure after pro-epileptogenic insult (e.g., genetic defect, brain injury, status epilepticus, or cancer). Epileptogenesis is a brain-wide circuit rewiring process comprising reorganization of microcircuits to long-range circuits, as well as gliosis, blood-brain barrier damage, inflammation, and neurodegeneration[114].

Since epilepsy is the consequence of electrical changes, electrophysiological characteristics in animal models and epilepsy patients have been analyzed in an attempt to understand the epileptogenic mechanism. Studies have indicated that high-frequency oscillations on electroencephalogram are characteristic of FCD[20]. Organotypic brain slice analysis of resected FCD lesions has revealed an electrographic firing pattern of ictal-like discharges after 4-aminopyridine treatment[5], which decreased with treatment with $GABA_A$ receptor antagonist.

In brain slice cultures of hyperactive mTOR signaling, researchers have found both the frequency and amplitude of the miniature excitatory postsynaptic current to be increased in glutamatergic synaptic transmission of the hippocampus[89,138]. In GABAergic neurons, hyperactive mTOR signaling increased evoked synaptic responses in the hippocampus[148]. In a subset of auditory cortical neurons of *Pten* conditional KO, synaptic inputs from the long-range connections, including the contralateral auditory cortex and thalamus, and local connections were increased[152].

In an epilepsy animal model of brain somatic mutations in the mTOR pathway, mutation-carrying cortical neurons showed an increased capacitance, increased cell size, reduced input resistance indicative of a higher current needed to reach the voltage threshold of the action potential, and increased gain of firing frequency[120]. Also, spontaneous excitatory postsynaptic current frequency was decreased in the mTOR pathway mutation-carrying cortical neurons[84,120].

There have been tremendous advances in the identification of the contribution of mTOR downstream functions to neuronal hypertrophy, dendritic branching, axon length, and neuronal migration. Although numerous epilepsy animal models have been utilized to unravel the path to epileptogenesis, the downstream mechanism of mTOR signaling that accounts for epileptogenesis remains unclear. The complexity of the downstream output of the mTOR pathway has hampered attempts to understand the epileptogenic mechanism. Using genetic manipulation techniques, normalizing major downstream outputs of hyperactive mTOR signaling, including translation via overexpressing constitutively active 4E-BP or knockdown of S6Ks[84] and autophagy via knockdown of OFD1[110], have failed to prevent or even reduce epileptogenesis. Further studies defining the downstream pathway of mTOR in epileptogenesis will be necessary.

INHIBITION OF THE MTOR PATHWAY IN TREATMENT OF EPILEPTIC DISORDERS

Researchers have hypothesized that epilepsy resulting from mTOR pathway activation could be treated by mTOR inhibition, and mTOR inhibition therapies have been evaluated in several clinical trials for epilepsy (Table 3). Rapalogs, including sirolimus and everolimus, have been reported as promising new anti-epileptics because they can penetrate the blood brain-barrier[71]. Rapalogs have been extensively tested and have gained the US Food and Drug Administration (FDA) approval as anti-epileptics in TSC[27,47,73].

The first study to show the beneficial effect of the rapalogs on epilepsy did so in a mouse model of TSC[158]. In this study, early treatment with sirolimus prevented the development of epilepsy. The anti-epileptic effect of rapalogs has also been demonstrated in other genetic models of epilepsy with mTOR pathway hyperactivation, including *Pten*[87] KO and *Strada* KO[111]. Recently, intractable epilepsy mouse models with brain somatic mutation in the mTOR pathway were found to be cured by rapalogs[63,82,83]. Interestingly, epileptic seizure was almost completely suppressed by mTOR inhibitors in mouse models of TSC KO or mTOR activating mutation, but only partially suppressed in *Pten* KO mouse models[87]. These results suggest that an independent mechanism of epileptogenesis distinct from that in TSC KO or mTOR mutation is present in the Pten KO model. In nongenetic seizure models, including the pilocarpine-induced epilepsy model, kainic acid-induced epilepsy model, and absence epilepsy model, rapalogs partially reduced seizures. Citraro et al.[31] reported a representative studies of the anti-epileptic effect of rapalogs in an animal model of intractable epilepsy.

Everolimus have been approved by the FDA for treating

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Table 3. Clinical studies with the mTOR inhibitors for the anti-epileptic effects

Type of study	Disease	Drug and dose	Number and age of patient(s)	Duration of treatment	Anti-epileptic effect	Refs
Prospective, open-label, phase I/II clinical trial	TSC	Everolimus 4.7–5.6 mg/m^2/day	16 patients; 3 year-old or older	Median duration : 21.5 months (range, 4.7–34.4)	Reduction in seizure frequency in 9/16 patients	11)
Case report	TSC	Everolimus 4.5 mg/m^2/day	1 patient; 10-year-old man	12 months	Cessation of seizure	20)
Prospective, multicenter, open-label, phase I/II clinical trial	TSC	Everolimus 5 mg/m^2/day	20 patients; median age : 8 years (age range, 2–21)	12 weeks	Reduction in seizure frequency in 17/20 patients, 4 of these patients were seizure-free at 12 weeks	24)
Prospective, double-blind, parallel-group, placebo-controlled, multicenter phase III	TSC	Everolimus 4.5 mg/m^2/day	8 patients, children under the age of 3	35 months (range, 33–38)	Cessation of seizures in 1 patient, significant (at least a 50%) reduction in the number of seizures in 2 patients	10)
Case study series	TSC	Everolimus 5–7 mg/day	6 patients; median age : 5 years (age range, 2–12)	36 weeks	Reduction in seizure frequency in 4/6 patients	29)
Open-label, single center case series	TSC	Everolimus 5 mg/day	1 patients; 14-year-old female	18 months	25–50% seizure reduction	3)
Case study	TSC	Everolimus 5 mg/m^2/day	1 patient; 13.5-year-old female	12 days	Seizure aggravation	30)
Case study	TSC	Everolimus 5 mg/day	1 patient; 13-year-old female	1.5 year	Reduction in seizure frequency	28)
Prospective, open-label, phase I/II clinical trial	TSC	Everolimus 5 mg/m^2/day	14 patients; median age : 8 years (age range, 2.0–21.3)	48 months	Reduction in seizure frequency in 13/14 patients (over 50% seizure reduction)	12)
Core phase, phase III, randomized, double-blind, placebo-controlled	TSC	Everolimus Low exposure group; 5.2 mg/m^2/day (range, 1.3–14.5) High exposure group; 7.5 mg/m^2/day (range, 1.4–24.4)	366 patients (including placebo group); median age; 10.1 years (age range, 2.2–56.3)	18 weeks	Response rate; 15.1% with placebo 28.2% with low-exposure everolimus 40.0% with high-exposure everolimus	7)
Extension phase, phase III, randomized, double-blind, placebo-controlled	TSC	Everolimus 5–9 mg/m^2/day	294 patients; median age : 8.7 years (age range, 2.2–18.0)	1 year	Sustained seizure reduction in 48.05%; Median percentage reduction in seizure frequency : 48.2%	5)

Table 3. Continued

Type of study	Disease	Drug and dose	Number and age of patient(s)	Duration of treatment	Anti-epileptic effect	Refs
Case study series	TSC	Sirolimus 1.5 mg/kg/day	3 patients; 15 years (age range, 5.5–21 years)	Median 4 months (range, 3–5)	Reduction in seizure frequency in 2/3 patients	6)
Case report	TSC	Sirolimus 0.15 mg/kg/day	1 patient; 9-year-old female	10 months	Reduction in seizure frequency	18)
Case study series	PMSE	Sirolimus 1-5 mg/m^2/day	6 patients; median age : 3 years (age range, 5 months–5 year)	6 months	Reduction in seizure frequency	19)
Open-label, single center case	TSC	Sirolimus 1 mg/m^2/day	6 patients; median age : 6 years (age range, 3–17)	Median duration : 18 months (range, 6–36)	Over 50% reduction in seizure frequency in 5/6 patients	3)
Case report	HME	Sirolimus 1 mg/m^2/day	1 patient; 3-month-old man	3 months	Seizure reduction (over 50%)	31)

mTOR : mechanistic target of rapamycin, TSC : tuberous sclerosis, PMSE : polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome, HME : hemimegalencephaly

subependymal giant astrocytoma, kidney tumors, and partial epilepsy in TSC patients. The first trial to test the efficacy of everolimus to treat epileptic seizure was reported in 2010 for TSC patients[74]. In this study, seizure frequency was reduced in nine of 16 patients with everolimus. Following studies supported the anti-epileptic effects of everolimus or sirolimus (Table 3).

The prospective, randomized, multicenter, placebo-controlled study testing the seizure suppression effect of everolimus on seizure frequency in TSC patients with drug-resistant epilepsy reported positive results[49]. Recently, long-term results of the prospective, open-label, non-randomized study of everolimus for treating epileptic seizure in TSC indicated that reduction in seizure frequency is sustained for up to 4 years[75]. This effect has been confirmed in the long-term follow-up of the EXIST3 trial[35]. Large-scale studies on the anti-epileptic effect of everolimus have described response rates for everolimus and a median seizure frequency reduction in respondents of about 40%[35,75]. In 2017 and 2018, respectively, both the European Medicinal Agency in Europe and the US FDA approved everolimus as an adjunctive therapy in partial-onset seizures in TSC patients aged 2 years and older. However, there are reports of non-responders, seizure aggravation, or an withdrawal after everolimus administration[35,149]. Meanwhile, clinical trials for treating epileptic seizure in FCD type II with everolimus have recently started (clinicaltrials.gov identifier NCT03198949).

The first report of the anti-epileptic effect of sirolimus in TSC patients, which appears to be similar to that of everolimus[23], was in 2009[103]. In HME patients with brain somatic mutation in mTOR, sirolimus administration reduced seizures[153]. Interestingly, sirolimus has been shown to prevent epilepsy in PMSE patients[111].

Animal and human treatment studies reported that withdrawal of rapalogs provokes seizure recurrence[73]. In electrophysiological analysis of brain slices of resected epileptic foci, everolimus was found to reduce spontaneous excitatory postsynaptic activity, burst discharges, and epileptiform activity in TSC, FCD, and HME cases; effects were subtle in epilepsy patients without mTOR mutations[27].

While rapalogs have been approved for use in various human diseases, concerns have been raised for their long-term use and their adverse effect profile, including potentially serious adverse effects. The adverse effects of rapalogs include im-

munosuppression, mucositis, hyperlipidemia, hyperglycemia, diabetes-like syndrome, and fatal pneumonitis[42,76,109,139]. In a phase III clinical trial with everolimus, over 90% of 111 patients experienced an adverse effects of any grade[48]. With everolimus treatment for over 1 year, grade 3 or 4 adverse events, which are severe adverse events, were reported in 45% of younger patients and 38% of older patients among 150 patients[35]. We should stress that developmental delay caused by rapalogs[63] should be a concern, considering that the onset of intractable epilepsy with mTOR pathway mutation is usually before adolescence[4]. Additionally, treatment with rapalogs has been found to induce adverse nervous system-specific effects altering sociability[129], learning and memory[16,25], and anxiety[56] in mouse models.

For epilepsy caused by brain somatic mutations, therapeutic regimens must seek to avoid perturbations outside of the CNS, and in this regard, antisense oligonucleotide (ASO) drugs appear to be promising therapeutic options. ASO is a complementary oligonucleotide sequence of sense mRNA sequences that hampers normal gene expression processes, including splicing, transcription, and translation[136]. The characteristics of the blood-brain barrier prevent ASO from permeating outside of the CNS when administered into the cerebrospinal fluid via intrathecal injection. Moreover, the half-life of ASO drugs is more than 3 months[19]. Recently, ASO drugs have been approved by the FDA for treating various types of neurodegenerative disorders[45]. We suspect that intrathecal injection of ASO drugs targeting mTOR itself, mTOR mutation, or downstream targets of mTOR will prove effective in treating intractable epilepsies while avoiding the adverse effects of rapalogs outside of the CNS.

CONCLUSION

Over the last two decades, extensive research to outline the roles of the mTOR pathway, to identify mutations in the mTOR pathway in human epilepsy patients, and to develop mTOR inhibitors have led to the discovery of novel strategies for diagnosing and treating intractable epilepsy. The overarching goal of this clinical research is attaining seizure-free status with few to no adverse effects in epilepsy patients. While rapalogs have proven to be effective in controlling seizures, complete seizure cessation has not been recorded in most of patients[35,75]. Also, about half of all TSC patients fail to respond to rapalogs. These discrepancies suggest that there might be an unknown biological mechanism at play. Considering about 40% of patients treated with rapalogs experience a severe adverse effect, it will be necessary to develop more potent and tolerable therapies.

While FMCD patients with drug-resistant epilepsy typically undergo surgical resection of the epileptic focus for treating the seizure, up to 50% of patients do not respond to therapy. Based on preclinical and clinical studies of TSC with rapalogs, it may no longer be necessary to perform highly invasive surgical resection for seizure treatment. However, there is a problem in that diagnosing FMCD patients with low-frequency brain somatic mutation in the mTOR pathway requires analyzing DNA from brain tissue after surgical resection. To alleviate this issue, minimally invasive diagnosis through biomarkers in patient cerebrospinal fluid would prove invaluable.

CONFLICTS OF INTEREST

J.H.L is a co-founder of SoVarGen, Inc. that develops new diagnostics and therapeutics for brain disorders. The remaining authors declare no competing financial interests.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in this study.

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- **Supplementary materials**

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