mTORopathy & Focal Epilepsy

Tayard Desudchit

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Cold Spring Harbor Perspectives in Medicine

mTOR Signaling in Epilepsy: Insights from Malformations of Cortical Development

Peter B. Crino

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Over the past decade enhanced activation of the mammalian target of rapamycin (mTOR)-signaling cascade has been identified in focal malformations of cortical development (MCD) subtypes, which have been collectively referred to as "mTORopathies."

Mutations in mTOR regulatory genes (TSC1, TSC2, AKT3, DEPDC5) -> focal MCD

- tuberous sclerosis complex (TSC),
- hemimegalencephaly (HME)
- focal cortical dysplasia.

mTOR plays important roles in the regulation of cell division, growth, and survival,

aberrant activation during cortical development -> alterations in cell size, cortical lamination, and axon and dendrite outgrowth often observed in focal MCD.

hyperactivated mTOR signaling -> structural alterations ->epileptogenesis,

hyperactivated mTOR signaling w/o Str. Changes -> enhance neuronal excitability.

Clinical trials with mTOR inhibitors -> Rx focal MCD seizures .

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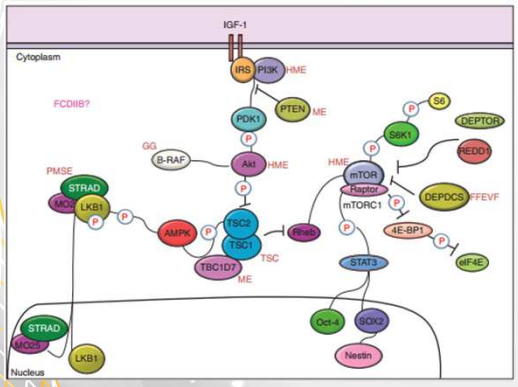
mTOR Signaling in Epilepsy



- Schematic depicting the mammalian target of rapamycin (mTOR)-signaling cascade.
- insulin-like growth factor-1 (IGF-1) receptor +epidermal growth factor(EGF) receptor,
- -> PI3kinase(PI3K),->phosphoinositide-dependent kinase-1 (PDK-1) modulated by PTEN
- ->Akt
- ->TSC1:TSC2:TBC1D7 complex.
  - Modulated by →LKB1:STRADA:MO25 complex via AMPK.
  - Serves to inhibit mTOR via Rheb transduction-> mTORC1 depicted
  - Downstream targets of mTORC1 :
    - p70S6kinase (S6K1),
    - elongation-binding protein1 (4E-BP1),
    - STAT3 modulate neural stem cell protein expression: SOX2, nestin
- mTOR modulated by REDD1,DEPTOR ,DEPDCS
- Malformation of cortical development (MCD)
  - polyhydramnios-megalencephaly-symptomatic-epilepsy (PMSE) (STRADA),
  - hemimegalencephaly (HME), megalencephaly (ME) (MPPH, MCAP; PI3K, AKT, MTOR),
  - ME/intellectual disability (ID) (TBC1D7),
  - tuberous sclerosis complex (TSC) (TSC1, TSC2),
  - ganglioglioma (GG) (B-RAF).
  - A gene mutation causing focal cortical dysplasias type IIB (FCDIIB) has not been identified.

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The physiologic role of mTOR

- mTOR is ubiquitously expressed in mammalian tissues +very important in the nervous system.
- mTOR is a serine-threonine protein kinase -> 2x intracellular signaling complexes, mTORC1 and mTORC2
- TSC1 and TSC2 genes proteins -> hamartin and tuberin complex -> inhibit Rheb GTP-binding protein -> inhibit mTORC1
- mTORC1 -> cell growth, proliferation and survival, via ribosomal biogenesis + protein translation.
- Upstream regulators of the hamartin/tuberin complex or mTORC1 -> cells respond to different metabolic conditions.
- Energy surplus, insulin and other growth factors stimulate the PI3K/Akt pathway, which in turn activates the mTOR pathway -> cell growth and metabolism.
- High levels of amino acids esp leucine, => DEPDC5 pathway => mTOR activation.
- Energy deficiency => LKB1/AMPK pathway => inhibits mTORC1 => limits cell growth.
- mTORC2, => maintenance of actin cytoskeleton, somatic size and dendritic growth.
- mTORC1 & mTORC2 : signaling mechanisms + feedback loops : connecting to each other and to upstream and downstream regulators=> sensitive modulation of cell metabolism to different environments.
- In the brain, mTORC1 & mTORC2 collectively regulate dendritic growth & morphology, synaptic transmission and plasticity, neurogenesis and neural network activity. => normal cortical brain development+ the maintenance of neuronal cells and neurotransmission across the lifespan.

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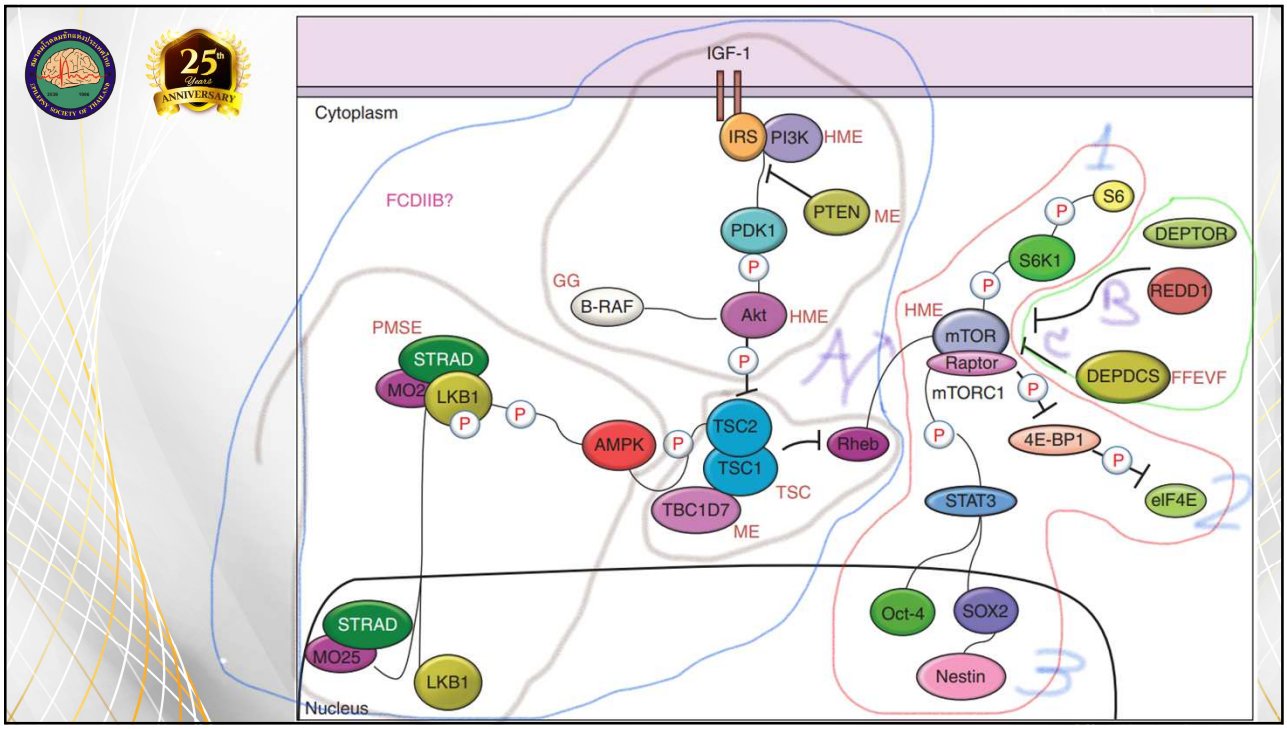

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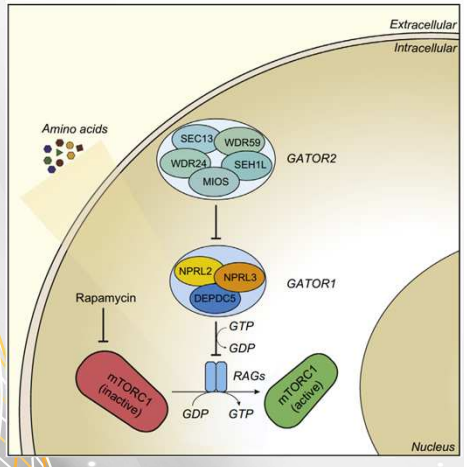
GATOR1/2-mTORC1 pathway



- The GATOR1 complex,(proteins DEPDC5, NPRL2, and NPRL3) => inhibits mTORC1 via GAP activity toward the GTPases RagA/C.
- The GATOR2 complex (proteins MIOS,SEH1L, WDR24, WDR59, and SEC13)=> inhibit the GATOR1 complex.

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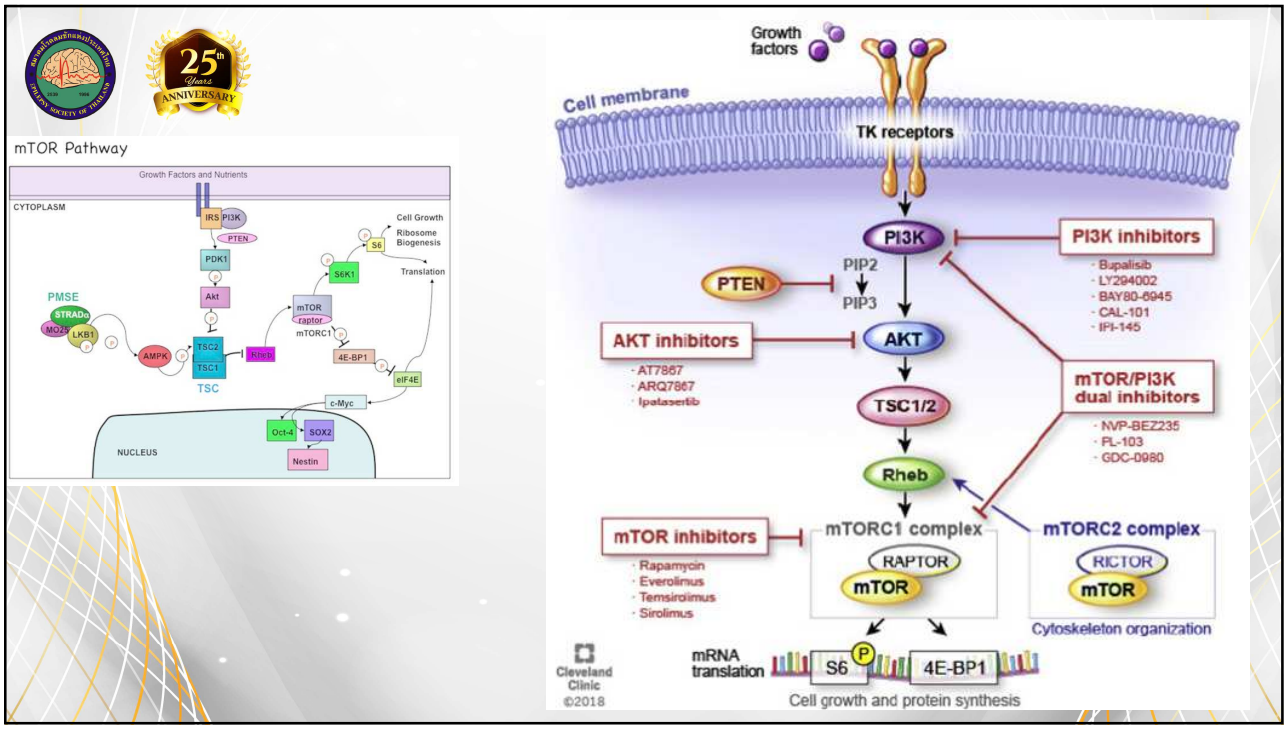

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The mTOR pathway in epilepsy

- dysregulation of the mTOR cascade => numerous neurological diseases.
- 'mTORopathies' has been used to group a number of clinical syndromes in which mTOR regulation is altered.
- 1) tuberous sclerosis complex (TSC), a genetic disorder characterized by benign tumors in multiple organs, including brain, kidneys, lungs, heart, eyes and skin.
- Mutations in the TSC1 or TSC2 genes => loss of normal inhibition of mTORC1, leading to cell overgrowth and disruptions in synaptogenesis
- => cortical malformations and subependymal giant cell astrocytomas (SEGAs) + drug-resistant epilepsy.
- Mutations in TSC1 or TSC2 => epilepsy multiple pathophysiological mechanisms
- deletions in PTEN, TSC1 or TSC2 genes => abnormal neuronal proliferation, differentiation and migration; altered expression of synaptic proteins; and abnormal axon and dendritic growth
- => decreased seizure threshold by promoting hyperexcitability of neuronal tissues at the cellular level, + altering cortical morphology and neuronal connectivity at the systems level.
- Patients with TSC1/TSC2 mutations develop cortical tubers, benign hamartomas found in cortex or subcortical white matter, which are thought to be the main structural abnormalities leading to development of refractory seizures, and surgical removal of tubers frequently reduces or eliminates seizures
- Tubers in TSC patients have abnormal cortical lamination, enlarged and dysmorphic neurons, and alteration in receptor expression=> hyperexcitability and seizures
- Perituberal and more remote cortex has similar histopathological abnormalities that may be epileptogenic.
- Dysregulated mTOR => molecular changes in gene and protein expression => affect neuronal excitability and promote seizures.
- Other mTORopathies share with TSC common clinical features of cortical developmental malformations and medically refractory seizures.

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Tuberous Sclerosis

- TSC1 and TSC2 genes proteins -> hamartin and tuberin complex -> inhibit Rheb GTP-binding protein -> inhibit mTORC1
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•Germline
Mutation

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Tuberous sclerosis complex (TSC)
Bourneville or Bourneville-Pringle disease

- classic clinical triad (vogt triad)
- Facial lesions ("adenoma sebaceum“)
- Seizure
- Mental retardation.

- Epilepsy affecting 80 – 90%
  - infantile spasms
  - simple or complex partial seizures
- EEG +ve in 75 % of patients
- Cognitive deficits 44 – 65%
- Autism and behavioral problems

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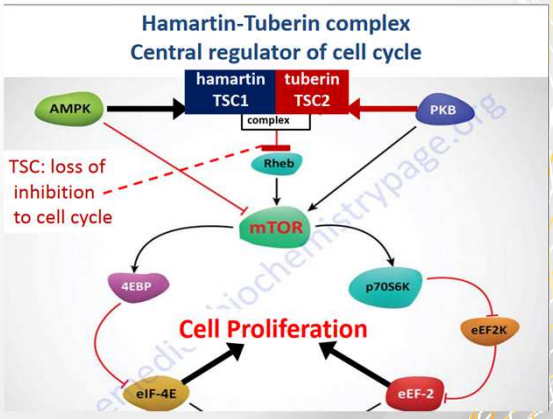
Tuberous Sclerosis : GENETICS

- Autosomal dominant
- Incidence 1 : 6000 livebirths
- Mutation in
  - TSC-1 (Hamartin) or
  - TSC-2 (Tuberin)
- +ve family history in 7 – 40%



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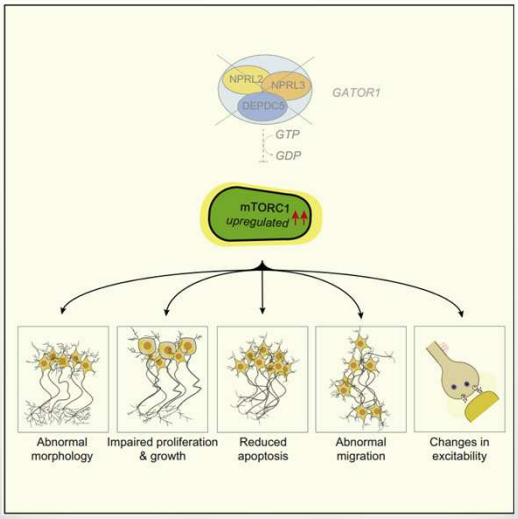
Neuronal consequences of an upregulation of mTORC1 pathway activity.



- Adapted from Curatolo, P., 2015. Mechanistic target of rapamycin (mTOR) in tuberous sclerosis complexassociated epilepsy. Pediatr. Neurol. 52, 281–289.

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Tuberous Sclerosis : Dx Criteria

Diagnostic criteria

Major Features

Identified clinically

- **Facial angiofibromas or forehead plaque**
- **Non-traumatic ungual or periungual fibroma**
- **Hypomelanotic macules**
- **Shagreen patch**
- **Multiple retinal nodular hamartomas**

Identified on imaging

- **Cortical tuber**
- **Subependymal nodule**
- **Subependymal giant cell astrocytoma**
- **Cardiac rhabdomyoma**
- **Lymphangio-myomatosis**
- **Renal angiomyolipoma**

Minor Features

- Multiple pits in dental enamel
- Hamartomatous rectal polyps
- Bone cysts,
- Cerebral white matter migration lines
- Gingival fibromas
- Non-renal hamartoma
- Retinal achromic patch
- Multiple renal cysts

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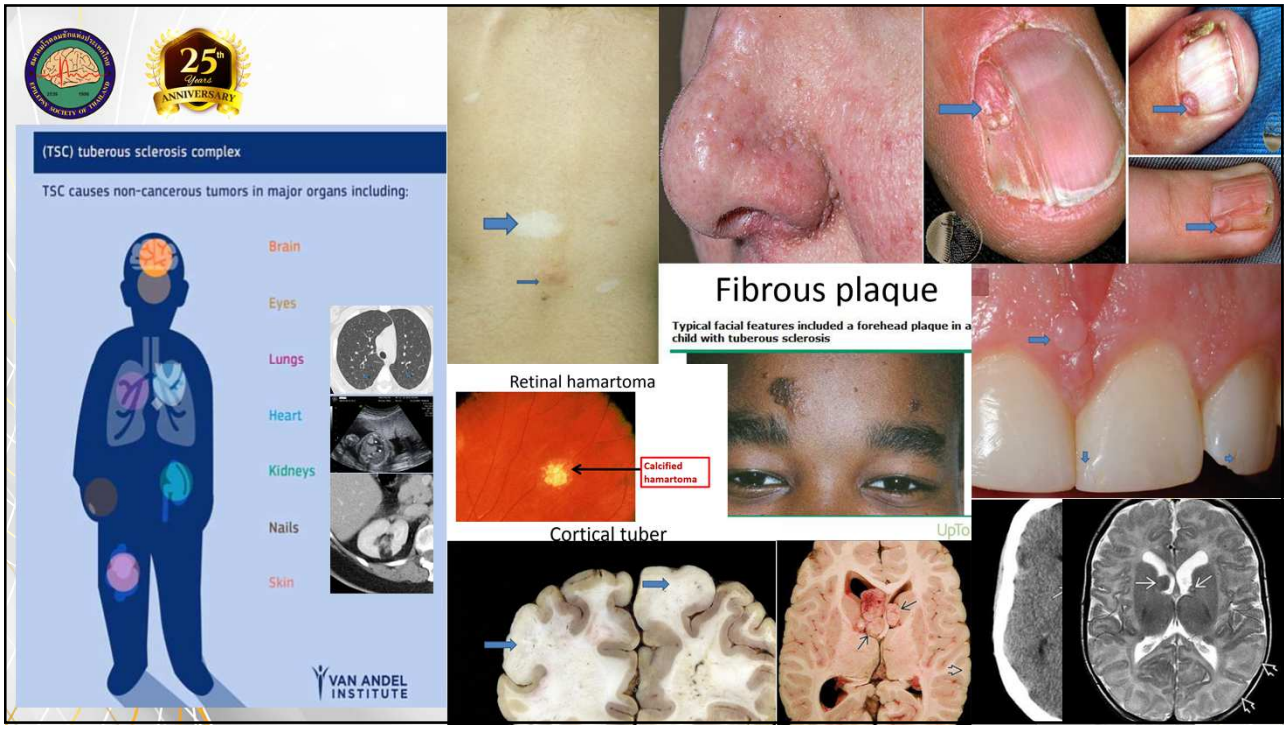

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The mTOR pathway in epilepsy

- Hemimegalencephaly (HME) is a disorder of cortical development in which all or part of one cerebral hemisphere is abnormally enlarged, and can occur in isolation or as part of some neurocutaneous syndromes.
- Studies of brain tissue from HME patients have shown mTOR hyperactivation in some, but not all, neural tissue, suggesting that HME occurs following somatic mutation=> hyperactivation of mTOR pathway during brain development
- More recent studies have shown specific causative somatic mutations in AKT3, PI3K, PTEN or mTOR itself in subsets of HME patients.

•Somatic Mutation

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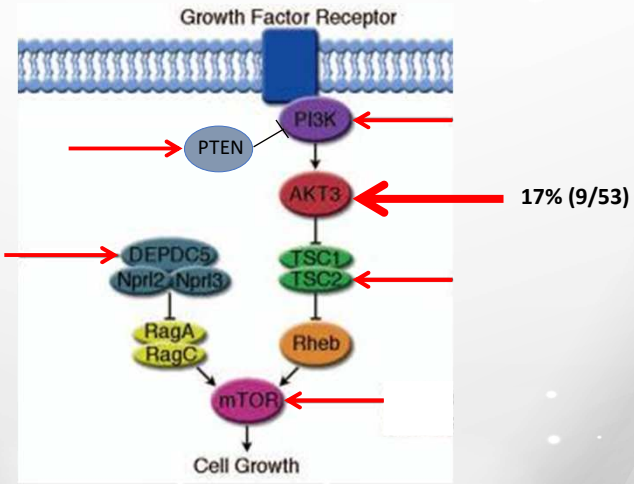
HME: 36% mTOR Pathway genes



Adapted: Ann Neurol. 2015 Apr; 77(4): 720–725.

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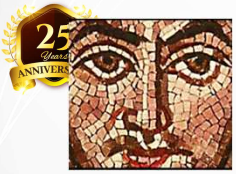
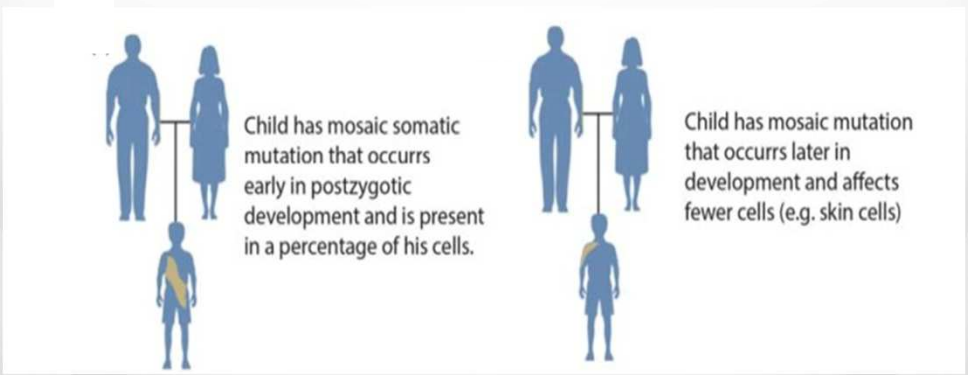


osaic Somatic Mutation



Genes (Basel). 2014 Dec; 5(4): 1064–1094.

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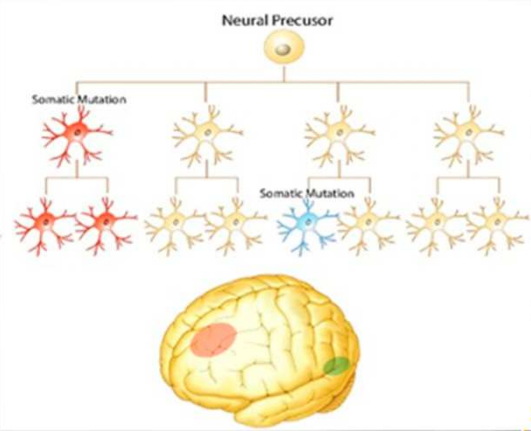
Brain Development

- Antenatal:
  - >100K divisions/min
- Postnatal:
  - 700 neuron/day
  - 1/3 hippocampal exchange over life



Exp Mol Med. 2016 Jun; 48(6): e239.

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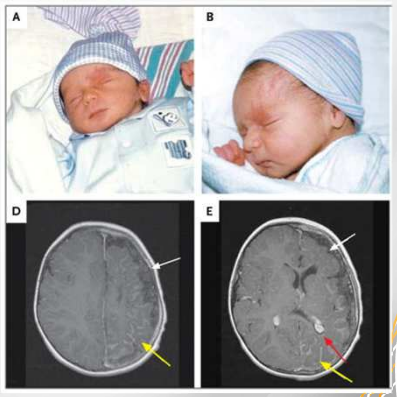
Somatic Mosaicism: Timing

- When = Where
- Example: Sturge-Weber Syndrome

- Port-wine stain
  - *GNAQ* gene (WGS, 2013)
  - 6% = S-W (26% if V1 distr.)
    - EARLY mutation
  - 94% = non-syndromic
    - LATE mutation



N Engl J Med. 2013; 368(21): 1971-9.

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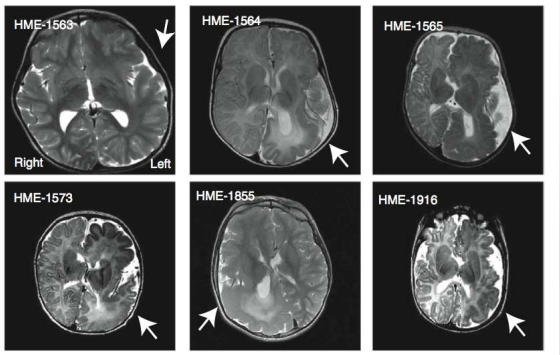
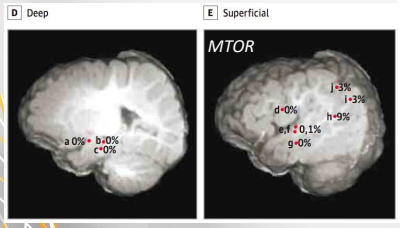
Hemimegalencephaly (HME)

- Blood vs brain
- PI3K-AKT-mTOR pathway (30%)
  - 20-80% HME cells
  - Region variable



	Blood		Brain		
	Mut counts	Ref counts	Mut counts	Ref counts	Mut allele (%)
PIK3CA c.1633G>A (HME-1573)	0	121	9	47	16%
AKT3 c.49C>T (HME-1565)	0	49	9	23	28%
MTOR c.4448C>T (HME-1563)	0	298	17	159	9.7%



JAMA Neurol. 2016;73(7):836-845.

Nature Genetics 2012; 44(8):941-6.

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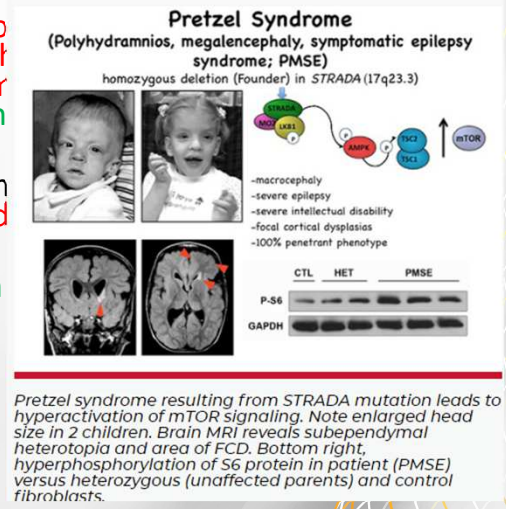
Pretzel syndrome,

polyhydramnios-megalencepahly-symptomatic epilepsy (PMSE)

- Megalencephaly is also seen in Pretzel syndro also known as polyhydramnios-megalencepal symptomatic epilepsy (PMSE), a rare autosom recessive disorder described in Mennonite ch from the area around Lancaster, PA,USA.
- In addition to megalencephaly, these children dysmorphic features, severe developmental d and intractable epilepsy.
- PMSE results from a homozygous deletion in STRADα gene.
- Loss of STRADα function => decreased hamartin/tuberin inhibition of mTORC1, => hyperactivation of the mTOR pathway.



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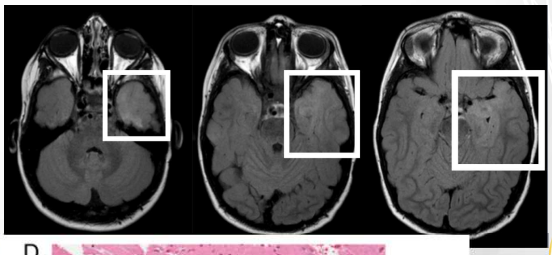
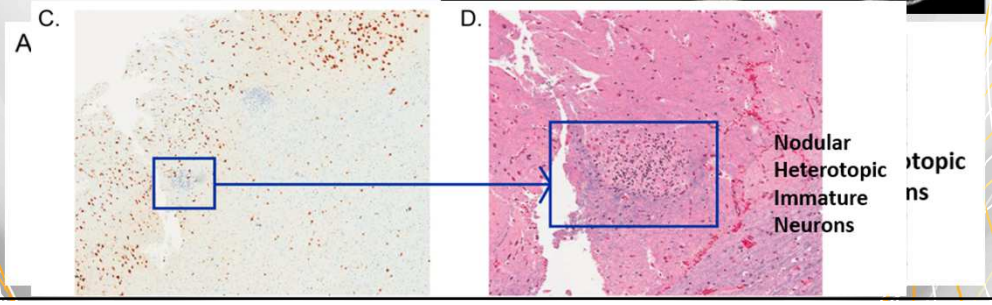
Focal cortical dysplasias (FCDs)

- : Collective term for a diverse group of cortical developmental malformations, all highly associated with medically intractable epilepsy.
- Both Germline VS Somatic mutations in the mTOR pathway => FCDs
- somatic mutations => 15% of patients with a subtype of FCD, a remarkably high yield, given that most if not all of these patients would be unlikely to have a causative genetic mutation found by conventional clinical testing.
- Many of the same somatic AKT3/PI3K mutations => HME + FCDs. A single somatic mutation => a spectrum of cortical abnormalities.
- Somatic mutations in PTEN gene => FCDs w/o tumor typically seen in PTEN-related syndromes .





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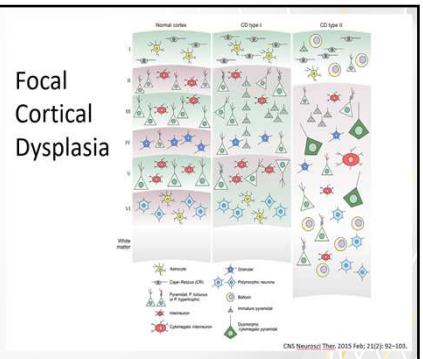
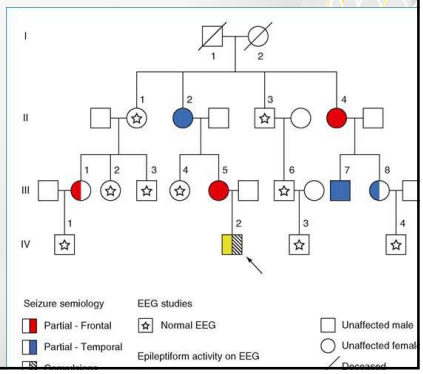
Focal cortical dysplasias (FCDs)

- FCDs have also been described in patients with mutations of the DEPDC5 gene. => GATOR1 complex (Protein DEPDC5+ NPRL2, NPRL3): inhibitor of mTORC1.
- DEPDC5 mutations first described in Australia Familial Focal Epilepsy with Variable Foci,
- Pts develop focal epilepsies localizing to different lobes.
- Some, but not all family members have focal lesions associated with their epilepsies, and the same mutations were found in both lesional and nonlesional family members.
- More recent studies have shown additional mutations in TSC1/2, DEPDC5, NPRL2 and NPRL3 that cause FCDs and nonlesional focal epilepsies, in both familial and sporadic forms,+ as well as epileptic spasms
- While mTOR-related syndromic epilepsies with associated non-neurological manifestations, such as TSC and PMSE, have been established as caused by germline mutations,
- somatic mutations in the mTOR pathway in cases of isolated, non syndromic cortical malformations, such as HME + FCDs.
- These Somatic mutation would be missed in standard genetic testing in blood, these exciting discoveries have revolutionized concepts of the pathophysiology of a spectrum of cortical malformation as genetic mTORopathies and have suggested the possibility of treatment of many of these disorders with mTOR inhibitors.





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FAMILIAL FOCAL EPILEPSY WITH VARIABLE FOCI: A NEW AND PROTEAN mTORopathy

- As previously suggested, new focal MCD syndromes will likely be identified that result from mutations in novel or known mTOR regulatory genes (Crino 2005, 2011).
- Most recently, nonsense and missense mutations were identified in DEPDC5 encoding a protein with tandem amino-terminal DEP (dishevelled, egl-10, pleckstrin) domains across several large Australian pedigrees in which the clinical phenotype was
- characterized by focal epilepsies arising from distinct lobar locations in different family members (Dibbens et al. 2013).
- FFEVF shows recurrent and at times intractable seizures in association with variable intellectual and neuropsychiatric disorders (e.g., depression, anxiety) with an autosomal dominant inheritance pattern.
- A subset of these patients shows radiographically apparent focal MCD seen on brain MRI suggesting a link between DEPDC5 mutations and altered brain development (Baulac et al. 2015). These focal MCDs often appear to be
- "bottom-of-the-sulcus" dysplasias, although, in a few cases, more expansive malformations were detected (e.g., focal band heterotopia) (Scheffer et al. 2014).
- Interestingly, in another report,DEPDC5 mutations were associated with non lesional focal epilepsies (Lal et al. 2014).
- DEPDC5 is an important component of the GATOR complex, which regulates mTORC1 activity in response to cellular levels of amino acid levels.
- Several studies have shown that, like TSC1 and TSC2, knockdown of DEPDC5 leads to enhanced mTORC1 signaling,
- Furtherstudies will be needed to define the role of DEPDC5 in cortical lamination and epileptogenesis and whether mTOR inhibitors can alter seizure frequency in FFEVF

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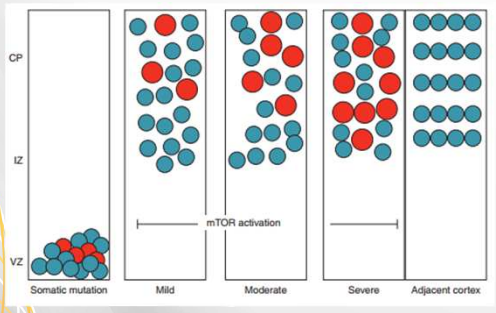

- Schematic depiction of how somatic mutations cause focal malformations of cortical development (MCD) during fetal brain development.
- Neuroglial progenitor cells (as early as 8- to 10-week gestation) sustaining a somatic mutation undergo an early cytopathic change (red cells). mTOR pathway activation.
- cellular enlargement (cytomegaly: enhanced cell soma size) and altered migration into the cortical plate.
- The admixture of affected cells (red) and adjacent cells (teal) form the focal MCD, surrounded by the unaffected cortex (all teal cells, far right).
- Hemimegalencephaly (HME), there may be differential somatic mutational load within individual MCDs causing mild, moderate, or severe pathological changes.
- Similarly, across subjects, there may be different levels of mosaicism for each MCD subtype

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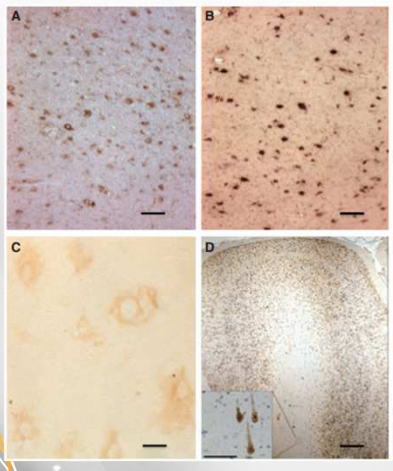
Histopathology of mammalian target of rapamycin (mTOR) activation in focal malformations of cortical development (MCD)



- (A) Focal cortical dysplasias type IIB (FCDIIB) showing phospho-S6 labeling.
- (B)Tuber showing phospho-S6 labeling.
- (C) Bottom-of-the-sulcus dysplasia showing phospho-S6 labeling.
- (D) Heterotopic neurons in white matter in Pretzel syndrome. (Adapted from Orlova et al. 2010a.) Scale bars, 300 mm (A,B); 80 mm (C); 1 mm (D); 50 mm (inset).

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mTOR inhibitors in Epilepsy Rx

- As a common pathway by which numerous sporadic and familial mutations cause epilepsy, mTOR represents a novel molecular target for antiseizure drugs.
- The compound rapamycin, an inhibitor of mTORC1, => antimicrobial and antitumor effects.
- Approved as sirolimus in 1999, => immunosuppressant to prevent rejection of organ transplants.
- In 2010, mTOR inhibitor, everolimus, was approved for treatment of SEGAs in TSC.
- 2013-2016 -> mTOR inhibitors as potential antiseizure medications.
- Rapamycin's antiseizure effects in multiple animal models
- Decrease seizure frequency or prevent development of epilepsy.
  - inhibiting abnormal cell growth and proliferation,
  - inhibition of mossy fiber sprouting
  - restoration of normal glutamate signaling
  - Mechanisms on cell morphology and proliferation=> less relevant in humans
  - mTOR inhibitors likely do not reverse preexisting structural lesions, such as tubers of TSC.

Summary of clinical evidence for mTOR inhibitors as adjunctive therapy in epilepsy.

Study (year)	Study type	Study drug	Patients	Duration of treatment	Summary of results	Ref.
TSC						
Wiegand *et al.* (2013)	Open-label case study/series	Everolimus	Seven patients, mean age 5 years (range 2–12 years)	36 weeks, with extended treatment in six patients up to 36 months	One patient stopped treatment due to rash. One patient achieved seizure freedom, three had reduction in seizure frequency between 25 and 59%, and two had no change from baseline	[36]
Cardamone *et al.* (2014)	Open-label case series	Sirolimus	13 patients, seven of which had seizures at baseline patients, median age 6 years (range 3–17 years)	Median 18 months (range 6–36 months)	One patient had more than 90% reduction in seizure frequency; four had 50–90% reduction, two had less than 50% reduction	[37]
Krueger *et al.* (2010)	Prospective, open-label Phase I/II study (primary end point SEGA reduction; secondary end point seizure frequency)	Everolimus	28 patients, median age 11 years (range 2–34 years)	6 months, with extended treatment in 27 patients, median 21.2 months (range 4.7–34.4 months)	At 6-month follow-up, nine patients had a decrease in seizure frequency, six patients had no change in frequency (five of whom had no seizures at baseline or follow-up) and one patient had an increase in frequency. At 24-month follow-up, 15 of 23 patients receiving extended treatments in whom seizure data were available reported no seizures since last visit [38]	[34]
Franz *et al.* (2013), EXIST-1	Multicenter, Phase III, randomized, placebo-controlled trial (primary end point SEGA reduction; secondary end point seizure frequency)	Everolimus	78 patients received everolimus, median age 9.5 years (range 1.0–23.9 years)	Median 41.9 weeks	Both median number of seizures at baseline and median change in seizure frequency from baseline to follow-up was 0 in both treatment and placebo groups	[39]
Kotulska *et al.* (2013)	Subgroup analysis of EXIST-1 trial	Everolimus	Eight patients under the age of 3 years, five of whom had active epilepsy at baseline	Median treatment duration and follow-up 35 months (range 33–38 months)	One child achieved seizure freedom and two had at least 50% reduction in seizure frequency. One child had decrease, then increase in seizure frequency and another had no change from baseline. None of the three children who were seizure free at baseline developed epilepsy	[40]
Krueger *et al.* (2013)	Prospective, multicenter, open-label, Phase I/II study (primary end point seizure frequency)	Everolimus	20 patients, median age 8 years (range 2–21 years)	12 weeks	Seizure frequency was reduced in 17 patients, with median reduction 71% ($p < 0.001$), and 12 of 20 patients having seizure reduction more than 50%. In a follow-up analysis of 18 patients receiving extended treatment (median 50.9 months, range 2.7–53.9 months), all but one of the 14 patients completing the study had more than 50% reduction in seizure frequency at 48 months [41]	[41]
Samueli *et al.* (2016)	Open-label, single-center prospective trial	Everolimus	15 children, median age 6 years (range 1–18 years)	Median 22 months (range 6–50 months)	Twelve of 15 children had treatment response, with seven patients achieving seizure freedom at last follow-up	[42]
French *et al.* (2016), EXIST-3	Phase III, randomized, placebo-controlled trial (primary end point seizure frequency)	Everolimus, low-exposure (target serum trough 3–7 ng/ml) versus	366 patients, median age 10.1 years (range 2.2–56.3 years)	18 weeks	Forty percent of patients in the high-exposure group showed a clinical response (defined as greater than 50% reduction in seizure frequency from baseline), compared with 28% in low-exposure group and 15% in placebo group. Median percentage reduction in seizure	[43]

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GG: TUMOR OR MALFORMATION?

- GG is a most common neoplasm associated with pediatric epilepsy,
- 5% of brain tumors of childhood (Southwell et al. 2012).
- Approximately 30% of GGs may be associated with an adjacent or adjoined FCD and are thus classified as FCDIIIB according to the
- recent ILAE classification (Blumcke et al. 2011).
- CD34-positive cells are also seen in BCs (characteristic enlarged cell found in TSC, FCDIIB,and HME) of FCDIIB suggesting a possible molecular link between GG and FCDIIB (Blumcke et al. 1999; Becker 2006; Marucci et al. 2013) => inclusion of GG as an mTORopathy.
- Enhanced mTOR signaling has been reported in GG as evidenced by p70S6kinase and S6 phosphorylation (largely in ATGCs),
- GG shares similar mTOR-signaling pathology with FCDs and cortical tubers (Samadani et al. 2007).
- Profile of phosphorylated-PDK1, -AKT, -mTOR, -4E-BP1, -eIF4G, -p70S6K, and -S6, arenearly identicalto FCDIIB (Boer et al. 2010).
- Interestingly, asomatic V600E mutation in B-RAF, a known pathogenic gene for melanoma, has been identified in 18% (Schindler et al. 2011) and 58% (Koelsche et al. 2013) of resected GG specimens.
- The V600E mutation is detected primarily in the neuronal and ATGC cell component of GG (Koelsche et al. 2013).
- B-RAF has been linked to enhanced mTOR signaling (Faustino et al. 2012) via LKB1 and via mTORC2/Akt (Chen et al. 2012b), suggesting a functional link between the molecular etiology and consequent observed changes in mTOR signaling.
- Some GGs, however, do not express the V600E mutation and, thus, like HME, it is likely that other molecular etiologies for GGs will be defined in future studies.

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mTOR inhibitors in Epilepsy Rx

- The most extensive use of mTOR inhibitors as antiseizure drugs has been in patients with TSC.
- A case series of the use of everolimus on a compassionate use basis for seven patients with severe, drug-resistant epilepsy,
- -> 4/6 patients completing treatment decrease in seizure frequency,
- Response varied from a 25 to 100% reduction in seizures
- Another case series evaluating the use of sirolimus demonstrated a decrease in clinical seizure frequency in all patients, with 5/7 patients having reduction greater than 50%.

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mTOR inhibitors in Epilepsy Rx

- Phase I/II study of everolimus to treat SEGAs in TSC patients showed the drug-reduced seizure frequency in 50%
- Extended treatment for a median duration of 34.2 months (range 4.7–47.1 months)
- % no seizures since their last visit (or more than 6 m.) increased from 38.5% (10 /26 patients) at baseline to 65.2% (15 of 23 patients) at 24 months.
- In the subsequent Phase III trial of everolimus for SEGAs, the analysis of seizure control as a secondary end point was inconclusive, as both the median number of seizures at baseline and median change in seizure frequency from baseline to follow-up was zero in both treatment and placebo groups

- Phase I/II study of everolimus as adjunctive therapy in TSC
- patients with the primary end point of reduction in seizure frequency
- 20 patients treated with 12 weeks of everolimus,
- Sz dec 17/20 w/ median reduction 73% ($p < 0.001$),
- 12 /20 patients having seizure reduction greater than 50%.
- FU 18/20 patients everolimus treatment extended past 12 weeks,
- 13/14 patients had > 50% reduction in seizure at 48 months.

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mTOR inhibitors in Epilepsy Rx

- A separate openlabel, single-center prospective trial of everolimus for epilepsy in children with TSC ages 1 - 18 years
- 12 /15 children had treatment response,
- 7 patients seizure-free at median follow-up of 22 months.

- Phase III, randomized placebo-controlled trial of adjunctive everolimus treatment for epilepsy, the EXIST-3 trial.
- 366 patients between age 2 - 65 years were randomized to placebo, low-exposure everolimus or high-exposure everolimus,
- 40% of patients in the high-exposure group showed a clinical response (>50% decrease in seizure frequency),
- 28% in low-exposure group
- 15% in placebo group
- Median seizure reduction in the high-exposure group was 39.6%.

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mTOR inhibitors in Epilepsy Rx

- A randomized controlled trial of sirolimus in children with TSC
- => no effect of the drug on seizure frequency,
- the study did not reach its target enrollment of 30 patients.

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mTOR inhibitors in Epilepsy Rx

- Other mTORopathies are < 15 year , clinical data=> effect of mTOR inhibitors for non-TSC epilepsies are limited.
- A prospective, open-label trial of sirolimus as adjunctive therapy in five infants with PMSE showed that
- 4/5 were seizure-free at follow-up (median 36 months, range 5–52 months),
- 5th child had only one seizure in the prior 12-month period.
- Pts also had improved receptive language vs untreated historical controls,
- mTORopathies may have effects beyond preventing seizures.

- Evidence for mTOR inhibitors in other mTOR dysregulation is in animal models.
- Rapamycin-suppressed epileptiform activity in EEG recordings of a PTEN-knockout mouse model.
- In mouse models of TSC, the use of postnatal rapamycin reversed histopathological sequelae of mTOR hyperactivation and prevented development of epilepsy

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mTOR inhibitors in Epilepsy Rx

- Prospective clinical studies of their use in other mTORopathies, w/ severe refractory epilepsies, may be feasible.
- The mTORopathies provide clinical cohorts for studies of mTOR inhibitors
- As antiseizure drugs
- As possible preventative antiepileptogenic therapy.
- TSC Infants w/o neurological findings represent an ideal clinical population for testing antiepileptogenic potential of drug therapies, c/o the majority of patients with TSC will develop epilepsy.
- To date, however, there is limited published clinical evidence that mTOR inhibitors prevent epilepsy.
- FU study of eight children younger than age 3 years in EXIST-1 trial, 3/5 children w/ refractory epilepsy at baseline => significant improvement in seizures with treatment, 0/3 children developed epilepsy

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Conclusion

- The mTOR pathway plays a key role in normal neurologic cell morphology and physiology
- Implicated in TSC and other genetic epilepsies.
- Genetic mutations in mTOR and other key regulatory proteins => pathologic changes in cell size and function =>epileptogenesis.
- mTOR inhibitors => reverse some of these neuropathologic or functional abnormalities.
- Recent clinical trials of the mTOR inhibitors everolimus and sirolimus => potential for their use as antiseizure in TSC + other mTOR dysregulation epilepsies.
- Future studies => evaluate the long-term effects of mTOR inhibitors, as well as their role in other clinical syndromes.

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Cold Spring Harbor Perspectives in Medicine

mTOR Signaling in Epilepsy: Insights from Malformations of Cortical Development

Peter B. Crino

Cold Spring Harb Perspect Med 2015; doi: 10.1101/cshperspect.a022442

- Over the past decade enhanced activation of the mammalian target of rapamycin (mTOR)-signaling cascade has been identified in focal malformations of cortical development (MCD) subtypes, which have been collectively referred to as "mTORopathies."
- Mutations in mTORregulatory genes (e.g., TSC1, TSC2, AKT3, DEPDC5) have been associated with several focal MCD highly associated with epilepsy such as tuberous sclerosis complex (TSC), hemimegalencephaly (HME; brain malformation associated with dramatic enlargement of one brain hemisphere), and cortical dysplasia.
- mTOR plays important roles in the regulation of cell division, growth, and survival, and, thus, aberrant activation of the cascade during cortical development can cause dramatic alterations in cell size, cortical lamination, and axon and dendrite outgrowth often observed in focal MCD.
- Although it is widely believed that structural alterations induced by hyperactivated mTOR signaling are critical for epileptogenesis, newer evidence suggests that mTOR activation on its own may enhance neuronal excitability.
- Clinical trials with mTOR inhibitors have shown efficacy in the treatment of seizures associated with focal MCD.

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