mTORopathies: A Road Well-Traveled

Commentary on: Crino PB. Knockout of a Tuberous Sclerosis Gene Highlights Role of Glia in Epileptogenesis. Epilepsy Currents 2003;3:139-141. doi: 10.1046/j.1535-7597.2003.03411.x

Malformations of cortical development (MCD) including focal cortical dysplasias (FCD) and hemimegalencephaly (HME) are common causes of pediatric epilepsy. Viewed as neuropathological oddities in the first reports, recurrent detection of MCD in epilepsy surgery specimens through the 1980s and 1990s plus improved neuroimaging techniques and then fetal brain imaging presented MCD as a true challenge for investigative research into developmental pathogenesis, establishment of the epileptic circuit, and of course, actionable mechanisms for new seizure therapies. Malformations of cortical development moved conceptually from pathological curiosities to causative lesions around which clinical strategies for epilepsy surgical approaches were constructed.

In 2003, I provided commentary on an interesting paper that defined a role for astrocytes in epileptogenesis in tuberous sclerosis complex (TSC), a genetic or sporadic disorder caused by mutations in TSC1 or TSC2, and characterized histopathologically by MCD (“tubers”) histologically similar to FCD type 2A or 2B (FCD2). Here, a conditional Cre-LoxP knockout of Tsc1 under human glial fibrillary acidic protein (GFAP) promoter control led to brain enlargement, astrogliosis, an abnormally shaped hippocampus, and recurrent spontaneous seizures by 4 weeks of age in mice. Interestingly, in 2001 and 2002, Tsc1 and Tsc2 were shown to canonically modulate the serine–threonine kinase mechanistic target of rapamycin (mTOR), within the insulin growth factor (IGF) signaling pathway. Although Uhlmann et al did not address mTOR in their paper, it seemed logical that the changes in brain structure and function they reported could be linked to mTOR signaling.

mTORopathies and MCD

In 2004, my laboratory hypothesized that since TSC1 and TSC2 mutations appeared to be “loss-of-function” causing constitutive hyperactivation of mTOR kinase, human TSC MCD tissue specimens should exhibit mTOR hyperactivation. We also postulated that since FCD2 histopathology was similar, though not identical, to tubers in TSC, mTOR activation would be observed in FCD2; at this time, no genes had been linked with FCD and specifically, TSC1 or TSC2 mutations had not been identified in FCD patients. We and others found robust hyper-phosphorylation of the mTOR pathway substrate ribosomal S6 protein (P-S6) in tubers and FCD2 specimens, demonstrating for the first time that mTOR activation was central to TSC and FCD2 pathogenesis in human brain. These observations were extended to human HME specimens, thus linking mTOR with TSC, FCD2, and HME.

Understanding mTOR activation in MCD was greatly aided by TSC mouse models, for example, the Tsc1GFAP-cre strain, that assessed how loss of Tsc1 or Tsc2 led to changes in brain structure and seizures. Germline knockout of Tsc1 or Tsc2 was lethal and so conditional knockout strains were generated under various cell-specific promoters, that is, GFAP (astrocytes), synapsin (neurons), and nestin (neuroglial progenitor cells). Although these animals did not exhibit classic histological features of tubers and FCD2, that is, focal collections of balloon cells or cytomegalic dysmorphic neurons, they showed glial proliferation, neuronal enlargement, ectopic neurons, increased brain size, and variably, seizures. A unifying feature of all models was the presence of abnormally high numbers of P-S6 labeled cells suggesting a causal relationship between gene knockout and mTOR activation, and the observation that histological abnormalities and seizures could be prevented with mTOR inhibitors (mTORi) prompting aspirational clinical plans with mTORi.

Conceptualization of mTORopathies

An association between PTEN variants, macrocephaly, and autism had been recognized in both human linkage studies and mouse Pten models which showed cellular hypertrophy, altered laminar structure, enlarged brain size, and in some reports, spontaneous seizures. PTEN is an inhibitory regulator of PI3Kinase (PI3K) upstream of AKT and mTOR. In my view...
at the time, PTEN provided evidence and a theoretical framework that other gene variants in the mTOR pathway could confer phenotypic features similar to TSC1/TSC2. Then, in 2007, a new rare epilepsy syndrome ("Pretzel Syndrome") was identified among the Old Order Mennonites, characterized by megalencephaly and histological features similar to FCD2 with mTOR hyperactivation, and linked to mutations in STRADA, a modulator of mTOR via AMPK in a non-canonical arm of the mTOR pathway. Pretzel syndrome was pivotal to conceptualizing mTOR-associated MCD as it demonstrated yet another mTOR pathway gene (MPG) with TSC1, TSC2, and PTEN causing abnormal brain structure and seizures. Evaluating published MCD human tissue studies and MCD mouse models linked to enhanced mTOR signaling, in 2009, we proposed that TSC, FCD2, HME, and some forms of megalencephaly, represented a spectrum of MCD mechanistically linked to mTOR hyperactivation via MPG mutations occurring within progenitor cells during brain development, which we named "mTORopathies." A key gap in knowledge however was what genes caused mTOR activation in these MCD subtypes. The mTORopathy field exploded with the discovery of several epilepsy pedigrees linked to DEPDC5,17 a component of the GATOR 1 complex governing mTOR activation in response to cellular amino acid levels. Subsequent studies found DEPDC5 mutations associated with FCD2, and mouse models of Depdc5 loss were associated with abnormal brain structure and spontaneous seizures. The DEPDC5 discovery was exciting because, like STRADA, it moved mTORopathies out of the canonical IGF-mTOR signaling pathway into metabolomic regulation of mTOR. Soon, mutations in NPRL2 and NPRL3, encoding binding partners of DEPDC5, were identified in MCD pedigrees and were associated with mTOR hyperactivation in tissue samples and in vitro models. Subsequent studies have shown that DEPDC5, NPRL3, and NPRL2 mutations are the most common genetic causes of FCD and focal onset neocortical epilepsy. The mTORopathy field exploded with the discovery of several epilepsy pedigrees linked to 14 distinct genes may be approached therapeutically with a single drug target, for example, mTORi. These data present an exciting clinical inflection point where epilepsy linked to 14 distinct genes may be approached therapeutically with a single drug target, for example, mTORi. Additionally, there are other targets within the mTOR pathway that offer compelling new options for drug development.

Genomics Revolution

In 2011, there began a rapid-fire series of remarkable breakthrough studies that elucidated the molecular pathogenesis of mTORopathies. First, HME was linked to activating mutations in the brain-specific AKT3 isoform13 and then PI3K,14 both culminating in mTOR hyperactivation. A seminal finding15,16 was identification of somatic activating MTOR mutations in human FCD2 specimens. Both cell culture and mouse models of MTO R variants demonstrated changes in cell morphology and abnormal neuronal firing as pathogenic underpinnings for FCD2 and mTORopathies. A pivotal observation that changed our understanding of mTORopathies was the determination that somatic as well as germ line MPG mutations could cause FCD2 and HME. Historically, HME and FCD were rarely found in large pedigrees suggesting a mechanism that was unique to each affected individual. The detection of somatic MPG variants, that is, de novo mutations occurring in early neural progenitor cells and affecting a limited subset of neurons within the MCD, provided a logical mechanism to account for lack of large pedigrees and the focal nature of the MCD.

mTORopathies Are Common, Not Rare

The mTORopathy field exploded with the discovery of several epilepsy pedigrees linked to DEPDC5, a component of the GATOR 1 complex governing mTOR activation in response to cellular amino acid levels. Subsequent studies found DEPDC5 mutations associated with FCD2, and mouse models of Depdc5 loss were associated with abnormal brain structure and spontaneous seizures. The DEPDC5 discovery was exciting because, like STRADA, it moved mTORopathies out of the canonical IGF-mTOR signaling pathway into metabolomic regulation of mTOR. Soon, mutations in NPRL2 and NPRL3, encoding binding partners of DEPDC5, were identified in MCD pedigrees and were associated with mTOR hyperactivation in tissue samples and in vitro models. Subsequent studies have shown that DEPDC5, NPRL3, and NPRL2 mutations are the most common genetic causes of FCD and focal onset neocortical epilepsy. The mTORopathy field exploded with the discovery of several epilepsy pedigrees linked to 14 distinct genes may be approached therapeutically with a single drug target, for example, mTORi. Additionally, there are other targets within the mTOR pathway that offer compelling new options for drug development.

Looking Back and Looking Ahead: Unanswered Questions

Perceptions of MCD have indeed changed over time. Prior to the 2000s, MCD were viewed as curiosities and thought to result from intrauterine injury, hypoxia–ischemia, toxic exposure, or infection. Now, MCD are viewed more with the lens of brain cancer, that is, lesions with defined pathological features and known molecular genetic causes that may be actionable. Over the past decade, there has been a literal explosion of genetic studies, human tissue analyses, and animal models including Drosophila, Caenorhabditis elegans, zebra fish, mouse, and rat to study the effects of MPG on brain development. Numerous papers and funded grant applications devoted to MCD have resulted from this intellectual progress. mTORopathies have provided a compelling and fascinating journey from bedside to bench and back again, with the laudable deliverable of actual benefit to human patients for example, with the use of mTORi in TSC patients. Critical unanswered questions for mTORopathies over the next decade should focus on molecular pathogenesis, establishment of epileptic circuits, discovery of affected cell lineages, and of course, strategies to prevent mTORopathy-associated seizures. To date, there is no explanation for why mTORopathy gene mutations occur. Are these mutational “hotspots,” stochastic errors, or another causative agent; why these genes, why so common? The cells of origin in tubers, FCD2, and HME remain to be fully defined and unlike the Tsc1cre-GFAP mouse where Tsc1 was absent from astrocytes, most studies to date now recognize that somatic mutations lead to MPG loss or over-activation in neurons. We still do not understand epileptogenesis in mTORopathies and clearly, gene mutations affect both neurons and astrocytes, leading to abnormal circuit formation which extends beyond the interesting observations of Uhlmann et al. Finally, while several clinical trials, for example, EXIST-3, have demonstrated that mTORi may reduce seizures in...
mTORopathies, no study has shown epilepsy cure and as a corollary, improvements in behavioral and intellectual disabilities, often comorbid in mTORopathies, have not been realized with mTORi. Clearly, another decade of research is challenging us ahead.

By Peter B. Crino
Department of Neurology University of Maryland School of Medicine

ORCID iD
Peter Crino https://orcid.org/0000-0002-6232-3740

References
1. Taylor DC, Falconer MA, Bruton CJ, Corellis JA. Focal dysplasia of the cerebral cortex in epilepsy. J Neurol Neurosurg Psychiatry. 1971;34(4):369-387.
2. Crino PB. Knockout of a tuberous sclerosis gene highlights role of glia in epileptogenesis. Epilepsy Curr. 2003;3(4):139-141.
3. Uhllmann EJ, Wong M, Baldwin RL, et al. Astrocyte-specific TSC1 conditional knockout mice exhibit abnormal neuronal organization and seizures. Ann Neurol. 2002;52(3):285-296.
4. Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC, Blenis J. Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. Proc Natl Acad Sci U S A. 2002;99(21):13571-13576.
5. Baybis M, Yu J, Lee A, et al. mTOR cascade activation distinguishes tubers from focal cortical dysplasia. Ann Neurol. 2004;56(4):478-487.
6. Miyata H, Chiang AC, Vinters HV. Insulin signaling pathways in cortical dysplasia and TSC-tubers: tissue microarray analysis. Ann Neurol. 2004;56(4):510-519.
7. Ljungberg MC, Bhattacharjee MB, Lu Y, et al. Activation of mammalian target of rapamycin in cytogenic neurons of human cortical dysplasia. Ann Neurol. 2006;60(4):420-429.
8. Aronica E, Boer K, Baybis M, Yu J, Crino P. Co-expression of cyclin D1 and phosphorylated ribosomal S6 proteins in hemimegalencephaly. Acta Neuropathol. 2007;114(3):287-293.
9. Meikle L, Pollizz K, Egnor A, et al. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. J Neurosci. 2008;28(21):5422-5432.
10. Ljungberg MC, Sunnen CN, Lugo JN, Anderson AE, D’Arcangelo G. Rapamycin suppresses seizures and neuronal hypertrophy in a mouse model of cortical dysplasia. Dis Model Mech. 2009;2(7-8):389-398.
11. Puffenberger EG, Strauss KA, Ramsey KE, et al. Polyhydramnios, megalencephaly and symptomatic epilepsy caused by a homozygous 7-kilobase deletion in LYK5. Brain. 2007;130( Pt 7):1929-1941.
12. Crino PB. Focal brain malformations: seizures, signaling, sequencing. Epilepsia. 2009;50(suppl 9):3-8.
13. Poduri A, Evrony GD, Cai X, et al. Somatic activation of AKT3 causes hemispheric developmental brain malformations. Neuron. 2012;74(1):41-48.
14. Lee JH, Huynh M, Silhavy JL, et al. De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. Nat Genet. 2012;44(8):941-945.
15. Lim JS, Kim WI, Kang HC, et al. Brain somatic mutations in mTOR cause focal cortical dysplasia type II leading to intractable epilepsy. Nat Med. 2015;21(4):395-400.
16. Nakashima M, Saitou H, Takei N, et al. Somatic mutations in the MTOR gene cause focal cortical dysplasia type Ib. Ann Neurol. 2015;78(3):375-386.
17. Dibbens LM, De Vries B, Donatello S, et al. Mutations in DEPDC5 cause familial focal epilepsy with variable foci. Nat Genet. 2013;45(5):546-551.
18. Sim JC, Scerri T, Fanjul-Fernández M, et al. Familial cortical dysplasia caused by mutation in the mammalian target of rapamycin regulator NPRL3. Ann Neurol. 2013;74(1):132-137.
19. Baldassari S, Picard F, Verbeek NE, et al. The landscape of epilepsy-related GATOR1 variants. Genet Med. 2019;21(2):398-408.
20. French JA, Lawson JA, Yapaci Z, et al. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study. Lancet. 2016;388(10056):2153-2163.