



More is Different in mTORopathies

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The mTOR pathway genes *mTOR*, *Rheb*, *Depdc5*, *Pten*, and *Tsc1* have convergent and divergent impacts on cortical neuron development and function

Nguyen LH, Xu YF, Nair M, Bordey A. The mTOR pathway genes *MTOR*, *Rheb*, *Depdc5*, *Pten*, and *Tsc1* have convergent and divergent impacts on cortical neuron development and function. *Elife* 2024;12:RP91010. doi: 10.7554/eLife.91010

Brain somatic mutations in various components of the mTOR complex 1 (mTORC1) pathway have emerged as major causes of focal malformations of cortical development and intractable epilepsy. While these distinct gene mutations converge on excessive mTORC1 signaling and lead to common clinical manifestations, it remains unclear whether they cause similar cellular and synaptic disruptions underlying cortical network hyperexcitability. Here, we show that in utero activation of the mTORC1 activators, *Rheb* or *mTOR*, or biallelic inactivation of the mTORC1 repressors, *Depdc5*, *Tsc1*, or *Pten* in mouse medial prefrontal cortex leads to shared alterations in pyramidal neuron morphology, positioning, and membrane excitability but different changes in excitatory synaptic transmission. Our findings suggest that, despite converging on mTORC1 signaling, mutations in different mTORC1 pathway genes differentially impact cortical excitatory synaptic activity, which may confer gene-specific mechanisms of hyperexcitability and responses to therapeutic intervention.

Commentary

Low-frequency somatic mutations in key genes within the mechanistic target of rapamycin (mTOR) pathway have been frequently identified in patients with malformations of cortical development (MCD), such as focal cortical dysplasia type II (FCDII) and hemimegalencephaly (HME). These diseases are collectively referred as mTORopathies, one of the most common pathologies in patients with severe, progressive, and drug-resistant epilepsies. Most patients do not respond to anti-seizure medications that primarily modulate neuronal channels and synaptic activity. mTOR inhibitors, the proposed “precision medicine,” are not superior to traditional anti-seizure medications and only showed modest efficacy. Pathogenic variants in 14 distinct genes that converge on mTORC1 and lead to its hyperactivation have been linked to MCD and epilepsy.¹ Despite the staggering advance in gene discovery, the molecular, cellular, and circuit networks that make the malformed cortex highly epileptogenic remain largely unknown, making it challenging to develop mechanistic-based therapies.

Attempting to define the shared and differential functional roles of different mTOR pathway genes on neurodevelopment and neuronal excitability, Nguyen LH et al took advantage of in utero electroporation (IUE) to introduce somatic mutations in mouse prenatal forebrain to hyperactivate mTORC1 by

either overexpressing human pathogenic gain-of-function variants (RHEBY35L or mTORS2215Y) or deleting mTORC1 inhibitors (*Tsc1*, *Pten*, and *Depdc5*).² All conditions led to increased mTORC1 activity, cytomegaly, and neuronal migration defects (only *Pten* KO animals showed proper cortical lamination). At the functional level, cytomegalic neurons had increased membrane capacitance, conductance, and rheobase. When measured by their action potential firing (AP) response to depolarizing current injection, these neurons showed decreased intrinsic membrane excitability. To explain how a few “hypoexcitable” mutant neurons generate a hyperexcitable cortex, the authors have previously shown that neurons expressing RHEBS16H (referred as RHEB^{CA}), another mTOR-activating variant of RHEB, ectopically expressed HCN4 channels that are sensitive to intracellular cAMP and give rise to hyperpolarization-activated cation current (*I_h*), leading to a more depolarized neuronal resting membrane potential (RMP) and therefore cell-autonomous hyperexcitability.³ In the current study, the authors confirmed ectopically expressed HCN4 channels in all conditions. However, only mTORS2215Y overexpressing and *Tsc1* KO neurons showed a significantly increased *I_h* amplitude. Notably, as compared to the strikingly increased *I_h* that was previously reported in neurons expressing *Rheb*^{CA} (~400pA), the *I_h* change in *Tsc1*



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KO neurons (ctrl: -8.7 ± 12.1 pA vs KO: -51.2 ± 31.2 pA), although significant, was modest. Surprisingly, neurons expressing RhebY35L did not display significantly increased I_h . The RMP was more depolarized in RhebY35L, mTORS2215Y, and *Tsc1* KO neurons but was unchanged in *Pten* KO or *Depdc5* KO neurons. These data suggested that the ectopically expressed HCN4 in cytomegalic neurons have variable functional outcomes in mTORopathies that are caused by different genes or even different mutations of the same gene, raising the question of whether HCN4-mediated hyperexcitability is the convergent epileptogenic mechanisms. Of note, previous studies have shown RMP was unchanged or even slightly hyperpolarized in *Tsc1* or *Pten* knockout neurons or neurons expressing mTORL2427P.^{4–6} At the synaptic level, the authors measured sEPSC amplitude and frequency. They showed that all conditions, except RhebY35L, lead to increased synaptic excitability, with variable impact on sEPSC frequency and amplitude.

This unprecedented comparison study has provided valuable and compelling evidence to support mTORopathies as a unifying neurodevelopmental disorder. However, the subtle and sometimes overt distinct functional expression (e.g., I_h) raises important questions. First, it is unknown if these different animal models developed epilepsy and if their epilepsy phenotypes were different. Earlier studies have shown that RHEB^{CA} IUE only generated seizures in the prefrontal cortex, while RHEBP37L or MTORL2427P IUE induced seizures independent of cortical regions. Interestingly, Cre-IUE *Tsc1* KO in the mouse somatosensory cortex only showed a reduced seizure threshold,⁷ and only 30% of Cre-IUE *Depdc5* KO developed rare seizures.⁸ It is unclear why overexpression of mutant RHEB or mTOR caused the most severe and consistent seizure phenotype. Could mutations in mTOR inhibitor and activator have different cellular and molecular mechanisms of epileptogenesis? Second, it is surprising that different RHEB mutants have different functional outcomes. RHEBS16H, referred to as RHEB^{CA} in a series of previous studies, was identified through *in silico* analysis,⁹ and its overexpression caused a highly elevated I_h , depolarized RMP, and frequent seizures. RHEBP37L, similar to RHEBY35L in the current study, is a patient-based pathogenic mutant, and its overexpression caused severe seizures without RMP changes, suggesting an I_h -independent mechanism.¹⁰ Could different mutations of the same gene have different mechanisms of epileptogenesis, or could the *in silico*-based mutation be a much more potent mTORC1 activator than patient-based mutations? Third, whether the electrophysiology recordings on mutant neurons were performed during the latent period, chronic stage, or disease progression is unclear. The author has previously noted that RHEB^{CA} mice developed seizures between P21 and P28 but performed the patch-clamp recording in the current study between P26–P51 when seizure could have induced changes in the cellular and molecular landscape, such as neurodegeneration, astrogliosis, and microglia activation, leading to changes in membrane and synaptic excitability. In addition, the authors did not evaluate inhibitory synaptic

properties. Interestingly, Koh et al have shown that both inhibitory and excitatory inputs onto mTOR mutant neurons were increased in an IUE FCD mouse model.⁶ Last, Filamin A,¹¹ HCN4,³ ADK,⁶ and ciliogenesis¹² have all been proposed to play a central role in mTORopathies, and reversing the deficit could all lead to substantial or near-complete seizure control, raising questions of how these molecular architectures are constructed to converge on cortical hyperexcitability.

With advanced sequencing and biochemical technologies, neuroscientists will undoubtedly discover more genes and mutations in the mTOR pathway, hoping to decode the pathogenesis of mTORopathies. Francis Crick once claimed, “The ultimate aim of the modern movement in biology is to explain all biology in terms of physics and chemistry,” epitomizing the biology reductionism that genotype-to-phenotype relationships are the additive effects of genetic information. In this current study, Nguyen et al clearly showed that mTORopathies, due to different mutations, share similar cellular phenotypes, such as increased cell size, intrinsic excitability, and synaptic transmission. Meanwhile, Nguyen and other groups showed that mechanisms of cortical hyperexcitability in the malformed cortex could be far beyond the cytomegalic neurons caused by different genetic mutations, and its complexity may no longer be fully understandable at the level of individual mutations. In August 1972, Philip Anderson’s essay ‘More is different’ argued that “at each level of complexity entirely new properties appear” —for example, chemistry is subject to the laws of physics, but we cannot deduce chemistry from our knowledge of physics. Nguyen et al demonstrated that the epileptogenic cortex is not only more than but very different from the sum of a few mutant neurons.

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