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Getting Sucker Punched by *Depdc5* Really Hurts

Acute Knockdown of Depdc5 Leads to Synaptic Defects in mTOR-Related Epileptogenesis

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DEP-domain containing 5 (DEPDC5) is part of the GATOR1 complex that functions as key inhibitor of the mechanistic target of rapamycin complex 1 (mTORC1). Loss-of-function mutations in DEPDC5 leading to mTOR hyperactivation have been identified as the most common cause of either lesional or non-lesional focal epilepsy. However, the precise mechanisms by which DEPDC5 loss-of-function triggers neuronal and network hyperexcitability are still unclear. In this study, we investigated the cellular mechanisms of hyperexcitability by comparing the constitutive heterozygous Depdc5 knockout mouse versus different levels of acute Depdc5 deletion (≈40% and ≈80% neuronal knockdown of Depdc5 protein) by RNA interference in primary cortical cultures. While heterozygous Depdc5$^{+/-}$ neurons have only a subtle phenotype, acutely knocked-down neurons exhibit a strong dose-dependent phenotype characterized by mTOR hyperactivation, increased soma size, dendritic arborization, excitatory synaptic transmission, and intrinsic excitability. The robust synaptic phenotype resulting from the acute knockdown Depdc5 deficiency highlights the importance of the temporal dynamics of Depdc5 knockdown in triggering the phenotypic changes, reminiscent of the somatic second-hit mechanism in patients with focal cortical dysplasia. These findings uncover a novel synaptic phenotype that is causally linked to Depdc5 knockdown, highlighting the developmental role of Depdc5. Interestingly, the synaptic defect appears to affect only excitatory synapses, while inhibitory synapses develop normally. The increased frequency and amplitude of miniature excitatory postsynaptic currents, paralleled by increased density of excitatory synapses and expression of glutamate receptors, may generate an excitation/inhibition imbalance that triggers epileptogenesis.

Commentary

In May of 2013, a trio of papers greatly expanded our view of the mTOR signaling pathway and its relation to epilepsy. These papers first identified *DEPDC5* as both a frequent cause of familial focal epilepsies[1,2] and a negative regulator of mTOR activity.[3] The discovery of *Depdc5's* normal and pathological roles was made more significant by the fact that DEPDC5 was not in the already well-studied canonical PI3K-TSC-mTOR axis, but a member of a new complex called GATOR1 that was proposed to signal amino acid availability to mTORC1. This, together with the finding that somatic mutations in *Depdc5* and several other mTOR pathway genes caused cortical dysplasias and hemimegalencephaly,[4] established mTOR hyperactivation as one of the major genetic causes of intractable epilepsy.

Animal models of *Depdc5* loss of function soon followed but encountered a familiar problem in mTOR-related basic and preclinical research. Heterozygous deletion of the gene-mimicked patient genotypes but failed to recapitulate key disease features such as spontaneous seizures, while homozygous germline deletion was embryonic lethal.[5,6] As had previously been done for other mTOR repressors, researchers turned to neuron-specific gene disruption using both in utero electroporation and the Cre-lox system to circumvent the problem of embryonic lethality. These neuron-specific models of *Depdc5* loss produced rodents that recapitulated key features of the human disease, including enlarged neurons, cortical disorganization, focal interictal epileptiform discharges, and spontaneous seizures, paving the way for future mechanistic studies.[7-9]

Differences in gene dosage between humans and animal models is a common issue. Heterozygous loss of a gene in animals often produces an apparently milder phenotype than expected based on the human disease symptoms. Numerous species differences could account for this phenomenon. Indeed, in regard to mTOR pathway variants and cortical malformations, the longer and much more complex period of cortical development in humans than mice likely plays a large role in

the phenotypic differences. A new study by De Fusco et al, however, suggests that the temporal dynamics of gene loss may interact with gene dosage to regulate the severity of cellular phenotypes, at least in *Depdc5*-related disorders.[10]

The experimental paradigm used by the authors was to compare the effects of heterozygous germline deletion of *Depdc5* (*Depdc5*$^{+/-}$), which reduces the amount of DEPDC5 protein by 50%, with the effects of acute knockdown (KD) with 2 different shRNA constructs, which reduced DEPDC5 levels by 40% or 80% in cultured cortical neurons. In this model system, *Depdc5*$^{+/-}$ neurons had very mild phenotypes; cell size and synaptic transmission were normal, but they did show a small increase in neurite outgrowth. In contrast, neurons treated with the shRNA that caused a mean 80% reduction showed strong cellular phenotypes including increased cell size and neurite outgrowth and increased excitatory synaptic transmission and synapse number.

The surprising result was that neurons treated with the shRNA that caused a mean 40% reduction in DEPDC5 levels showed apparently stronger phenotypes than *Depdc5*$^{+/-}$ neurons. Forty percent KD neurons had increased levels of pS6, which correlates with mTOR hyperactivation, as well as increased cell size and strongly increased frequency of miniature excitatory postsynaptic currents (mEPSCs), phenotypes that were absent in *Depdc5*$^{+/-}$ neurons. Because the 40% of KD neurons had more DEPDC5 protein than the *Depdc5*$^{+/-}$ neurons, the authors concluded that the stronger phenotype was due to the temporal aspect of gene deletion. This suggests that *Depdc5*$^{+/-}$ neurons may compensate for their constitutive lack of sufficient DEPDC5 levels in ways that mitigate the phenotypes, while acute KD at a later developmental time point has more severe consequences.

Although the authors were primarily working in an artificial system (ie, lentiviral shRNA KD in primary cultured cortical neurons), the finding that the timing of DEPDC5 protein reduction affects the severity of the phenotypes may still be important to understanding human disease. This is because variants in *Depdc5* and other mTOR-related genes in humans are often somatic mutations, probably in dorsal telencephalic progenitor cells that become cortical astrocytes and excitatory neurons. A somatic mutation is a genetic change that occurs not in the germline, but at some point later in development, meaning that it affects only a minority of cells, and that these cells with somatic mutations are generated from cells that have gone through early development with a normal genome. One of the mysteries of somatic mutations is how a genetic change in a small number of neurons (they often affect only 2% to 10% of brain cells) can cause such strong changes in brain architecture and severe seizures. If cells at later developmental time points are more sensitive to reduction of DEPDC5 and mTOR hyperactivation than cells that have never had normal levels of DEPDC5, this may help explain why somatic mutations in *Depdc5* and other genes that regulate mTOR signaling have such strong effects on brain morphology and physiology.

Of course, there are some strong caveats to making this connection. The neurons in this study were mature neurons, not dorsal progenitors, thus the relative timing of KD and when a somatic mutation would occur in vivo are not well aligned. Also, the effects on excitatory synaptic transmission found in this study were strong. Forty percent or 80% of DEPDC KD caused approximate 3- and 4-fold increases, respectively, in the frequency of mEPSCs. Changes of this magnitude are higher than what is generally observed as a result of mTOR hyperactivation. They also contrast somewhat with a recent study that used in utero electroporation to knockout *Depdc5* in the developing cortex, which did not observe increases in spontaneous EPSC frequency, despite stronger increases in cell size and pS6 levels.[8] However, these 2 studies differ significantly in the means and timing of both the DEPDC5 reduction and the experimental measurements, meaning that the difference in results is not especially surprising. Like increases in neuron growth and altered migration, increases in excitatory synaptic transmission have been observed in multiple animal models of mTORopathies, including, now, *Depdc5* loss. The authors of this study argue that the strengthening they see of excitatory transmission, without a corresponding strengthening of inhibitory transmission, increases the excitation/inhibition ratio and contributes to seizure generation. The precise relationship between mTOR hyperactivation, increases in excitatory transmission, cell growth, and their contribution to disease phenotypes is still unclear. This new study suggests, however, that the timing of mTOR hyperactivation could be a key variable in determining their interactions.

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