Fil-lamin-ing in the Gap in Cortical Dysplasia

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Filamin A Inhibition Reduces Seizure Activity in a Mouse Model of Focal Cortical Malformations

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Epilepsy treatments for patients with mechanistic target of rapamycin (mTOR) disorders, such as tuberous sclerosis complex (TSC) or focal cortical dysplasia type II (FCDII), are urgently needed. In these patients, the presence of focal cortical malformations is associated with the occurrence of lifelong epilepsy, leading to severe neurological comorbidities. Here, we show that the expression of the actin cross-linking protein filamin A (FLNA) is increased in resected cortical tissue that is responsible for seizures in patients with FCDII and in mice modeling TSC and FCDII with mutations in phosphoinositide 3-kinase (PI3K)-ras homolog enriched in brain (Rheb) pathway genes. Normalizing FLNA expression in these mice through genetic knockdown limited cell misplacement and neuronal dysmorphogenesis, 2 hallmarks of focal cortical malformations. In addition, *Flna* knockdown reduced seizure frequency independently of mTOR signaling. Treating mice with a small molecule targeting FLNA, PTI-125, before the onset of seizures alleviated neuronal abnormalities and reduced seizure frequency compared to vehicle-treated mice. In addition, the treatment was also effective when injected after seizure onset in juvenile and adult mice. These data suggest that targeting FLNA with either short hairpin RNAs or the small molecule PTI-125 might be effective in reducing seizures in patients with TSC and FCDII bearing mutations in PI3K-Rheb pathway genes.

Commentary

Many intractable epilepsy disorders belong to the group of "mTORopathies," brain disorders that are characterized by dysregulated and increased signaling through the protein kinase mechanistic target of rapamycin (mTOR).[1] Mechanistic target of rapamycin is a signaling hub with a variety of functions throughout the body ranging from cell growth, mitosis, and survival to apoptosis. In the brain, mTOR regulates cell migration and neuronal and dendritic structure. Dysregulations in the mTOR signaling cascade are often associated with brain malformations, including macrocephaly and focal cortical dysplasia (FCD), which can lead to intractable epilepsy. These mTORopathies can be caused by activating mutations in mTOR itself or defects in upstream regulators such as tuberous sclerosis complex (TSC)1/2, PTEN, and phosphoinositide 3-kinase (PI3K). Recently, mTOR-inhibiting drugs, such as Everolimus and Sirolimus, which are approved for use in humans as cancer treatments or immunosuppressants after organ transplants, have been evaluated as disease mechanism-targeting therapeutic strategies in seizure disorders associated with mTORopathies, for example, TSC and refractory FCDs.[2] These clinical trials showed some success and promise but also revealed that mTOR inhibition only partially suppresses seizures and that some patients do not respond at all, leaving a need for alternative or additional treatments.

In a series of recent studies, Bordey and colleagues have begun to fill this gap by bringing a new protein into play, Filamin A (FLNA). Filamin A is increased in human FCDs and in mouse models and could thus serve as a treatment target to reduce seizures in intractable epilepsy associated with brain malformations and mTOR dysregulation.[3-5] Their most recent study, published in Science Translational Medicine, is an important step toward advancing these so far preclinical studies into clinical application.[5] Although still focusing on a mouse model of mTOR-associated FCD, they have now shown promising seizure-reducing effects with an inhibitor of FLNA, PTI-125.[6] PTI-125 is currently used in clinical trials with Alzheimer patients, thus moving a clinical trial in intractable epilepsy within reach.

Apart from pointing toward an alternative treatment strategy, Bordey and colleagues' work is also noteworthy because it provides novel insights into the signal transduction pathways underlying cellular dysmorphology and seizures in brain malformations. Their previous studies have shown that FLNA increase in TSC depends on the signaling molecule ERK1/2 (extracellular-regulated kinase 1/2) but not mTOR. Considering that ERK1/2 and mTOR signaling pathways are strongly

intertwined, regulating each other but also being regulated by the same upstream signaling receptors and effecting similar protein targets, it stands to reason that many mTORopathies, even those not directly affecting ERK1/2 could be characterized by increased FLNA. Vice versa, FCDs caused by other mutations that are not altering mTOR but only ERK1/2 could be amenable to a FLNA-targeted strategy. An interesting question to address in future studies will be if another group of neurological diseases caused by defects in a signaling hub regulating ERK1/2 signaling, RASopathies,[7] could likewise benefit from an FLNA-targeted treatment.

Another notable observation the authors made was that the seizure-suppressing effect of FLNA inhibition did not depend on correcting morphological defects or increased S6 phosphorylation. Filamin A belongs to a group of large scaffolding proteins that crosslink actin and are thus fundamental for cell structure. PTI-125 binds FLNA and blocks association with other proteins, such as actin. To test whether increased FLNA mediates the morphological alterations associated with mTORopathies, Bordey and colleagues used a mouse model for TSC- and FCD-associated brain malformations, in which an mTOR activator, namely constitutively active Rheb GTPase ($Rheb^{CA}$) is expressed in the cortex of mice starting at embryonic day 15. $Rheb^{CA}$ expression in a subset of cells during embryogenesis leads to cortical cell misplacement, cellular dysmorphology, and spontaneous seizures at 8 to 9 weeks of age, thus replicating many of the hallmarks of FCD. In an elegant within-subject controlled approach, the authors also showed that FLNA is increased in dysmorphic balloon cells but not the surrounding healthy cells, which enabled a detailed assessment of the contributions of FLNA to morphological changes and seizures. Introducing a short hairpin RNA to knock down FLNA simultaneously with $Rheb^{CA}$ expression only partially normalized soma size and cell misplacement and did not rescue S6 phosphorylation but significantly reduced seizure frequency in adult mice. Very similar results were obtained using the FLNA inhibitor PTI-125 at translationally relevant time points ranging from ages equivalent to human birth, adolescence, and adulthood, thus spanning periods before and after seizures had developed. At all ages, seizures were strongly and significantly reduced; however, cellular morphology was only partially rescued and S6 phosphorylation was unchanged after treatment. These results add to other recent findings that seizure reduction in mouse models of increased PI3K/mTOR activation can be achieved without normalizing S6 phosphorylation[8] and suggest that increased S6 phosphorylation downstream of mTOR may not mediate the seizure phenotype. Mechanistic target of rapamycin inhibitors currently assessed to treat epilepsy in mTORopathies are specifically targeting mTORC1, which is upstream of S6 phosphorylation, and levels of phospho-S6 are considered as a readout for efficiency. More studies are needed to further evaluate the role S6 activation plays in epileptogenesis and chronic epilepsy in brain malformations.

Interestingly, there were large variations in FLNA expression in human tissue from resected FCD compared with controls, with some showing strongly increased FLNA, while others were unchanged. This suggests that only a subset of FCDs are characterized by increased FLNA and might be responsive to an FLNA-targeted treatment. To be developed into a successful treatment, it will be essential to identify the type of mutations and FCDs that show dysregulated FLNA. As outlined above, it is conceivable that other mutations outside the classic mTORopathies may be important.

One caveat in interpreting the results of this study is that seizures were only monitored during treatment. It is thus unclear if PTI-125 has simply an anti-seizure effect, unrelated to the underlying mechanism, or is truly disease-modifying. Although the partial correction of some morphological defects argues in favor of a disease-modifying effect, further studies are needed to fully understand how inhibition of PTI-125 reduces seizures in this model before advancing these promising findings to clinical trials. The fact that FLNA reduction strongly reduced seizure frequency but only partially rescued cellular dysmorphology and was even effective without correcting cell displacement, suggests that FLNA's function in cell structure regulation may not be the most important factor mediating the seizure-suppressive effect. Apart from actin, FLNA binds to and regulates key signaling molecules, such as membrane receptors.[9] In line with a more far-reaching function, the FLNA inhibitor PTI-125 inhibits inflammation by reducing FLNA association with the toll-like receptor 4 (TLR4), which prevents TLR4-induced cytokine release.[6] Seizures, epilepsy, and epileptogenesis are associated with neuroinflammation, for example mediated through TLR4,[10] and strong evidence suggests that chronic neuroinflammation as a consequence of recurrent seizures is harmful to the brain and may exacerbate seizure-induced brain damage. In addition to reducing seizure burden in a subset of intractable epilepsy disorders not fully or not at all responsive to mTOR inhibition, PTI-125 may thus provide further benefits by ameliorating comorbidities associated with epilepsy, such as impaired cognition or depression.

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References

1. Crino PB. The mTOR signalling cascade: paving new roads to cure neurological disease. *Nat Rev Neurol*. 2016;12(7):379-392.
2. Overwater IE, Rietman AB, van Eeghen AM, de Wit MCY. Everolimus for the treatment of refractory seizures associated with tuberous sclerosis complex (TSC): current perspectives. *Ther Clin Risk Manag*. 2019;15:951-955.
3. Zhang L, Bartley CM, Gong X, et al. MEK-ERK1/2-dependent FLNA overexpression promotes abnormal dendritic patterning in

tuberous sclerosis independent of mTOR. *Neuron*. 2014;84(1): 78-91.
4. Zhang L, Huang T, Bordey A. Tsc1 haploinsufficiency is sufficient to increase dendritic patterning and Filamin A levels. *Neurosci Lett*. 2016;629:15-18.
5. Zhang L, Huang T, Teaw S, et al. Filamin A inhibition reduces seizure activity in a mouse model of focal cortical malformations. *Sci Transl Med*. 2020;12(531):eaay0289.
6. Wang H-Y, Bakshi K, Frankfurt M, et al. Reducing amyloid-related Alzheimer's disease pathogenesis by a small molecule targeting filamin A. *J Neurosci*. 2012;32(29):9773-9784.
7. Vithayathil J, Pucilowska J, Landreth GE. ERK/MAPK signaling and autism spectrum disorders. *Prog Brain Res*. 2018;241:63-112.
8. Chen CJ, Sgritta M, Mays J, et al. Therapeutic inhibition of mTORC2 rescues the behavioral and neurophysiological abnormalities associated with Pten-deficiency. *Nat Med*. 2019;25(11):1684-1690.
9. Stossel TP, Condeelis J, Cooley L, et al. Filamins as integrators of cell mechanics and signalling. *Nat Rev Mol Cell Biol*. 2001;2(2): 138-145.
10. Vezzani A, Balosso S, Ravizza T. Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. *Nat Rev Neurol*. 2019;15(8):459-472.