



PARVing the Way to Cap Translation for Seizure Control

4E-BP2-Dependent Translation in Parvalbumin Neurons Controls Epileptic Seizure Threshold

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The mechanistic/mammalian target of rapamycin complex 1 (mTORC1) integrates multiple signals to regulate critical cellular processes such as mRNA translation, lipid biogenesis, and autophagy. Germline and somatic mutations in mTOR and genes upstream of mTORC1, such as *PTEN*, *TSC1/2*, *AKT3*, *PIK3CA*, and components of GATOR1 and KICSTOR complexes, are associated with various epileptic disorders. Increased mTORC1 activity is linked to the pathophysiology of epilepsy in both humans and animal models, and mTORC1 inhibition suppresses epileptogenesis in humans with tuberous sclerosis and animal models with elevated mTORC1 activity. However, the role of mTORC1-dependent translation and the neuronal cell types mediating the effect of enhanced mTORC1 activity in seizures remain unknown. The eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and 2 (4E-BP2) are translational repressors downstream of mTORC1. Here we show that the ablation of 4E-BP2, but not 4E-BP1, in mice increases the sensitivity to pentylenetetrazole (PTZ)- and kainic acid-induced seizures. We demonstrate that the deletion of 4E-BP2 in inhibitory, but not excitatory neurons, causes an increase in the susceptibility to PTZ-induced seizures. Moreover, mice lacking 4E-BP2 in parvalbumin, but not somatostatin or vasoactive intestinal polypeptide (VIP) inhibitory neurons exhibit a lowered threshold for seizure induction and reduced number of parvalbumin neurons. A mouse model harboring a human *PIK3CA* mutation that enhances the activity of the PI3K-AKT pathway (*Pik3ca*^{H1047R-Pvalb}) selectively in parvalbumin neurons shows susceptibility to PTZ-induced seizures. Our data identify 4E-BP2 as a regulator of epileptogenesis and highlight the central role of increased mTORC1-dependent translation in parvalbumin neurons in the pathophysiology of epilepsy.

Commentary

Dysregulated signaling through mechanistic target of rapamycin (mTOR) has long been associated with neuronal dysfunction and epilepsy.¹ Mutations within this pathway lead to syndromic epilepsy disorders like Tuberous Sclerosis and are frequently found in focal cortical dysplasia. Mechanistic target of rapamycin is a signaling hub that functions through two major complexes, mTORC1 and mTORC2, and controls cell proliferation and survival, among many other processes. In particular the mTORC1-dependent arm of the pathway has drawn a lot of interest in the epilepsy field because of the availability of mTORC1-inhibiting drugs approved for use in humans, such as everolimus, that reduce seizures in mouse models and have shown some success in ameliorating the seizure phenotype in patients.² Not all patients are responders, though, and the molecular and cellular mechanisms that lead to the development of epilepsy when the mTOR pathway is overactive are still not fully understood. In fact, a few recent studies painted a more nuanced picture of mTOR signaling in epilepsy: Chen et al challenged the “mTORC1-centric” view of epilepsy by showing that genetic reduction of the other arm of mTOR signaling, mTORC2, rescues seizure phenotypes in a mouse model of epilepsy, while

inhibiting mTORC1 was less effective.³ In another study, Huang and colleagues illustrated the importance of cell type by demonstrating that mTOR signaling in microglia is neuroprotective during epileptogenesis, opposing the traditional view of overactive mTOR in the brain as a risk factor for seizures.⁴

In a tour de force employing 11 different mouse lines, Sharma and colleagues recently shed more light on molecular and cellular mediators of mTOR-related regulation of brain excitability.⁵ They provided support for neuronal mTORC1 signaling as a driver of brain excitability while at the same time emphasizing the importance of neuronal subtype.

Through activation of specific translation initiation factors, mTORC1 regulates the translation of a subgroup of mRNAs that contain a 5' cap structure. The role of overactive cap-dependent translation in seizure susceptibility and epilepsy is unknown. Sharma et al addressed this gap by inducing cell type-specific deletion of the translation initiation factor binding protein 4E-binding protein 2 (4E-BP2), a translation factor downstream of mTORC1, which, in its constitutive form, inhibits cap-dependent translation but releases translational repression when mTOR is activated. Somewhat surprisingly, they show that pre- or postnatal deletion of 4E-BP2 in inhibitory but not excitatory neurons increased the susceptibility to pharmacologically



induced seizures. This suggests that excessive cap-dependent translation in inhibitory neurons promotes brain excitability. Interestingly, they showed that deleting 4E-BP2 from just parvalbumin-positive interneurons but not somatostatin- or vasoactive intestinal polypeptide (VIP)-expressing interneurons is sufficient to increase seizure susceptibility.

There are several important questions remaining. A critical aspect to consider when comparing the effects of the different neuronal promoters used in this study is the number of cells with a 4E-BP2 deletion. Parvalbumin-positive interneurons constitute about 40% of all interneurons, and thus outnumber the other subtypes tested here. Given that a recent study showed that a minimum threshold of mTOR-activated granule cells in the dentate gyrus is needed to elicit seizures,⁶ the observed differences in the interneuron subtype-specific knockout mice might have rather been caused by the number than the type of interneurons affected.


Follow-up studies should also evaluate gross brain morphology in the 4E-BP2 knockout lines. Previous work showed that cell type-unspecific short hairpin RNA (shRNA)-mediated knockdown of 4E-BP2 in the brain starting at embryonic day 14.5 (E14.5) leads to defects in cortical lamination and neuron misplacement.⁷ Given that a few of the promoters used in the present study start inducing gene expression even earlier (Emx1 for excitatory neurons at E12.5, and Nkx2.1 for inhibitory neurons at E10.5), effects on cortical lamination in these mice should be assessed. In the above-mentioned study by Chen et al, reducing mTORC2 ameliorated the seizure phenotype in epileptic mice despite the fact that they were still macrocephalic.³ In light of this study, it would be interesting to investigate if deletion of 4E-BP2 in excitatory neurons during embryogenesis or early postnatal development leads to structural defects in the cortex without altering seizure susceptibility. Conversely, increasing cap-dependent translation in parvalbumin-positive neurons during early development may induce alterations in cortical lamination, which may underlie the increased seizure susceptibility. Of note, the authors show reduced numbers of parvalbumin positive neurons in the hippocampus along with increased intensity of the surrounding perineuronal nets, which could be an indication of additional changes of the cytoarchitecture in other brain areas. However, the fact that an earlier onset of 4E-BP2 loss during embryogenesis or even a constitutive knockout, which are both expected to cause more severe morphological defects, did not further increase seizure susceptibility speaks against a crucial role of cortical dysplasia in reducing seizure threshold in this model.

Nonetheless, the loss of parvalbumin positive interneurons might be key to the observed phenotypes: Loss of GABAergic interneurons leads to spontaneous recurrent seizures that persist over months if the amount and spatial spread of initial inhibitory neuron loss is sufficient.^{8,9} Although it was not evaluated in these previous studies, the occurrence of spontaneous recurrent seizures suggests a heightened network excitability that would

lower seizure threshold. Sharma et al did not detect spontaneous seizures in any of their mouse models, but longer follow-up studies with continuous EEG/video monitoring are needed to confidently rule out the development of epilepsy with loss of 4E-BP2 in interneurons.

Another open question is how increased cap-dependent translation in parvalbumin positive interneurons leads to their loss. Is the generation of the neurons inhibited or is there increased cell death? Alternatively, is this a secondary effect, in which increased overall network activity caused by loss of 4E-BP2 leads to parvalbumin interneuron death as observed in epilepsy¹⁰? Sharma et al suggest that alterations in the perineuronal net surrounding parvalbumin positive interneurons may have caused loss of interneurons, but their causal role and underlying mechanisms remain to be established. Future experiments should analyze the transcriptome of 4E-BP2 knockout cells to reveal how increased cap-dependent translation in parvalbumin-positive interneurons could lead to changes in the perineuronal net and the loss of interneurons.

In conclusion, Sharma et al.'s work emphasizes the importance of specific cell types and molecular mechanisms in the pathophysiology of mTORopathies, but also highlights the need for additional detailed studies to better understand the molecular and cellular aspects of mTOR signaling in epilepsy to identify the optimal treatment targets and modalities.

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