EPILEPSY CURRENTS

Current Literature

In Basic Science

"TOR"-ing Down the Dentate Gate in Temporal Lobe Epilepsy

Excessive Activation of mTOR in Postnatally Generated Granule Cells Is Sufficient to Cause Epilepsy.

Pun RY, Rolle IJ, Lasarge CL, Hosford BE, Rosen JM, Uhl JD, Schmeltzer SN, Faulkner C, Bronson SL, Murphy BL, Richards DA, Holland KD, Danzer SC. *Neuron* 2012;75:1022–1034.

The dentate gyrus is hypothesized to function as a "gate," limiting the flow of excitation through the hippocampus. During epileptogenesis, adult-generated granule cells (DGCs) form aberrant neuronal connections with neighboring DGCs, disrupting the dentate gate. Hyperactivation of the mTOR signaling pathway is implicated in driving this aberrant circuit formation. While the presence of abnormal DGCs in epilepsy has been known for decades, direct evidence linking abnormal DGCs to seizures has been lacking. Here, we isolate the effects of abnormal DGCs using a transgenic mouse model to selectively delete PTEN from postnatally generated DGCs. PTEN deletion led to hyperactivation of the mTOR pathway, producing abnormal DGCs morphologically similar to those in epilepsy. Strikingly, animals in which PTEN was deleted from ≥ 9% of the DGC population developed spontaneous seizures in about 4 weeks, confirming that abnormal DGCs, which are present in both animals and humans with epilepsy, are capable of causing the disease.

Commentary

The dentate gyrus (DG) is thought to serve as a gate regulating the spread of excitatory input from the entorhinal cortex into the hippocampus (1). Breakdown of this gating function in the DG has been hypothesized to promote development of epileptogenesis in temporal lobe epilepsy (1, 2). A variety of pathological changes in DG granule cells in animal models and patients with temporal lobe epilepsy may contribute to disrupted DG function, including somatic hypertrophy, formation of basilar dendrites, ectopic granule cells within the hilus, and mossy fiber sprouting (3). These and other cellular and molecular abnormalities within the DG may lead to the formation of aberrant, excitatory circuits that result in temporal lobe epilepsy. However, as previous studies linking DG dysfunction and epileptogenesis have primarily been correlative in nature, direct proof that such DG abnormalities can definitively cause temporal lobe epilepsy has been lacking.

In an elegant but conceptually straightforward study, Pun and colleagues provide compelling evidence that pathological disruption of the dentate gyrus is capable of causing temporal lobe epilepsy. To test this longstanding hypothesis, they took advantage of a specific genetic manipulation involving activation of the mammalian target of rapamycin complex 1 (mTORC1) pathway within DG granule cells. The mTORC1 pathway regulates a number of important cellular processes and has been implicated in promoting epileptogenesis in a variety of types of epilepsy (4). This is especially well-established in animal models of the genetic epilepsy, tuberous sclerosis complex; however, there is also some evidence for a role of mTORC1 in models of acquired epilepsy, such as due to brain injury following status epilepticus or trauma. Using targeted genetic techniques, Pun and colleagues inactivated the *phosphatase and tensin homolog* (*PTEN)* gene primarily in DG granule cells in 2-week-old mice, as well as incidentally in a small population of inhibitory interneurons in the olfactory bulb. As *PTEN* acts as an upstream regulator of the mTORC1 pathway, loss of *PTEN* led to abnormal hyperactivation of the mTORC1 pathway in the targeted neurons of the knock-out mice.

Remarkably, epilepsy occurred in almost all of the *PTEN* knock-out mice within 4–6 weeks of inducing the *PTEN* inactivation. As documented by intracranial EEG recordings, the seizures appeared to originate focally within the hippocampus, not neocortex. Quantitative assessment found that *PTEN* inactivation in as few as 9% of DG granule cells was enough to cause epilepsy. Furthermore, the DG granule cells in these mice developed a number of pathological abnormalities seen in human patients and other animal models of temporal lobe epilepsy, including neuronal hypertrophy, basal dendrite formation, increased dendritic spine density, ectopic neurons, and mossy fiber sprouting. Importantly, treatment with the mTORC1 inhibitor, rapamycin, significantly attenuated the development of epilepsy and DG pathological changes, indicating that abnormal mTORC1 pathway activation mediated epileptogenesis in the *PTEN* knock-out mice. Thus, this study provides direct evidence that mTOR-mediated pathological abnormalities in DG granule cells are sufficient to cause temporal lobe epilepsy.

Given the potential significance of this finding, this study was thorough in including a number of control experiments to evaluate for alternative interpretations and mechanisms. The incidental inactivation of *PTEN* in inhibitory granule cells in

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olfactory bulb (which share the same genetic promoter as hippocampal granule cells used to drive *PTEN* inactivation) had surprisingly little effect on the morphology of these olfactory granule cells, as well as no evidence of abnormal EEG activity in the olfactory bulb. Although mTOR activation in astrocytes can promote epileptogenesis in mouse models of tuberous sclerosis complex (5), there were no significant abnormalities in the number, morphology (e.g., reactive gliosis), or *PTEN* expression of astrocytes in the *PTEN* knock-out mice in this study. Thus, the source of epileptogenesis in these mice can most likely be localized to the DG granule cells.

Although the findings from this study support the concept that abnormalities in DG granule cells are capable of causing epilepsy, the specific pathophysiological defect(s) in the DG granule cells that promote epileptogenesis in the *PTEN* knock-out mice remains to be determined. Consistent with pathological specimens from human patients and other animal models of temporal lobe epilepsy, a variety of histological abnormalities in DG granule cells were identified in the *PTEN* knock-out mice and could potentially contribute to a breakdown of the DG gate leading to epilepsy. Based purely on the correlative pathological observations in the current and previous studies, it is impossible to distinguish which granule cell abnormalities are more critical for epileptogenesis and which may be compensatory mechanisms or epiphenomena. However, unlike most of the other morphological abnormalities in DG granule cells, the degree of mossy fiber sprouting was poorly correlated with the presence or absence of *PTEN* inactivation. Thus, while mossy fiber sprouting has been a longstanding, leading candidate hypothesized to promote excitatory recurrent circuits in temporal lobe epilepsy, this finding supports other recent studies indicating that mossy fiber sprouting may not be necessary for epileptogenesis in temporal lobe epilepsy (6).

Finally, proving that pathological abnormalities in DG granule cells are sufficient to cause epilepsy does not prove that these abnormalities are necessarily involved in temporal lobe epilepsy, especially in other models or the human condition. More targeted future approaches—selectively reversing specific aspects of DG granule cell dysfunction—will be needed to determine whether and which of these abnormalities are truly necessary for epileptogenesis in this and other models. Similarly, with regard to the involvement of the mTORC1 pathway in epileptogenesis, this and other recent studies provide strong evidence that mTORC1 hyperactivation is sufficient to cause epilepsy (7,8), but further work is needed to determine the conditions under which abnormal mTORC1 activity is necessary for epileptogenesis in acquired temporal lobe epilepsy. mTORC1 may have numerous downstream effects relevant to epileptogenesis and has been implicated in a variety of different models of epilepsy (4). Although in the present study, mTORC1 activation was used primarily as a tool for triggering epileptogenesis and DG granule cell dysfunction, the mechanistic link between mTORC1 and morphological properties of neurons, such as DG granule cells, may be critical for other types of epilepsy beyond the classic mesial temporal lobe epilepsy.

by Michael Wong, MD, PhD

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