Not So Innocent Bystanders in Focal Cortical Dysplasia

Non-Cell Autonomous Epileptogenesis in Focal Cortical Dysplasia

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Objective: Low-level somatic mosaicism in the brain has been shown to be a major genetic cause of intractable focal epilepsy. However, how a relatively few mutation-carrying neurons are able to induce epileptogenesis at the local network level remains poorly understood. Methods: To probe the origin of epileptogenesis, we measured the excitability of neurons with MTOR mutation and nearby nonmutated neurons recorded by whole-cell patch-clamp and array-based electrodes comparing the topographic distribution of mutation. Computational simulation is used to understand neural network-level changes based on electrophysiological properties. To examine the underlying mechanism, we measured inhibitory and excitatory synaptic inputs in mutated neurons and nearby neurons by electrophysiological and histological methods using the mouse model and postoperative human brain tissue for cortical dysplasia. To explain non–cell-autonomous hyperexcitability, an inhibitor of adenosine kinase was injected into mice to enhance adenosine signaling and to mitigate hyperactivity of nearby nonmutated neurons. Results: We generated mice with a low-level somatic mutation in MTOR presenting spontaneous seizures. The seizure-triggering hyperexcitability originated from nonmutated neurons near mutation-carrying neurons, which proved to be less excitable than nonmutated neurons. Interestingly, the net balance between excitatory and inhibitory synaptic inputs onto mutated neurons remained unchanged. Additionally, we found that inhibition of adenosine kinase, which affects adenosine metabolism and neuronal excitability, reduced the hyperexcitability of nonmutated neurons. Interpretation: This study shows that neurons carrying somatic mutations in MTOR lead to focal epileptogenesis via non–cell-autonomous hyperexcitability of nearby nonmutated neurons.

Commentary

Somatic mutations in key genes within the mechanistic target of rapamycin (mTOR) pathway have been discovered in patients with focal cortical dysplasia type II (FCDII), the most important cause of medically refractory epilepsy in children, as well as other malformations of cortical development. However, the mechanistic understanding of its epileptogenesis fails to keep pace with genetic discoveries. Clinically, MRI characteristics of focal “clonal” FCD could be explained by the inside-out radial migration of post-mitotic pyramidal neurons arising from dorsal progenitors to populate the cerebral cortex. Experimentally, animal models have also demonstrated that mTOR hyperactivation in dorsal progenitors but not ventral progenitors that generate interneurons is sufficient to induce pathognomonic cellular changes and overall cortical overgrowth. Electrophysiologically, however, these cytomegalic mutant neurons showed hyperpolarized resting membrane potential, reduced current density, shifted input-output curve, and lack of spontaneous depolarization, suggesting that they alone do not have the intrinsic membrane properties necessary to generate epileptic events.

Koh HY et al. took advantage of episomal plasmid-based in utero electroporation (IUE) to probe the origin of epileptogenesis in dysplastic brains. They generated a FCD mouse model with ∼2% cells in the IUE site ectopically overexpressing mutant MTOR. Their previous study has shown that these mice recapitulated the pathological features of FCDII and developed frequent generalized tonic–clonic seizures around P21. Although brain slices from these mice showed a higher spontaneous firing rate than wildtype, topographic correlation analysis suggested that hyperactivity is highly associated with GFP-negative wildtype (WT) neurons but not with GFP-positive mutant neurons. At the synaptic level, the authors showed that mutant neurons had increased inhibitory and excitatory synaptic activities to similar levels, concordant with the increased vGLUT1 and vGAT puncta on the mutant neurons in FCDII mice and HME patients. Additionally, the density of all interneurons or parvalbumin+ or somatostatin+ interneuron subtype was not altered in these mice. The authors then confirmed that the intrinsic excitability of mutant neurons was significantly decreased in the presence of synaptic blockers. However, the intrinsic excitability of nearby WT neurons was increased considerably. Mechanistically, the authors have
previously shown that mTOR hyperactivation increased the translation of adenosine kinase (ADK), and therefore could lower the extracellular level of adenosine, an endogenous anticonvulsant that exerts an inhibitory tone on neuronal activity via activating adenosine receptors A1 and A2A. The authors then demonstrated that either CCAP, a selective adenosine A1 receptor agonist, or 5-ITU, an ADK-specific inhibitor, could rescue the increased excitability of WT neurons. Furthermore, the authors have previously shown that co-electroporation of mutant MTOR and shRNA targeting ADK could dramatically decrease seizure frequency, and 5-ITU at 2.6 mg/kg could almost abolish seizures. These results together suggested that the upregulated ADK in MTOR mutant neurons plays a critical role in the epileptogenesis and ictogenesis of FCDII via non–cell-autonomous mechanisms affecting WT neurons.

Previous studies have shown reduced intrinsic excitability and spontaneous activity in neurons with mTOR hyperactivation. Unlike germline mutations affecting both inhibitory and excitatory neurons, FCDII is caused by somatic mutations in dorsal progenitors that give rise to excitatory neurons but not interneurons. Therefore, what drives the hyperexcitability in the focal dysplastic cortex becomes intriguing. Koh’s work confirmed mutant neurons’ intrinsic/cell-autonomous HYPOexcitability and demonstrated that the disrupted adenosine homeostasis non-cell-autonomously generated HYPERexcitability in nearby WT neurons. However, several important questions need to be further explored. Firstly, because plasmids remain episomal in dorsal neural progenitors, the standard IUE used in Koh’s study would result in the form of transfection in which only pyramidal excitatory neurons that do not undergo a second cell division express mutant MTOR. However, somatic mutations in human FCDII affect the complete cell lineage of dorsal progenitors, including excitatory neurons, astrocytes, and oligodendrocytes. Does mTOR hyperactivation in glial cells generate non–cell-autonomous effects on WT neurons and how? A transposon-based overexpression system to affect both neurons and glial cells would be required to answer this question. Secondly, because of copy-number variability and overexpression artifacts like cytotoxicity and transcriptional squelching, the transgene overexpression system often introduced significant confounding factors such as genotypic and phenotypic variability. For example, Hiesh et al. used the same IUE platform to overexpress mutant Rheb, a canonical mTOR activator, but observed intrinsic hyperexcitability in mutant neurons, in sharp contrast to Koh’s study. Do overexpression artifacts contribute to these contradictory data? Therefore, more physiological and clinically relevant models, such as CRISPR-IUE knockout for mTOR inhibitors or mosaic analysis by dual recombinase-mediated cassette exchange (MADR) for mTOR activators, should be used. Thirdly, although interneurons are generated by ventral progenitors that do not harbor somatic mutations, their development is sculptured by pyramidal excitatory neurons and glial cells arising from dorsal progenitors. It is therefore conceivable that cortical inhibitory circuitry could be also non–cell-autonomously impaired. Interestingly, interneuron loss has been well documented in histopathologic studies on human tissues but not in Koh’s study. A more detailed analysis of genetically intact interneurons, particularly parvalbumin+ fast-spiking cells, should be conducted in future studies. Fourthly, the intracellular injection of ADK in individual neurons did not modify extracellular adenosine concentration, suggesting that multiple neurons must have their metabolism altered to allow modification of adenosine levels. In Koh’s study, less than 2% of cells carried mutant MTOR, of which 30% were positive for ADK. How did a tiny fraction of cells (~0.6%) disrupt the basal extracellular adenosine level to generate severe seizures? Koh’s work, however, did not provide direct evidence for altered adenosine level. In addition, ADK is predominately expressed in astrocytes and was initially established as a critical player in the epileptogenesis of a kainate epilepsy model. Unlike the IUE-based FCDII animals with a low mutation rate in excitatory neurons, mice with kainate-induced temporal lobe epilepsy have a widespread and profound gliosis. To better understand the role of ADK in FCDII, further biochemical analysis of adenosine release, transport, and metabolism is needed. In summary, Koh’s study demonstrated non–cell-autonomous hyperexcitability in FCDII and proposed potential underlying molecular mechanisms that could pave ways to novel therapeutic development.

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References
1. D’Gama AM, Woodworth MB, Hossain AA, Bizzotto S, Hatem NE, LaCoursiere CM, et al. Somatic mutations activating the mTOR pathway in dorsal telencephalic progenitors cause a continuum of cortical dysplasias. Cell Rep. 2017;21:3754-3766.
2. Bateup HS, Johnson CA, Denefrio CL, Saulnier JL, Kornacker K, Sabatini BL. Excitatory/inhibitory synaptic imbalance leads to hippocampal hyperexcitability in mouse models of tuberous sclerosis. Neuron. 2013;78:510-522.
3. Koh HY, Jang J, Ju SH, Kim R, Cho GB, Kim DS, et al. Non-cell autonomous epileptogenesis in focal cortical dysplasia. Ann Neurol. 2021;90:285-299.
4. Kim JK, Cho J, Kim SH, Kang HC, Kim DS, Kim VN, et al. Brain somatic mutations in MTOR reveal translational dysregulations underlying intractable focal epilepsy. *J Clin Invest*. 2019;129:4207-4223.

5. Chen F, Maher BJ, LoTurco JJ. piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by in utero electroporation. *Cold Spring Harb Protoc*. 2014;28:741-749.

6. Kim GB, Rincon Fernandez Pacheco D, Saxon D, Yang A, Sabet S, Dutra-Clarke M, et al. Rapid generation of somatic mouse mosaics with locus-specific. *Stably Integr Transgenic Elem Cell*. 2019;179:251-267 e224.

7. Hsieh LS, Wen JH, Nguyen LH, Zhang L, Getz SA, Torres-Reveron J, et al. Ectopic HCN4 expression drives mTOR-dependent epilepsy in mice. *Sci Transl Med*. 2020;54:12.

8. Wong FK, Bercsenyi K, Sreenivasan V, Portales A, Fernandez-Otero M, Marin O. Pyramidal cell regulation of interneuron survival sculpts cortical networks. *Nature*. 2018;557:668-673.

9. Latini S, Pedata F. Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J Neurochem*. 2001;79:463-484.

10. Gouder N, Scheurer L, Fritschy JM, Boison D. Overexpression of adenosine kinase in epileptic hippocampus contributes to epileptogenesis. *J Neurosci*. 2004;24:692-701.