mTOR Strikes Again: mTORC1 Activation Causes Epilepsy Independent of Overt Pathological Changes

TORC1-Dependent Epilepsy Caused by Acute Biallelic *Tsc1* Deletion in Adult Mice.

Abs E, Goorden SMI, Schreiber J, Overwater IE, Hoogeveen-Westerveld M, Bruinsma CF, Aganović E, Borgesius NZ, Nellist M, Elgersma Y [published online ahead of print May 30, 2013]. *Ann Neurol*. doi:10.1002/ana.23943

OBJECTIVE: Seizure development in Tuberous Sclerosis Complex (TSC) correlates with the presence of specific lesions called cortical tubers. Moreover, heterozygous *Tsc* animal models do not show gross brain pathology and are seizure-free, suggesting that such pathology is a prerequisite for the development of epilepsy. However, cells within TSC lesions show increased activity of the target of rapamycin complex 1 (TORC1) pathway, and recent studies have implicated this pathway in non-TSC-related animal models of epilepsy and neuronal excitability. These findings imply a direct role for TORC1 in epilepsy. Here, we investigate the effect of increased TORC1 signaling induced by acute biallelic deletion of *Tsc1* in healthy adult mice. METHODS: Biallelic *Tsc1* gene deletion was induced in adult *Tsc1* heterozygous and wild-type mice. Seizures were monitored by EEG and video recordings. Molecular and cellular changes were investigated by Western blot analysis, immunohistochemistry and electrophysiology. RESULTS: Mice developed epilepsy a few days after biallelic *Tsc1* deletion. Acute gene deletion was not accompanied with any obvious histological changes, but resulted in activation of the TORC1 pathway, enhanced neuronal excitability and a decreased threshold for protein-synthesis-dependent long-term potentiation preceding the onset of seizures. Rapamycin treatment after seizure onset reduced TORC1 activity and fully abolished the seizures. INTERPRETATION: Our data indicate a direct role for TORC1 signaling in epilepsy development, even in the absence of major brain pathology. This suggests that TORC1 is a promising target for treating seizures not only in TSC but also in other forms of epilepsy, which result from increased TORC1 activation.

Commentary

The mammalian target of rapamycin (mTOR) is a ubiquitous protein kinase that has received extensive attention in recent years as a potential mediator of epilepsy. mTOR, particularly a specific complex of proteins called mTORC1, regulates numerous cellular and physiologic functions, including protein synthesis, cell growth, proliferation, autophagy, and metabolism. Abnormal regulation of mTORC1 is most strongly implicated in promoting epilepsy in the genetic disease, tuberous sclerosis complex (TSC). However, there is now abundant evidence that mTORC1 may be involved in epileptogenesis in a variety of other types of epilepsies, including infantile spasms, neonatal hypoxic seizures, absence epilepsy, posttraumatic epilepsy, and acquired temporal lobe epilepsy (1).

In TSC, the association between mTORC1 and epilepsy is strongly rooted in the primary genetic defect that causes this disease. Two genes, *TSC1* and *TSC2*, have been linked to TSC. Since the protein products of *TSC1* and *TSC2*, hamartin and tuberin, normally function to inhibit mTORC1, mutations in *TSC1* or *TSC2* lead to disinhibited or abnormally elevated mTORC1 activity. Given the role of mTORC1 in stimulating cell growth and proliferation, hyperactivation of mTORC1 promotes excessive cell growth and tumor formation in various organs in TSC patients, including the skin, heart, kidneys, and brain. Identification of this pathophysiological mechanism has already led to the use of mTORC1 inhibitors for treating brain and kidney tumors in TSC patients.

While the relationship of mTORC1 to tumors is relatively straightforward, the link to epilepsy in TSC is more complicated (1). Cortical tubers, the pathological hallmark of TSC, are most closely associated with epilepsy, as surgical removal of tubers can eliminate seizures in some TSC patients. Cortical tubers are considered malformations of cortical development, akin to focal cortical dysplasia. While tubers do not grow like true tumors, they do feature some tumor-like cellular features, such as glial proliferation and poorly differentiated cytomegalic cells, as well as biochemical evidence of increased mTORC1 activity. Although mTORC1 might also promote epileptogenesis through tuber-independent mechanisms, the connection

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between mTORC1 and epilepsy almost certainly involves, at least in part, these classic pathological lesions. mTORC1 inhibitors have already been shown to prevent or decrease seizures, as well as reverse associated pathological abnormalities, in animal models of TSC (1). Currently, clinical trials are ongoing to determine whether mTORC1 inhibitors are effective therapies for epilepsy in TSC patients.

Besides TSC, mTORC1 may contribute to acute seizure generation or chronic epileptogenesis in animal models of a variety of other genetic and acquired epilepsies. Evidence for mTORC1 involvement primarily derives from the effects of rapamycin or other mTORC1 inhibitors in decreasing existing seizures (antiseizure) or inhibiting the subsequent development of epilepsy (antiepileptogenic). Limited, selective antiseizure effects of rapamycin have been found in the classic preclinical anticonvulsant screening tests of convulsant- or electrical stimulation–induced acute seizures (2). More robust antiseizure effects of mTORC1 inhibitors occur in animal models of infantile spasms, absence seizures, pilocarpine/status epilepticus-induced temporal lobe epilepsy, and *Pten*–knock-out mice (3–6). Furthermore, potential antiepileptogenic effects of mTORC1 inhibitors in preventing or decreasing future seizures have been reported in kainate/status epilepticus–induced temporal lobe epilepsy, angular bundle electrical stimulation–induced temporal lobe epilepsy, neonatal hypoxia-induced epilepsy, and both in vivo and in vitro models of posttraumatic epilepsy (7–11), although there are other models of temporal epilepsy in which mTORC1 inhibitors have been found to have no effect (12). Finally, genetic activation of mTORC1 in dentate granule cells was recently shown to be sufficient to induce pathological changes and temporal lobe epilepsy in previously normal mice (13).

In almost all the cases in which mTORC1 has been implicated as contributing to epilepsy, there are concurrent gross pathological abnormalities, including neuronal death, cellular proliferation and hypertrophy, axonal sprouting, and astrogliosis. Thus, analogous to TSC, mTORC1's effect on epileptogenesis may be dependent on the initial formation of pathological lesions, which then secondarily causes seizures. Nevertheless, mTORC1 has a diversity of cellular and molecular functions that may not involve gross histologic changes, such as regulation of the expression of ion channels and other proteins. Even in TSC, there is increasing evidence that the areas of cortex that appear grossly normal on magnetic resonance imaging have cellular and molecular abnormalities that may contribute to epilepsy and other neurologic deficits in this disease.

The recent study by Abs and colleagues provides strong evidence that abnormal mTORC1 activation is sufficient to cause epilepsy independent of overt pathological lesions. Utilizing advanced genetic targeting techniques, they inactivated the *Tsc1* gene in previously normal, adult mice. Remarkably, all mice developed epilepsy within 10 days of *Tsc1* gene inactivation. This induced epilepsy was preceded by abnormal mTORC1 activation and inhibited by rapamycin, indicating that mTORC1 mediates epileptogenesis in this model. Of importance, although there were rare cytomegalic neurons, detailed histologic analysis revealed no gross pathological changes in the brains of these mice.

A few caveats from this article deserve further attention. Along with developing epilepsy, all mice died within a couple of weeks from unexplained causes. One potential explanation for the dramatic lethality is that the genetic technique involved global loss of *Tsc1* throughout the body, which may have detrimental systemic effects similar to embryonic demise that results from homozygous germline *Tsc1* deletion in mice. Thus, there could be other systemic consequences of *Tsc1* inactivation that secondarily contributed to the development of seizures. A more targeted inactivation of *Tsc1* selectively within neurons would eliminate the possibility of a confounding systemic effect. Furthermore, in the absence of gross pathological lesions, the specific mechanisms of epileptogenesis in this model were not identified. Cellular electrophysiological studies of hippocampal neurons in the mice did find changes in neuronal excitability. A mTORC1-mediated regulation of voltage-gated ion channels is a rational hypothesis to account for increased neuronal excitability and seizures in this model, but future studies are needed to identify these mechanisms more definitively. Finally, proving that mTORC1 activation is sufficient to cause epilepsy is not the same as establishing that it is necessary for epileptogenesis in any given type of epilepsy. Continued studies of various acquired and genetic epilepsies will help to determine when mTORC1 is most clinically relevant.

Nevertheless, the demonstration that mTORC1-mediated epilepsy does not require gross pathological changes in the brain has important clinical implications. In TSC, while tubers are critically involved in causing seizures in many TSC patients, this study supports the recent trend that implicates nontuber brain regions in epileptogenesis. Beyond TSC, medically intractable nonlesional epilepsy is one of the greatest therapeutic challenges, as many of these patients are not good candidates for epilepsy surgery. This study suggests the possibility of targeting mTORC1 in other types of epilepsy, with or without pathological lesions.

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