



## A Tuber-ful Animal Model of Tuberous Sclerosis At Last?

### Single-Cell *Tsc1* Knockout During Corticogenesis Generates Tuber-Like Lesions and Reduces Seizure Threshold in Mice.

Feliciano DM, Su T, Lopez J, Platel JC, Bordey A. *J Clin Invest* 2011;121:1596–1607.

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by mutations in *Tsc1* or *Tsc2* that lead to mammalian target of rapamycin (mTOR) hyperactivity. Patients with TSC suffer from intractable seizures resulting from cortical malformations known as tubers, but research into how these tubers form has been limited because of the lack of an animal model. To address this limitation, we used in utero electroporation to knock out *Tsc1* in selected neuronal populations in mice heterozygous for a mutant *Tsc1* allele that eliminates the *Tsc1* gene product at a precise developmental time point. Knockout of *Tsc1* in single cells led to increased mTOR activity and soma size in the affected neurons. The mice exhibited white matter heterotopic nodules and discrete cortical tuber-like lesions containing cytomegalic and multinucleated neurons with abnormal dendritic trees resembling giant cells. Cortical tubers in the mutant mice did not exhibit signs of gliosis. Furthermore, phospho-S6 immunoreactivity was not upregulated in *Tsc1*-null astrocytes despite a lower seizure threshold. Collectively, these data suggest that a double-hit strategy to eliminate *Tsc1* in discrete neuronal populations generates TSC-associated cortical lesions, providing a model to uncover the mechanisms of lesion formation and cortical hyperexcitability. In addition, the absence of glial reactivity argues against a contribution of astrocytes to lesion-associated hyperexcitability.

### Commentary

Tuberous sclerosis complex (TSC) is one of the most common genetic etiologies of epilepsy. Epilepsy in TSC patients may involve multiple seizure types and is often intractable to medication. TSC is caused by mutation of either the *TSC1* or *TSC2* gene and is characterized pathologically by the development of hamartomas or tumors in multiple organs. Cortical tubers, hamartomatous lesions of the brain, are most closely associated with epilepsy and other neurologic symptoms of TSC, such as autism and developmental delay. Tubers represent focal cortical malformations, consisting of loss of normal cortical lamination, dysmorphic neurons, astrogliosis, and unique giant cells with immature neuronal and glial features. The importance of tubers in causing seizures is supported by the fact that surgical removal of tubers can eliminate seizures in a subset of TSC patients (1).

Direct pathologic analysis of tubers resected from TSC patients during epilepsy surgery has provided abundant information about the histologic, cellular, and molecular features of these lesions. However, the specific mechanisms of epileptogenesis and seizure generation (ictogenesis) in TSC continue to be poorly understood. First, it's often debated as to whether seizures originate from within the tubers themselves or the surrounding perituberal cortex. Although traditionally tubers were assumed to generate seizures directly, recent invasive

electrical recordings from TSC patients suggest that epileptiform activity primarily arises from the perituberal regions (2). Other unresolved issues include defining the relative involvement of aberrant networks (tuberal or perituberal), abnormal cell types (e.g., giant cells, dysmorphic neurons, astrogliosis), and molecular defects (e.g., abnormal glutamate or GABA receptor expression) in promoting epileptogenesis and seizure generation. In addition, the role of abnormal activation of the mTOR pathway, the major biochemical pathway regulated by the TSC genes, in triggering various downstream mechanisms of epileptogenesis is under intense investigation.

A number of animal models of TSC have been developed that have yielded insights into the pathophysiology of neurologic manifestation of TSC, including epilepsy. Several of these rodent models, involving inactivation of either the *Tsc1* or *Tsc2* gene in various subsets of brain cells, have recapitulated some of the cellular aspects of human tubers, such as dysmorphic or cytomegalic neurons and astrogliosis (3–5). Furthermore, some models have identified novel molecular defects, such as in astrocyte glutamate transporters, that likely relate to epileptogenesis and have subsequently been confirmed in human tubers (3, 6). However, despite these advances, a major criticism of existing animal models of TSC is the failure to replicate focal tubers. Most of these animal models exhibit widespread cellular abnormalities and megalencephaly but not discrete cortical lesions, with the exception of very rare tuberlike lesions in the Eker rat (7). Thus, while “tuber-less” models have revealed some important information about the neurological phenotype of TSC (8), a more complete understanding of the pathophysiology of



epilepsy in TSC will likely depend on having an animal model that faithfully recapitulates tubers.

The failure of previous animal models to reproduce tubers probably relates to the temporal and spatial specificity of the effects of *Tsc1/Tsc2* gene mutations during cortical development. A leading hypothesis about the pathogenesis of cortical tubers is that embryonic progenitor cells in the brain of TSC patients suffer a somatic *TSC1* or *TSC2* mutation during a specific stage of cortical development (9). In combination with the germline mutation of these patients, the resulting “double-hit” of the *TSC1* or *TSC2* gene leads to complete loss of function of the respective gene product, hamartin or tuberin, in the affected embryonic cells. As hamartin and tuberin normally function together to control cell growth and proliferation, tuber formation may then result from dysregulated cell growth, proliferation, and migration in focal areas of embryonic cortex. It may only require a second hit in a limited number or possibly even a single, progenitor cell to generate a tuber. Although some debate exists as to the frequency of second-hit events documented in human tubers (9,10), in animal models it appears that two hits are necessary to induce significant pathologic changes, as models involving only heterozygous (single hit) *Tsc1* or *Tsc2* gene inactivation exhibit minimal to no pathologic abnormalities. However, cortical abnormalities in previous two-hit models are diffuse, not focal, because these models affect a large population of embryonic cells in the developing brain with little temporal or spatial specificity.

The study by Feliciano and colleagues represents a significant advance in the field in generating an animal model featuring focal, tuberlike lesions. Compared with previous models, this model involves more controlled temporal and spatial regulation of *Tsc1* gene inactivation, utilizing the innovative technique of in utero electroporation to knock out the *Tsc1* gene at specific times and locations in the embryonic brain. As a result, discrete cortical tuberlike lesions as well as white matter heterotopic nodules subsequently develop in adult mice. These focal lesions contain some of the cellular and histologic features of human tubers, such as dysmorphic or cytomegalic neurons and loss of normal cortical lamination. However, true giant cells appear to be lacking, as molecular markers indicate that most abnormal cells are consistent with neurons, not immature cells. Furthermore, in contrast to human tubers, an intriguing finding is a lack of astrogliosis. The absence of astrogliosis in this animal model raises the provocative question of whether astrogliosis is really a primary pathogenic feature of human tubers or just a secondary phenomenon, perhaps caused by seizures, but it also indicates limitations of the model in not reproducing all features of human tubers. Although the authors conclude from this model that astrocytes do not contribute to epileptogenesis, this conclusion might not be justified, as these mice have not been documented to have epilepsy and other models suggest that astrocyte abnormalities are sufficient to generate seizures (3).

The behavioral and functional characteristics of this novel TSC model have not yet been fully investigated. These mice did have a decreased seizure threshold to the convulsant drug,

pentylentetrazole, but no spontaneous seizures were observed. However, there was a limited observation period, and video-EEG monitoring was not employed; thus, spontaneous seizures could have gone unrecognized.

Thus, although this animal model does not recapitulate all the features of human tubers, it is a significant advance in the right direction and a useful tool for the future. More detailed electrophysiologic, biochemical, and molecular studies should help address unanswered questions about the localization (tuber versus perituberal region) of seizure activity and the contribution of specific abnormal cell types and molecules to epileptogenesis. Furthermore, modifications of the in utero electroporation technique will allow additional insights into the pathogenesis of tuber formation and further refinement of the model. Finally, preclinical studies of potential therapeutic approaches, such as mTOR inhibitors, may be more accurately assessed in this model.

by Michael Wong, MD, PhD

#### References

1. Madhavan D, Schaffer S, Yankovsky A, Arzimanoglou A, Renaldo F, Zaorff CM, LaJoie J, Weiner HL, Andermann E, Franz DN, Leonard J, Connolly M, Cascino GD, Devinsky O. Surgical outcome in tuberous sclerosis complex: a multicenter survey. *Epilepsia* 2007;48:1625–1628.
2. Major P, Rakowski S, Simon MV, Cheng ML, Eskandar E, Baron J, Leeman BA, Frosch, MP, Thiele EA. Are cortical tubers epileptogenic? Evidence from electrocorticography. *Epilepsia* 2009;50:147–154.
3. Uhlmann EJ, Wong M, Baldwin RL, Bajenaru ML, Onda H, Kwiatkowski DJ, Yamada KA, Gutmann DH. Astrocyte-specific *TSC1* conditional knockout mice exhibit abnormal neuronal organization and seizures. *Ann Neurol* 2002;52:285–296.
4. Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, Kwiatkowski DJ. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci* 2008;28:5422–5432.
5. Way SW, McKenna J 3rd, Mietzsch U, Reith RM, Wu HC, Gambello MJ. Loss of *Tsc2* in radial glia models the brain pathology of tuberous sclerosis complex in the mouse. *Hum Mol Genet* 2009;18:1252–1265.
6. Wu X, Sosunov AA, Tikoo R, Weiner HL, Crino PD, McKhann GM. Glutamate transport is impaired in the human tuberous sclerosis tissue. *Epilepsia* 2005;46(suppl 8):279.
7. Mizuguchi M, Takashima S, Yamanouchi H, Nakazato Y, Mitani H, Hino O. Novel cerebral lesions in the Eker rat model of tuberous sclerosis: cortical tuber and anaplastic ganglioglioma. *J Neuropath Exp Neurol* 2000;59:188–196.
8. Wong M. The utility of tuber-less models of tuberous sclerosis. *Epilepsia* 2007;48:1629–1630.
9. Crino PB, Aronica E, Baltuch G, Nathanson KL. Biallelic *TSC* gene inactivation in tuberous sclerosis complex. *Neurology* 2010;74:1716–1723.
10. Qin W, Chan JA, Vinters HV, Mathern GW, Franz DN, Taillon BE, Bouffard P, Kwiatkowski DJ. Analysis of TSC cortical tubers by deep sequencing of *TSC1*, *TSC2* and *KRAS* demonstrates that small second-hit mutations in these genes are rare events. *Brain Pathol* 2010;1095–1105.



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