



DO WE HAVE A CURE FOR TUBEROUS SCLEROSIS COMPLEX?

Peter B. Crino, MD, PhD

Department of Neurology, PENN Epilepsy Center, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, USA

The recent development of several mouse models for tuberous sclerosis complex (TSC) provides in vivo systems to test new therapies for the neurological manifestations of TSC. Rapamycin is known to antagonize the effects of loss of TSC protein function in vitro and in mouse TSC models, rapamycin can prevent seizures and improve learning task performance. These findings provide new hope for TSC patients suffering from intractable seizures and possibly, for those with autism and cognitive disabilities.

Tuberous sclerosis complex (TSC) is an autosomal dominant, multisystem disease associated with mutations in the *TSC1* or *TSC2* genes (1). While renal, cardiac, dermatological, or pulmonary manifestations of TSC provide daunting challenges for many patients, the neurological features of TSC, including infantile spasms, intractable epilepsy, cognitive disabilities, brain tumors, and autism account for significant morbidity. The dizzying array of antiepileptic drugs for refractory seizures, psychotropic medications for anxiety, agitation, depression, or self-injurious behaviors, and the very real possibility of facing intracranial EEG recordings and resective epilepsy or tumor surgery has rendered the neurological landscape for many TSC patients quite bleak.

Genetic Research on Tuberous Sclerosis Complex

In a truly compelling example of bedside-to-bench-to-bedside translational research, studies that began with TSC patient cohorts to identify the two TSC genes (i.e., *TSC1* and *TSC2*) and their corresponding proteins TSC1 (hamartin) and TSC2 (tuberin), ultimately led to genetic manipulations of *Tsc* genes

in *Drosophila melanogaster* (2,3). Knockout of either *dTsc1* or *dTsc2* (the *Drosophila* orthologs) revealed that loss of *Tsc* gene function was directly related to enhanced cell size, altered cell proliferation, and abnormal organogenesis. Investigators discovered that loss of TSC1 or TSC2 protein function led to constitutive activation of the mammalian target of rapamycin (mTOR) cascade, a well-recognized and highly evolutionarily conserved signaling pathway known to govern regulation of cell growth (4). TSC1 and TSC2 combine to form a functional heteromer that negatively modulates mTOR (part of a larger protein complex, mTORC1). Loss of either TSC1 or TSC2 as a consequence of mutation leads to constitutive mTOR activation by phosphorylation, via an intermediary G-protein known as Ras homolog enriched in brain (Rheb). Activated mTOR serves as a kinase and phosphorylates several downstream proteins, including S6 kinase, ribosomal S6 protein, and several components of the translational apparatus. These proteins, and in effect the mTOR pathway, exert control over cell size, via regulation of gene transcription and protein translation, and in particular govern assembly of the cellular translational apparatus. Thus, a central tenet in understanding the pathogenesis of TSC is that loss of TSC function leads to relentless mTOR pathway signaling, which is well demonstrated in renal, skin, and brain lesions. This major breakthrough provided a backdrop to develop strategies to pharmacologically manipulate mTOR. Fortuitously, the macrolide antibiotic rapamycin was known as a powerful inhibitor of mTOR, and thus, the logical connection was made to study the effects of rapamycin both *in vitro* and in several animal TSC models. Since mutations in the *TSC1* or *TSC2* genes lead to constitutive activation of mTOR, rapamycin provides an off-the-shelf and readily available antidote to test in human TSC patients.

Preclinical Studies with Rapamycin

Three recent preclinical studies have shown clearly that administration of rapamycin to mice lacking one or both copies of a *Tsc* gene can reverse the neurological phenotype of that strain. As reviewed by Merlin in this issue of *Epilepsy Currents*, Zeng and colleagues studied the effects of rapamycin in a conditional knockout TSC (*Tsc1*^{GFAP}CKO) mouse in which loss of *Tsc1* is engineered under the control of a human glial fibrillary acidic protein (GFAP) promoter driving cre recombinase (5). These animals exhibit progressive astrocytosis in the cortex, altered hippocampal cytoarchitecture, and diminished survival. A majority of the mice develop spontaneous and electrographically documented seizures by the 4th week of postnatal life. Administration of rapamycin to the animals, prior to onset of seizure

Address correspondence to Peter B. Crino, MD, PhD, Department of Neurology, PENN Epilepsy Center, University of Pennsylvania Medical Center, 3400 Spruce St, Philadelphia, PA 19104. E-mail: peter.crino@uphs.upenn.edu

Epilepsy Currents, Vol. 8, No. 6 (November/December) 2008 pp. 159–162
Wiley Periodicals, Inc.

© American Epilepsy Society

induction, prevented the development of seizures and structural changes in the brain. Of particular interest, interictal spikes disappeared, and there was normalization of the interictal EEG following rapamycin. When given to these mice after the onset of seizures, rapamycin led to a cessation of clinical seizures and enhanced survival. However, the seizures returned, and survival curves revert when the rapamycin was discontinued.

Also reviewed by Merlin in this issue, Meikle et al. demonstrate that in a conditional knockout TSC (*Tsc1^{syn}CKO*) mouse in which loss of *Tsc1* is engineered under the control of a synapsin promoter-driven cre recombinase, rapamycin and a related compound, RAD001 [40-*O*-(2-hydroxyethyl)-rapamycin], reversed the behavioral and cellular phenotypes (6). For example, rapamycin treatment led to a decrease in pathological clasping behavior, postural kyphosis, and tremor. Rapamycin also attenuated aberrant mTOR signaling in the brain, as evidenced by decreased numbers of cortical neurons expressing phosphorylated S6 protein. Cortical myelination defects were improved, and there was partial reduction in the number of neurofilament labeled cytomegalic neurons in the cerebral cortex. Interestingly, while the number of enlarged cortical neurons was diminished following rapamycin, subtle cortical laminar defects in these animals did not dramatically change. Although a previous report revealed seizures in a subset of these mice (7), seizures and EEG changes were not evaluated in the paper by Meikel and colleagues. In addition, the investigators demonstrated that both chronic administration and transient treatment for 23 days (between postnatal days 7 and 30) improved long-term survival, suggesting that lifetime maintenance rapamycin therapy might not be required for TSC patients. This is an important finding since the side effects of chronic rapamycin, including aphthous oral ulcers, hyperlipidemia, and an increased rate of systemic infections, may lead to cessation of therapy in some patients. As discussed in the following section, *Potential Limitations of Rapamycin Therapy*, the effect of long-term rapamycin therapy specifically for TSC patients is unknown, and nearly 50% of TSC patients receiving rapamycin in a recent clinical study reported adverse effects (8).

Ehninger et al. showed that *Tsc2^{+/-}* heterozygous animals exhibit deficits in hippocampal-dependent learning, using a hippocampus-dependent win-shift version of the eight-arm radial maze paradigm (9). Since the mTOR cascade plays a role in the establishment of late-phase long-term potentiation (L-LTP) in the hippocampus, Ehninger et al. demonstrated that L-LTP was deficient in acute hippocampal slices from *Tsc2^{+/-}* mice compared with wild type mice. L-LTP corresponds to the later phase of classic long-term potentiation and is partially dependent on protein synthesis. Although the *Tsc2^{+/-}* mice exhibit only a 25% reduction in *Tsc2* protein levels, analysis of Western blots revealed enhanced mTOR signaling as evidenced by increased phosphorylation of S6 protein on the

active sites Ser 235/236. These findings suggest that even in the absence of a complete *Tsc2* gene knockout, there is abnormally enhanced mTOR pathway signaling. Treatment with rapamycin prior to the learning paradigm exposure rescued the phenotype of these mice such that the learning paradigm performance, L-LTP deficits, and aberrant phosphorylation of S6 protein were corrected to near wild-type levels. Then, a second mouse model was tested in which the floxed *Tsc1* mice were crossed with mice expressing cre recombinase under the control of the Ca(2+)/calmodulin-dependent protein kinase II (CaMKII) promoter (CaMKII-Cre). The phenotype of these mice was quite severe with pronounced macroencephaly, neuronal hypertrophy, and astrogliosis. These animals exhibit early mortality, hypoactivity, and a pathological hindlimb clasp reflex. Early treatment with rapamycin enhanced survival and led to a decrease in pathological reflex expression. Seizures and EEG were not evaluated in these mice.

Potential Mechanisms for Phenotypic Changes

These three preclinical studies in four distinct animal model systems demonstrate that aberrant activation of the mTOR cascade, as a consequence of diminished TSC1 or TSC2 function, is associated with neurological manifestations and that these abnormalities can be abrogated by rapamycin administration. How might rapamycin lead to changes in neurological phenotype? First, there is direct evidence that the mTOR pathway plays a pivotal role in establishment of long-term synaptic plasticity (10), regulation of dendritic protein synthesis (11), and modulation of potassium channel expression (12). Indeed, the *Tsc1^{GFAP}CKO* mice exhibit impaired inward rectifier potassium currents (13). These control points are likely to be pivotal in regulating cellular excitability and thus, constitutive mTOR activation may result in a cascade of events leading to seizures. In resected tubers from human TSC epilepsy patients, there is clear evidence for hyperactive mTOR signaling (14,15), so it seems plausible that mTOR is at least partially linked to seizures. Furthermore, in these specimens there is dramatic alteration in glutamate and GABA receptor subunit expression that may foster seizure initiation (16,17). Ample evidence exists to demonstrate that the mTOR pathway is an important component of the cellular learning mechanism in hippocampus (18), amygdala (19), and cortex (20). Since it is virtually axiomatic that learning and memory are dependent to some extent on protein synthesis, aberrant mTOR modulation of the translational apparatus may play a critical role in altered memory-task performance in both TSC animal models and human patients. What is tantalizing is that in most of the studies on mTOR and learning, mTOR signaling *promotes* learning and rapamycin *inhibits* performance on a learning task. Thus, the role of rapamycin

in TSC, in which mTOR signaling is left unchecked, may be different than in the absence of TSC mutations.

Potential Limitations of Rapamycin Therapy

It is evident from the preclinical studies that early treatment, prior to phenotypic expression of loss of TSC protein function, provides the most robust clinical effect. So, is enough known about rapamycin to begin clinical trials? What are the limitations to widespread off-label use of rapamycin for TSC? First, there is a growing body of evidence that dysregulation of the mTOR cascade may not be the whole cellular story in TSC—other cellular cascades may be co-activated. For example, there is evidence that aberrant activation of mitogen-activated protein kinase (MAPK), glycogen synthetase kinase 3 (GSK3), and AMP-dependent kinase (AMPK) may occur in cells lacking TSC1 or TSC2 function. These kinases act both upstream and in parallel to mTOR and thus, may not be affected by rapamycin. In addition, while loss of TSC protein function in vitro provides a relatively pure mechanistic effect on mTOR activation, TSC gene mutations in whole tissues or organs can result in far more complex cellular effects, which is in part due to secondary effects on surrounding cells, inflammatory responses, cell death, and humoral factors. For example, tubers can have a robust inflammatory response evidenced by activation of complement protein, invasion by CD68⁺ macrophages, and expression of proinflammatory cytokines, such as TNF- α and interleukin- β (21). These events have cellular consequences that may reverberate within a tuber or subependymal giant cell astrocytomas far in excess of mTOR cascade activation. Second, it is well recognized that both TSC1 and TSC2 bind to additional proteins, such as 14-3-3, Pam, and cyclins (22), and thus, it is likely that yet undefined cellular functions of TSC1 and TSC2 may be altered in TSC. Third, mTOR functions in two protein complexes: mTORC1 and mTORC2. While mTORC1 is rapamycin sensitive, mTORC2 is rapamycin insensitive, and in fact, recent evidence suggests that TSC1 and TSC2 positively regulate mTORC2 (23). Finally, and perhaps most cautionary, is that the effect of TSC1 and TSC2 on mTOR is not binary or “on-off.” Rather, like many cellular protein cascades, it serves as a dynamic sliding scale that is differentially activated as a function of development, cell cycle, cellular energy demands, nutrient availability, cell type, and even subcellular localization. Because there are feedback controls to other protein pathways, the effects of pharmacologically maintaining mTOR in an “off” position are not yet known.

Future Clinical Role for Rapamycin in TSC

The current view among TSC clinicians and researchers is that rapamycin will likely provide hope for TSC patients. In animal models and in in vitro systems, rapamycin seems to accom-

plish what it is supposed to do, and a broad clinical literature indicates that rapamycin can be used safely in pediatric renal transplant recipients. While the side effect profile is not benign, the most prominent side effects, such as aphthous oral ulcers and hyperlipidemia, can be effectively managed. However, there are several compelling questions that must factor into the long-term vision of rapamycin therapy for patients with TSC. First, should rapamycin be used for TSC patients with neurological features only or prophylactically in all TSC patients? The preclinical data in mice suggest that appropriately timed rapamycin therapy could stop seizures altogether. A particularly tantalizing hope is that rapamycin might yield a highly targeted therapy for infantile spasms in TSC. Should rapamycin be given to infants as a presumptive strike against infantile spasms? Once started, how long should treatment be continued? A recent clinical study in a cohort of TSC patients demonstrated that rapamycin therapy for 1 year diminished the radiographic size of renal angiomyolipomas and may yield improved pulmonary function in patients with lymphangiioleiomyomatosis (8). Interestingly, like some of the preclinical data, when rapamycin was discontinued, there was some regrowth of the angiomyolipomas. These findings echo a prior clinical case series of five TSC patients with subependymal giant cell astrocytomas, in which rapamycin led to shrinkage of the lesions, but discontinuation led to lesion regrowth in one of the patients (24).

Conclusion

There are no data yet in the clinical neurology literature that support use of rapamycin for epilepsy, cognitive disability, or behavioral dyscontrol in TSC. However, normalization of the interictal EEG in mouse models could herald improvement in cognition, and Ehninger et al. show that rapamycin can reverse not only learning deficits but also the cellular processes (e.g., L-LTP) that underlie memory consolidation. At several recent national and international meetings, papers were presented by investigators who are planning clinical trials to document the benefit of rapamycin for seizures, cognition, and behavior. The vision now is for implementation of rapamycin therapy in well-designed, multicenter trials: 1) to accurately document its effect on seizures, cognition, or autism, 2) to ensure a safe risk/benefit profile for TSC, and 3) to determine the length of therapy in TSC patients. Indeed, there yet may be a cure for TSC.

References

1. Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. *N Engl J Med* 2006;355:1345–1356.
2. Potter CJ, Huang H, Xu T. Drosophila Tsc1 functions with Tsc2 to antagonize insulin signaling in regulating cell growth, cell proliferation, and organ size. *Cell* 2001;105:357–368.
3. Gao X, Pan D. TSC1 and TSC2 tumor suppressors antagonize insulin signaling in cell growth. *Genes Dev* 2001;15:1383–1392.

4. Huang J, Manning BD. The TSC1-TSC2 complex: A molecular switchboard controlling cell growth. *Biochem J* 2008;412:179–190.
5. Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol* 2008;63:444–453.
6. 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.
7. Meikle L, Talos DM, Onda H, Pollizzi K, Rotenberg A, Sahin M, Jensen FE, Kwiatkowski DJ. A mouse model of tuberous sclerosis: Neuronal loss of Tsc1 causes dysplastic and ectopic neurons, reduced myelination, seizure activity, and limited survival. *J Neurosci* 2007;27:5546–5558.
8. Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, Schmithorst VJ, Laor T, Brody AS, Bean J, Salisbury S, Franz DN. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. *N Engl J Med* 2008;358:140–151.
9. Ehninger D, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ, Ramesh V, Silva AJ. Reversal of learning deficits in a Tsc2(+/-) mouse model of tuberous sclerosis. *Nat Med* 2008;14:843–848.
10. Tang SJ, Reis G, Kang H, Gingras AC, Sonenberg N, Schuman EM. A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proc Natl Acad Sci U S A* 2002;99:467–472.
11. Tsokas P, Ma T, Iyengar R, Landau EM, Blitzer RD. Mitogen-activated kinase upregulates the dendritic translation machinery in long-term potentiation by controlling the mammalian target of rapamycin pathway. *J Neurosci* 2007;27:5885–5894.
12. Raab-Graham KF, Haddick PC, Jan YN, Jan LY. Activity- and mTOR-dependent suppression of Kv1.1 channel mRNA translation in dendrites. *Science* 2006;314:144–148.
13. Jansen LA, Uhlmann EJ, Crino PB, Gutmann DH, Wong M. Epileptogenesis and reduced inward rectifier potassium current in tuberous sclerosis complex-1-deficient astrocytes. *Epilepsia* 2005;46:1871–1880.
14. Baybis M, Yu J, Lee A, Golden JA, Weiner H, McKhann G 2nd, Aronica E, Crino PB. mTOR cascade activation distinguishes tubers from focal cortical dysplasia. *Ann Neurol* 2004;56:478–487.
15. Miyata H, Chiang AC, Vinters HV. Insulin signaling pathways in cortical dysplasia and TSC-tubers: Tissue microarray analysis. *Ann Neurol* 2004;56:510–519.
16. White R, Hua Y, Scheithauer B, Lynch DR, Henske EP, Crino PB. Selective alterations in glutamate and GABA receptor subunit mRNA expression in dysplastic neurons and giant cells of cortical tubers. *Ann Neurol* 2001;49:67–78.
17. Talos DM, Kwiatkowski DJ, Cordero K, Black PM, Jensen FE. Cell-specific alterations of glutamate receptor expression in tuberous sclerosis complex cortical tubers. *Ann Neurol* 2008;63:454–465.
18. Horwood JM, Dufour F, Laroche S, Davis S. Signalling mechanisms mediated by the phosphoinositide 3-kinase/Akt cascade in synaptic plasticity and memory in the rat. *Eur J Neurosci* 2006;23:3375–3384.
19. Parsons RG, Gafford GM, Helmstetter FJ. Translational control via the mammalian target of rapamycin pathway is critical for the formation and stability of long-term fear memory in amygdala neurons. *J Neurosci* 2006;26:12977–12983.
20. Schicknick H, Schott BH, Budinger E, Smalla KH, Riedel A, Seidenbecher CI, Scheich H, Gundelfinger ED, Tischmeyer W. Dopaminergic modulation of auditory cortex-dependent memory consolidation through mTOR. *Cereb Cortex* 2008 Mar 6. [Epub ahead of print doi: 10.1093/cercor/bhn026].
21. Boer K, Jansen F, Nellist M, Redeker S, van den Ouweland AM, Spliet WG, van Nieuwenhuizen O, Troost D, Crino PB, Aronica E. Inflammatory processes in cortical tubers and subependymal giant cell tumors of tuberous sclerosis complex. *Epilepsy Res* 2008;78:7–21.
22. Rosner M, Hanneder M, Siegel N, Valli A, Hengstschläger M. The tuberous sclerosis gene products hamartin and tuberlin are multifunctional proteins with a wide spectrum of interacting partners. *Mutat Res* 2008;658:234–246.
23. Huang J, Dibble CC, Matsuzaki M, Manning BD. The TSC1-TSC2 complex is required for proper activation of mTOR complex 2. *Mol Cell Biol* 2008;28:4104–4115.
24. Franz DN, Leonard J, Tudor C, Chuck G, Care M, Sethuraman G, Dinopoulos A, Thomas G, Crone KR. Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. *Ann Neurol* 2006;59:490–498.