Rational Therapy From Bench to Bedside for a Rare Epilepsy

Rapamycin Prevents Seizures After Depletion of STRADA in a Rare Neurodevelopmental Disorder.


A rare neurodevelopmental disorder in the Old Order Mennonite population called PMSE (polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome; also called Pretzel syndrome) is characterized by infantile-onset epilepsy, neurocognitive delay, craniofacial dysmorphism, and histopathological evidence of heterotopic neurons in subcortical white matter and subependymal regions. PMSE is caused by a homozygous deletion of exons 9 to 13 of the LYK5/STRADA gene, which encodes the pseudokinase STRADA, an upstream inhibitor of mammalian target of rapamycin complex 1 (mTORC1). We show that disrupted pathfinding in migrating mouse neural progenitor cells in vitro caused by STRADA depletion is prevented by mTORC1 inhibition with rapamycin or inhibition of its downstream effector p70S6 kinase (p70S6K) with the drug PF-4708671 (p70S6Ki). We demonstrate that rapamycin can rescue aberrant cortical lamination and heterotopia associated with STRADA depletion in the mouse cerebral cortex. Constitutive mTORC1 signaling and a migration defect observed in fibroblasts from patients with PMSE were also prevented by mTORC1 inhibition. On the basis of these preclinical findings, we treated five PMSE patients with sirolimus (rapamycin) without complication and observed a reduction in seizure frequency and an improvement in receptive language. Our findings demonstrate a mechanistic link between STRADA loss and mTORC1 hyperactivity in PMSE, and suggest that mTORC1 inhibition may be a potential treatment for PMSE as well as other mTOR-associated neurodevelopmental disorders.

Commentary
The Mammalian (also called Mechanistic) Target of Rapamycin (mTOR) is a protein kinase critical for integrating extracellular and intracellular signals to control multiple cell functions, including autophagy, RNA expression and translation, mitochondrial and lipid metabolism, cell polarity and growth, and cytoskeletal organization. mTOR hyperactivation has been linked to many human diseases, among them diabetes, cancer, and developmental brain malformations (1, 2).

mTOR exerts its function through two different multiprotein complexes, mTORC1 and mTORC2. Human samples and animal models of focal cortical dysplasia, hemimegalencephaly, and tuberous sclerosis complex (TSC) have confirmed increased activity of mTORC1 by immunohistochemistry or Western Blot (2). Little is known about the activity of mTORC2 in these disorders. Rapamycin, an inhibitor of mTORC1 clinically used as an immunosuppressant after organ transplantation and in the treatment of certain cancers (3), is capable of reverting the immunohistochemical markers of mTORC1 hyperactivity and some of the associated neurological deficits in TSC and other animal models (4). Currently, rapamycin and its analogs are also approved for the treatment of TSC, where they have been shown to reduce the size of renal, pulmonary, and CNS tumors. However, their effect on seizure frequency and severity in TSC remain under investigation (5, 6).

Polyhydramnios, Megalencephaly, and Symptomatic Epilepsy (PMSE, also called Pretzel Syndrome, OMIM 611087) is an autosomal recessive disease that occurs in the Old Order Mennonite community. It is caused by homozygous deletion of the LYK5/STRADA gene, which encodes the pseudokinase STRADA. We show that disrupted pathfinding in migrating mouse neural progenitor cells in vitro caused by STRADA depletion is prevented by mTORC1 inhibition with rapamycin or inhibition of its downstream effector p70S6 kinase (p70S6K) with the drug PF-4708671 (p70S6Ki). We demonstrate that rapamycin can rescue aberrant cortical lamination and heterotopia associated with STRADA depletion in the mouse cerebral cortex. Constitutive mTORC1 signaling and a migration defect observed in fibroblasts from patients with PMSE were also prevented by mTORC1 inhibition. On the basis of these preclinical findings, we treated five PMSE patients with sirolimus (rapamycin) without complication and observed a reduction in seizure frequency and an improvement in receptive language. Our findings demonstrate a mechanistic link between STRADA loss and mTORC1 hyperactivity in PMSE, and suggest that mTORC1 inhibition may be a potential treatment for PMSE as well as other mTOR-associated neurodevelopmental disorders.
Strad α during radial cortical migration in the developing brain, they electroporated the Strada shRNA in utero at E14. Cells with Strad α depletion accumulated at the ventricular and subventricular zones 5 days later, unable to migrate into the cortical plate (8).

In the present study, the authors sought to test whether histological abnormalities seen in the Strad α-depleted animals and patient fibroblasts are rescued with mTORC1 pathway inhibitors, and the effects of rapamycin on seizures and cognitive function in PMSE patients.

The authors first explored the abnormal migration of mNPCs caused by the loss of Strad α. They established stably transfeatured mNPC lines that expressed Strada or scrambled control shRNA. As expected, cells expressing Strada shRNA displayed markers of increased mTORC1 activation, which were normalized with the use of rapamycin (reported previously) or the p70S6 kinase inhibitor PF-4708671, a blocker of the downstream target of mTORC1 p70S6K. Using a wound-healing assay, they found that Strad α-depleted mNPCs have reduced migration in vitro compared with cells expressing the scrambled shRNA. This defect was due to an erratic migration of the Strad α-depleted cells and was also associated with abnormal cell polarization, measured by Golgi apparatus compaction around the nucleus. The migratory parameters and Golgi compaction were normalized with the use of mTORC1 pathway inhibitors.

To explore if rapamycin could correct the migration abnormalities seen in vivo, the authors repeated their experiments electroporating Strada shRNA in utero to induce loss of function (8). The dams in the treatment group then received intraperitoneal rapamycin for the next four days, while the control group received intraperitoneal vehicle injections. The controls again demonstrated an inability of Strad α-depleted cells to migrate into the cortical plate, while animals from rapamycin-treated dams showed rescue of the migration deficit in electroporated cells. The data are very interesting and informative, although there are some limitations: First, the authors did not test for potential phenotypes of the mice that develop in the setting of mosaic Strada depletion. Second, it remains uncertain how the abnormalities seen in cells with hyperactivation of mTORC1 contribute to the development of epilepsy. Future studies will need to focus in these questions to allow for a better understanding of disease pathogenesis.

The authors then focused on the ability of rapamycin to rescue abnormalities in cells from PMSE patients. Fibroblasts were extracted from skin punch biopsies of PMSE subjects, heterozygous parents, and controls. Not surprisingly, the fibroblasts from PMSE patients show increased mTORC1 activity compared to controls and the asymptomatic heterozygous parents. Wound-healing assays on fibroblast monolayers showed less migration of PMSE fibroblasts. Treatment with both rapamycin and PF-4768071 normalized the levels of mTORC1 activity and the migration defect. However, potential effects of the compounds on the heterozygous parent or control fibroblasts were not reported.

In the most significant portion of the paper, the authors reported their experience treating PMSE patients with Sirolimus, the commercial preparation of rapamycin. Five PMSE patients (ranging in age from 3–8 months) received Sirolimus once daily, with the dose adjusted to achieve a blood concentration between 5 and 15 ng/ml. Treatment was stopped temporarily during mild infectious illnesses (common in children) to avoid its immunosuppressive effects and was restarted upon recovery. Subjects were followed for 5 to 52 months. No deaths occurred, and the drug was well tolerated. Four of the treated patients had seizure-free periods of at least 12 months, including one that had experienced multiple episodes of status epilepticus prior to Sirolimus treatment. All five required fewer antiepileptic drugs when compared with a historical cohort. The treated group was also evaluated for psychomotor development. Compared to the historical cohort, treated patients showed better receptive language and social domain skills. This assessment was confirmed by the parents, who also reported them to be more attached emotionally. No effect of Sirolimus was demonstrated on expressive language, gross motor function, or adaptive learning. One caveat is that the treated cohort was younger than the historic one, making comparisons of cognitive development difficult. Subsequent reporting of longer follow-up will help to provide a clearer picture of the medication effect on psychomotor development of the patients. Another caveat of the study is the use of a historical cohort, as opposed to a placebo group, which limits the generalizability of the findings. Nonetheless, this approach was an important first step that should be followed by subsequent trials with appropriate controls to provide clinicians with the highest level of evidence.

This report is a great example of translational research, building on the description of the cellular abnormalities secondary to mTORC1 hyperactivity seen in PMSE, and their rescue with rapamycin in human samples and murine cells both in vivo and in vitro. It is also an important contribution to understanding potentially effective therapy for this very severe and treatment-resistant epilepsy, including the possibility of Sirolimus preventing disease progression as well as acting as an anti-seizure agent. Along with the ongoing reports on the use of Sirolimus in TSC, the present work helps to better define the role of mTORC1 inhibition in epilepsy treatment both for mTORepathies and for epilepsy syndromes in general.

by Gustavo A. Patino, MD PhD, Jack Parent, MD

References


Disclosure of Potential Conflicts of Interest

Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. **Identifying information.**
   Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. **The work under consideration for publication.**
   This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. **Relevant financial activities outside the submitted work.**
   This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (DGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

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Section #1 Identifying Information

1. Today's Date: February 25, 2014

2. First Name Jack Last Name Parent Degree MD

3. Are you the Main Assigned Author?  Yes No

If no, enter your name as co-author:

4. Manuscript/Article Title: Rational Therapy from Bench to Bedside for a Rare Epilepsy

5. Journal Issue you are submitting for: 14.5

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

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<th>Type</th>
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<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
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<td>7. Payment for manuscript preparation.</td>
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<td>12. Travel/accommodations/meeting expenses unrelated to activities listed.**</td>
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<td>Yes</td>
<td>Various</td>
<td>Travel as a speaker to various scientific meetings (only academic, non-pharmaceutical)</td>
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<td>13. Other (err on the side of full disclosure)</td>
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*  This means money that your institution received for your efforts.
**  For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

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