



# mTORC2 Steals the Spotlight

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## Therapeutic Inhibition of mTORC2 Rescues the Behavioral and Neurophysiological Abnormalities Associated With PTEN-Deficiency

Chen C, Sgritta M, Mays J, et al. *Nat Med.* 2019;25(11):1684-1690. doi:10.1038/s41591-019-0608-y.

Dysregulation of mammalian target of rapamycin (mTOR) signaling, which is mediated by 2 structurally and functionally distinct complexes, mTORC1 and mTORC2, has been implicated in several neurological disorders. Individuals carrying loss-of-function mutations in the phosphatase and tensin homolog (*PTEN*) gene, a negative regulator of mTOR signaling, are prone to developing macrocephaly, autism spectrum disorder (ASD), seizures, and intellectual disability. It is generally believed that the neurological symptoms associated with loss of *PTEN* and other mTORopathies (eg, mutations in the tuberous sclerosis genes *TSC1* or *TSC2*) are due to hyperactivation of mTORC1-mediated protein synthesis. Using molecular genetics, we unexpectedly found that genetic deletion of mTORC2 (but not mTORC1) activity prolonged life span, suppressed seizures, rescued ASD-like behaviors and long-term memory, and normalized metabolic changes in the brain of mice lacking *Pten*. In a more therapeutically oriented approach, we found that administration of an antisense oligonucleotide targeting mTORC2's defining component *Rictor* specifically inhibits mTORC2 activity and reverses the behavioral and neurophysiological abnormalities in adolescent *Pten*-deficient mice. Collectively, our findings indicate that mTORC2 is the major driver underlying the neuropathophysiology associated with *Pten*-deficiency, and its therapeutic reduction could represent a promising and broadly effective translational therapy for neurological disorders where mTOR signaling is dysregulated.

## Commentary

Genetic mutations that result in unrestrained activation of the cellular PI3K-AKT-mTOR pathway (the “mTORopathies”) are significant causes of epilepsy, developmental disability, and autism.<sup>1</sup> These disorders can result either from loss of function mutations in genes that normally inhibit the pathway (ie, *TSC1*, *TSC2*, *DEPDC5*, *PTEN*) or from gain of function mutations in genes that activate the pathway (ie, *AKT3*, *PIK3CA*, *MTOR*). Mammalian target of rapamycin (mTOR) is a part of 2 separate protein complexes with distinct functions; mTORC1, which contains the protein Raptor, and mTORC2, which instead contains the protein Rictor. Mammalian target of rapamycin complex 1 (mTORC1) functions to regulate such cellular processes as messenger RNA translation, metabolism, protein turnover, and autophagy, while mTORC2 regulates cytoskeletal arrangement, glucose metabolism, and apoptosis.<sup>2</sup> In animal models of mTOR pathway dysregulation, seizures and other neurologic phenotypes are often prevented or reversed by administration of rapamycin or related compounds (rapalogs), which exhibit relative selectivity for inhibition of mTORC1.<sup>3</sup> These findings have led to the hypothesis that many of the neurologic consequences of mTOR pathway dysfunction are due to

dysregulation of mTORC1 activity rather than of mTORC2. However, the study reported by Chen et al may prompt reconsideration of this dogma.

In this article, Chen et al<sup>4</sup> report a selective approach to investigating the relative roles of mTORC1 and mTORC2 in the pathogenesis of disease in the mTORopathy caused by loss of function of *PTEN*. *PTEN* is an upstream inhibitor of the PI3K/AKT/mTOR pathway, serving as a phosphatase that opposes the action of PI3K in the growth factor-stimulated phosphorylation of PIP<sub>2</sub> to PIP<sub>3</sub>. *PTEN* loss of function mutations result in unrestrained activity of both mTORC1 and mTORC2. The mouse model utilized in Chen et al is a conditional homozygous knockout of *Pten* generated using Cre-lox technology, with Cre under the control of the *Camk2α* promoter (*Pten* fb-KO mice). This results in postnatal loss of *Pten* expression in a portion of excitatory neurons in the forebrain, with phenotypes of increased brain size, shortened survival, spontaneous seizures, increased electrophysiologic measures of excitatory synaptic transmission, and impairments in social behavior and memory. Elevations in both mTORC1 and mTORC2 activity were demonstrated in hippocampus of *Pten* fb-KO mice through detection of increased expression of



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phosphorylated ribosomal protein S6 (marker of mTORC1 activity) and of S473-phosphorylated Akt (marker of mTORC2 activity). The investigators then analyzed the phenotypes of mice with conditional double knockout of *Pten* and either *Rptor* (mTORC1-deficient) or *Rictor* (mTORC2-deficient). Importantly, the brain overgrowth phenotype of *Pten* fb-KO mice was prevented by loss of *Rptor*, but the survival, seizure, behavioral, and electrophysiological phenotypes were only ameliorated by loss of *Rictor*. These results suggest that the neurologic phenotypes of postnatal loss of *Pten* function in forebrain excitatory neurons are primarily mediated by dysregulated activity of mTORC2 rather than mTORC1.


These findings were supported by additional experiments in which antisense oligonucleotides (ASO) targeting *Rictor* were injected intraventricularly in *Pten* fb-KO mice at 4 weeks of age. When analyzed 1 to 3 weeks later, *Rictor*-ASO injected animals were found to exhibit improvements in seizure frequency and behavioral measures as compared to those with control-ASO injections. However, not surprisingly, these improvements were often not as dramatic as those seen in the *Pten-Rictor* double knockout animals. These findings led to the suggestion that *Rictor*-ASO may serve as a therapeutic strategy in the treatment of the mTORopathies.

At first glance, the findings of Chen et al may appear to contradict previous studies, in which treatment with the mTORC1-selective inhibitor rapamycin was able to both prevent and reverse abnormalities in brain size, behavior, and seizures in *Pten* conditional knockout mice.<sup>5-7</sup> However, close reading of these articles reveals that the rapamycin dosing paradigms used resulted in not only inhibition of mTORC1 activity but also significant inhibition of mTORC2 activity. While short-term rapamycin exposure may selectively inhibit mTORC1, longer term exposure has been demonstrated to also inhibit mTORC2 activity, potentially through a reduction of mTOR availability for formation of the complex.<sup>2</sup> However, the genetic manipulation used by Chen et al was also not completely selective for the targeted mTOR complex, as significant *increases* in mTORC2 activity were seen in the *Pten-Rptor* DKO animals with reduction in mTORC1 activity. This may have contributed to the lack of effect of *Rptor* knockout on the examined phenotypes.


Another important consideration is in regard to the timing and cell types affected in the *Pten* fb-KO mice. These animals were generated using Cre under the control of the *Camk2α* promoter, which only causes recombination and loss of *Pten* function after postnatal day 14, and only in a portion of forebrain excitatory neurons.<sup>8</sup> This model therefore lacks the migration abnormalities and neuronal hypertrophy found in patients with *PTEN* mutations and in other *Pten* mouse models. *PTEN* loss in inhibitory neurons and glia also likely contributes to the neurologic phenotypes of the *PTEN* hamartoma tumor syndrome. These observations suggest that selective mTORC2 inhibition may be less effective in clinically relevant situations than in *Pten* fb-KO mice.

However, the most critical factor in assessing the impact of this work is whether it is generalizable to other clinically important mTORopathies.<sup>1</sup> *PTEN* loss of function mutations result in unrestrained activation of both mTORC1 and

mTORC2, as do gain of function mutations in the *PIK3CA* and *AKT* kinases. In contrast, loss of function mutations in *TSC1*, *TSC2*, and the GATOR1 complex components (such as *DEPDC5*) result in activation of mTORC1 with feedback *inhibition* of mTORC2 activity.<sup>9</sup> Interestingly, gain of function mutations in *MTOR* itself may result in activation of either mTORC1, mTORC2, or both complexes depending on the specific mutation.<sup>10</sup> Therefore, inhibition of mTORC2 is unlikely to be of therapeutic benefit (and may actually worsen symptoms) in tuberous sclerosis complex, while it may be beneficial (in combination with inhibition of mTORC1) in megalencephaly-capillary malformation syndrome due to *PIK3CA* mutation. These findings highlight the importance of consideration of the specific pathogenic mutation and its role in the mTOR pathway when developing new therapies for the mTORopathies.

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