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Raptor Preys on mTOR Imbalance in Tuberous Sclerosis

Raptor Downregulation Rescues Neuronal Phenotypes in Mouse Models of Tuberous Sclerosis Complex

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Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder caused by mutations in the *TSC1* or *TSC2* genes, which encode proteins that negatively regulate mTOR complex 1 (mTORC1) signaling. Current treatment strategies focus on mTOR inhibition with rapamycin and its derivatives. While effective at improving some aspects of TSC, chronic rapamycin inhibits both mTORC1 and mTORC2 and is associated with systemic side-effects. It is currently unknown which mTOR complex is most relevant for TSC-related brain phenotypes. Here we used genetic strategies to selectively reduce neuronal mTORC1 or mTORC2 activity in mouse models of TSC. We find that reduction of the mTORC1 component Raptor, but not the mTORC2 component Rictor, rebalanced mTOR signaling in Tsc1 knock-out neurons. Raptor reduction was sufficient to improve several TSC-related phenotypes including neuronal hypertrophy, macrocephaly, impaired myelination, network hyperactivity, and premature mortality. Raptor downregulation represents a promising potential therapeutic intervention for the neurological manifestations of TSC.

Commentary

The mechanistic target of rapamycin (mTOR) pathway is an important signaling pathway that regulates cellular metabolism, proliferation, growth, and survival. Pathogenic variants in genes that regulate mTOR signaling cause a broad spectrum of malformations of cortical development, collectively called mTORopathies.[1] These include tuberous sclerosis (TS), the most well-characterized mTORopathy, caused by loss-of-function variants in *TSC1* and *TSC2*.[2,3] Tuberous sclerosis is characterized by nonmalignant tumors in various organs of the body, including tubers in the brain. Clinically, TS is characterized by uncontrolled seizures, developmental delay, and intellectual disability.[4] Due to the uncontrolled nature of these seizures, novel precision therapeutics are urgently needed.

Mechanistic target of rapamycin signaling is effectuated by two protein complexes, mTORC1 and mTORC2. Both complexes have a common member, mTOR. The proteins that primarily distinguish the two complexes are Raptor (*Rptor*) in mTORC1 and Rictor (*Rictor*) in mTORC2. mTOR signaling is up- or down-regulated as necessary to maintain cellular homeostasis. However, in TS, mTOR signaling is constitutively active due to loss of the upstream regulators encoded by *TSC1/2*. While mTORC1 hyperactivity in TS is well-known, recent findings point to abnormal mTORC2 signaling as well. There has thus been much interest in mTOR inhibition to treat TS via rapamycin and its derivatives, and these inhibitors have shown some efficacy in reducing seizures.[5] However, chronic usage of mTOR inhibitors results in severe side effects including immunosuppression.[6] This warrants the search for alternate strategies for more precise modulation of mTOR signaling. In this study, Karalis et al dissect the mechanisms of aberrant mTOR signaling in TS and discover that downregulation of mTORC1 protein, Raptor, was effective not only at restoring mTOR balance but also in rescuing some TS phenotypes in a mouse model.[7]

To model TS, the authors established primary hippocampal cultures from *Tsc1* floxed mice, and then treated the cultured cells with a cre-recombinase-expressing adeno-associated viral construct to delete the floxed *Tsc1* gene, as germline *Tsc1* deletion is embryonically lethal. Tsc1-cKO cultures exhibited increased levels of mTORC1 proteins Raptor and p-S6, suggesting increased mTORC1 signaling. However, a decrease in p-Akt was also observed, despite unaltered levels of mTORC2 protein Rictor, suggesting decreased mTORC2 activity. This bidirectional change of mTORC1 and mTORC2 signaling prompted the authors to try to reestablish the balance of mTOR activity. Rapamycin treatment of Tsc1-cKO cultures suppressed both mTOR pathways, thus normalizing mTORC1 signaling, but further reducing mTORC2 activity. Hence the authors attempted to modulate mTOR signaling using conditional knockouts of *Rptor* and *Rictor*. Loss of *Rictor* suppressed mTORC2 activity but showed no effects in overall mTOR

rebalancing, nor rescue of *Tsc1*-loss related cellular hypertrophy. Conversely, Raptor loss in Tsc1-cKO hippocampal cultures normalized elevated p-S6 levels (mTORC1 signaling) while boosting reduced p-Akt levels (mTORC2 signaling) and restoring reduced soma size. Overall, these results suggest that Raptor inhibition could rescue constitutive mTOR activity in TS. Moreover, even a small amount of Raptor (20%) was sufficient and necessary to restore cellular levels of mTORC1 and mTORC2 activity. This likely explained why heterozygous *Rptor* cKO was more effective than homozygous *Rptor* cKO in restoring mTOR balance associated with *Tsc1* loss. These results also suggest that potential therapies to equilibrate mTOR signaling should not fully eliminate cellular Raptor levels but instead must be finessed just enough to allow for retention of low levels of this protein, which will be technically challenging in humans.

To further explore the potential of a partial reduction in Raptor protein, the authors examined the effects of Raptor heterozygous loss on TS-related phenotypes *in vivo* using an Emx1-Cre mouse model, which enables targeted and timed (~embryonic day 9.5) deletion of both *Tsc1* and *Rptor* in cortical and hippocampal neurons and glia. Heterozygous loss of *Rptor* improved survival rates and overall body weight. A normal brain size and thickness of cortex and hippocampus were also observed, indicating rescue of the macrocephaly phenotype. At the molecular level, p-S6 (mTORC1 signaling) returned to baseline levels, and neuronal hypertrophy as well as impaired neuronal myelination and hippocampal lamination defects seen with Tsc1-cKO mice were also improved with heterozygous *Rptor* loss. In addition, high glial fibrillary acidic protein levels seen in astrocytes of Tsc1-cKO mice were partially reduced in cortical astrocytes and nearly normal in hippocampal astrocytes from Rptor-cHet mice.

Finally, to evaluate hyperactivity, the authors used hippocampal cultures from animals with *Tsc1*-cKO and *Tsc1*-cKO + *Rptor*-cHet deletion, to demonstrate that Raptor loss mitigated neuronal hyperexcitability and epileptiform activity seen with *Tsc1* loss. However, future studies need to determine whether this rescue also correlates with a reduction in seizures, given that this is one of the major morbidities in individuals with TS. This is perhaps the most notable limitation in this study.

The rescue of mTOR signaling imbalances, as well as cellular and some morphological and survival characteristics with Raptor heterozygous loss in this TS mouse model is encouraging, but it is important to contrast with similar studies in the mTORopathies. For instance, loss of the negative mTOR regulator *PTEN* is also associated with a spectrum of clinical presentations, including epilepsy. Consistent with the present results, loss of Raptor and downregulation of mTORC1 signaling improved neuronal morphology, hypertrophy, and synaptic functions in a model of Pten loss.[8] However, another study showed downregulation of mTORC2 signaling via loss of Rictor improved Pten-loss associated synaptic functions while also rescuing seizure and other associated behavioral phenotypes.[9] These results highlight the complexities of this signaling pathway, but also that mTORC1/2 may both play a role in downstream clinical phenotypes of the mTORopathies. This emphasizes how important it is to study both mTORC1/2 in the same model system in order to dissect their roles and associated phenotypes, but also to ensure that mTOR signaling is balanced, toward the highest likelihood of restoring physiological conditions and precision therapy success.

Targeting ubiquitous cellular pathways with precision therapies is extremely challenging; the mTOR signaling pathway is one such with multiple downstream targets. Crosstalk between upstream regulators and feedback pathway loops to maintain signaling homeostasis add another layer of difficulty in modulating the highly complex mTOR pathways, highlighted by the examples of TSC/PTEN. Additionally, the task of regulating mTOR signaling not only entails identifying a target that can be easily genetically modulated but also evaluating the dosage effects of this target. Overall, Karalis et al demonstrate that reduction of the mTORC1 protein Raptor is an effective approach at improving mTOR signaling imbalance and TS-related phenotypes using mouse models, including postnatally. As the authors acknowledge, Raptor downregulation alone cannot be effective in restoring mTOR signaling in therapy and must be considered in conjunction with modulating other targets of this signaling pathway. However, as the authors elegantly elucidate, a more mechanistic dissection of mTOR signaling, and identification of specific modulators, is much more likely to be an effective strategy than broad inhibition strategies such as rapamycin.

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Declaration of Conflicting Interests

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References

1. Crino PB. Focal brain malformations: seizures, signaling, sequencing. *Epilepsia*. 2009;50:38. doi:10.1111/J.1528-1167.2009.02289.X
2. Nellist M, Janssen B, Brook-Carter PT, et al. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell*. 1993;75(7):1305-1315. doi:10.1016/0092-8674(93)90618-Z
3. van Slegtenhorst M, de Hoogt R, Hermans C, et al. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science*. 1997;277(5327):805-808. doi:10.1126/SCIENCE.277.5327.805

4. Northrup H, Koenig MK, Pearson DA, Au KS. *Tuberous Sclerosis Complex*. GeneReviews®; 2021.
5. Moloney PB, Cavalleri GL, Delanty N. Epilepsy in the mTORopathies: opportunities for precision medicine. *Brain Commun*. 2021;3(4):fcab222. doi:10.1093/BRAINCOMMS/FCAB222
6. Luo C, Ye WR, Shi W, et al. Perfect match: mTOR inhibitors and tuberous sclerosis complex. *Orphanet J Rare Dis*. 2022;17(1):106. doi:10.1186/s13023-022-02266-0
7. Karalis V, Caval-Holme F, Bateup HS. Raptor downregulation rescues neuronal phenotypes in mouse models of tuberous sclerosis complex. *Nat Commun*. 2022;13(1):4665. doi:10.1038/S41467-022-31961-6
8. Tariq K, Cullen E, Getz SA, et al. Disruption of mTORC1 rescues neuronal overgrowth and synapse function dysregulated by *Pten* loss. *Cell Rep*. 2022;41(5):111574. doi:10.1016/J.CELREP.2022.111574
9. Chen CJ, Sgritta M, Mays J, et al. Therapeutic inhibition of mTORC2 rescues the behavioral and neurophysiological abnormalities associated with *Pten*-deficiency. *Nat Med*. 2019;25(11):1684-1690. doi:10.1038/s41591-019-0608-y