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"Oligodendrocytes Require mTOR to Maintain Myelin Integrity and Efficiently Remyelinate in the Adult Brain"

by
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ABSTRACT

In demyelinating diseases such as multiple sclerosis (MS), primary loss of myelin and subsequent neuronal degeneration throughout the central nervous system (CNS) impair patient functionality and decrease quality of life. While endogenous myelin repair by newly generated oligodendrocytes (OLs) occurs during early stages of MS, eventual failure to remyelinate leads to accumulation of pathological symptoms, including tremors, tingling, and partial paralysis. Therefore, it is critical to determine mechanisms to limit demyelination and promote remyelination.

We have previously shown that mTOR deletion from the OL lineage using ',3'-cyclic nucleotide 3' phosphodiesterase (Cnp)-Cre excision of mTOR floxed alleles (Cnp-mTOR cKO) during development results in hypomyelination of the spinal cord. Conversely, the brain displays normal myelin thickness however, myelin protein expression is still moderately reduced, suggesting that mTOR deletion may affect the formation of healthy myelin sheaths that can be maintained throughout life. Additionally, previous work has demonstrated that systemic pharmacological mTOR inhibition during cuprizone (CPZ) demyelination of the brain results in severely delayed remyelination, suggesting that mTOR signaling in OLs may promote remyelination of the brain. We therefore tested the hypothesis that mTOR signaling supports normal myelin maintenance, limits demyelination, and promotes remyelination in the adult CNS.

In order to examine myelin integrity over time, we examined Cnp-mTOR cKO and control littermate brains at 8 weeks of age, when myelin previously appeared normal by electron microscopy, and at 12 weeks of age. At 8 weeks of age, Cnp-mTOR cKO mice displayed significantly reduced expression of cholesterol biosynthesis enzymes and myelin proteins in the corpus callosum, as well as a trend towards increased apoptotic OLs. By one month later at 12 weeks of age, the mice with OL-specific deletion of mTOR displayed significantly reduced mature OL numbers and myelination in the corpus callosum, indicating an important role for mTOR in preventing endogenous demyelination. The sudden demyelination in early adulthood corresponded with severe axonal impairment when examined by compound action potential callosal slice recordings, highlighting the importance of myelin maintenance to support CNS function.

In order to determine whether the observed demyelination in adult Cnp-mTOR cKO brains was the result of impaired myelin maintenance, we acutely deleted mTOR from mature OLs in the adult brain using a tamoxifen-inducible Cre driven from the proteolipid protein (*Plp*) promoter to excise mTOR (Plp-mTOR cKO). Only long-term myelin maintenance was impaired in Plp-mTOR cKO brains, with thinner myelin observed in the aged callosum. However, because Plp-mTOR cKO brains did not entirely recapitulate the Cnp-mTOR cKO phenotype that shows short-term deficits in myelin maintenance, our data suggest that the loss of mTOR in developmental OLs results in changes that lead to sudden demyelination in early adulthood.

To determine whether mTOR signaling limits the extent of demyelination in the CPZ model, we administered CPZ via dietary intake over the course of 4 weeks. We found that CPZ-mediated demyelination is unaffected by the loss of mTOR in mature OLs. Plp-mTOR cKO mice displayed similar loss of OL lineage cells during demyelination, and a similar number of myelinated axons in the callosum at the peak of demyelination. These data suggest that mTOR signaling does not protect against CPZ-mediated demyelination of the brain, despite its role in myelin maintenance.

In order to examine the function of mTOR in OLs during remyelination, we used a tamoxifen-inducible Cre driven from the *Ng2* promoter to conditionally delete *mTOR* (Ng2-mTOR cKO) from adult oligodendrocyte precursor cells (OPCs) that give rise to new OLs responsible for remyelination. CPZ was administered via dietary intake over 6 weeks to induce demyelination, then removed to allow endogenous remyelination. Proliferation and differentiation of OPCs, as well as area of remyelination, were unaffected by cell autonomous loss of mTOR. However, mice lacking mTOR in adult OPCs had thinner myelin that recovered by late-stage repair, suggesting a delay in remyelination. Interestingly, we observed no effect on remyelination in lysophosphatidylcholine lesions in either the spinal cord or the brain, suggesting that mTOR deletion specifically affects remyelination after CPZ demyelination. *In vitro* experiments indicate that both mTOR inhibition and CPZ treatment in differentiating OLs impair mitochondrial function, suggesting an important role for mTOR signaling in promoting remyelination through supporting normal mitochondrial dynamics.

Taken together, our data support the conclusions that mTOR signaling is critical for short-term and long-term myelin maintenance in the adult brain, and promotes efficient remyelination of the brain after CPZ demyelination. These results may lead to future identification of therapeutic targets downstream of mTOR to support healthy myelin maintenance and promote remyelination.