mTOR promotes BBB breakdown in a model of Alzheimer's disease

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INTRODUCTION

Cerebral amyloid angiopathy (CAA) is characterized by fibrillary amyloid (Aβ) association with cerebrovascularity, which leads to impaired brain vascular function, and is present in 87% of people with Alzheimer's disease (AD). Previously, it has been shown that inhibition of mTOR by rapamycin prevents vascular leakage in 18-19 month old Tg2576 mice – a mouse model that mimics AD-associated CAA. This finding suggests that mTOR plays a role in regulating the integrity and permeability of the blood brain barrier (BBB). To further expand on this study, the abundance of tight junction proteins, zonula occludens 1 (ZO-1), occludin, and claudin-5 were examined using immunofluorescent confocal microscopy on frozen brain tissue sections of the same Tg2576 mice from the previous study.

Together, these studies demonstrate that attenuation of mTOR by rapamycin preserves BBB integrity by decreasing the amount of vascular Aβ accumulation, which also showed a reduced likelihood of cerebral microbleeds, reduced fibrinogen extravasation, and an increase in tight junction protein abundance in our transgenic Tg2576 mice. Therefore, these data suggest that mTOR is a key component in vascular Aβ accumulation, BBB breakdown, vascular dysfunction, and BBB permeability in the Tg2576 mice. Thus, the data suggests that using mTOR inhibitors such as rapamycin – an FDA approved drug, may be therapeutic in the pathogenesis of AD and other dementias with related cerebrovascular dysfunction.

METHODS

- 18-19 month old male and female transgenic/Alzheimer's disease (AD) Tg2576 mice and their nontransgenic/wildtype (WT) littermates were fed either rapamycin containing diet (140ppm) or Rapamycin control chow beginning at 4 months of age.
- Two-photon microscopy through a thinned skull cranial window was used to visualize cortical vasculature (i.e. Texas-red Dextran) and fibrillary amyloid (i.e. Methoxy-XO4, green) in NtG/WT and Tg/AD Tg2576 mice at 18-19 months of age.
- Brains were harvested after two-photon optical imaging, snap frozen on dry ice, and sectioned into 10μm thick slices on a cryostat for subsequent immunohistochemistry and immunofluorescent analysis.
- All images were measured in Fiji/ImageJ. Statistical analyses were performed in GraphPad Prism 7. Two-way ANOVAs were used to assess both genotype x treatment and group x amyloid effects, followed by Tukey's multiple comparison post hoc tests among all means.

CONCLUSIONS

- mTOR activity contributes to cerebrovascular amyloid pathogenesis (Figure 1).
- Chronic suppression of mTOR by rapamycin reduces cerebrovascular fibrillary amyloid accumulation (Figure 1), and preserves BBB integrity (Figure 2A-2C).
- The frequency of cerebral microbleeds associated with CAA are reduced by chronic mTOR inhibition (Figure 2B).
- Preservation of cerebrovascular integrity by mTOR inhibition in Alzheimer's disease, including maintenance of the BBB, could be mediated by increased expression of tight junction proteins ZO-1, occludin, and claudin-5 with rapamycin treatment (Figure 3).
- Inhibitors of mTOR may have therapeutic potential for diseases involving cerebrovascular pathology.

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