mTOR-driven Alterations in the Brain Microvasculature Proteome of Mice Modeling Alzheimer's Disease

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Introduction

Alzheimer’s disease (AD) is the leading cause of dementia. The development of effective therapies for AD remains limited due to an incomplete understanding of the molecular mechanisms of its onset and progression. Attenuation of mTOR, a key regulator of aging, by rapamycin treatment attenuates and reverses AD-like disease by restoring cerebral blood flow and cerebrovascular integrity1,2 and protecting the blood-brain barrier3.

To define the mechanisms by which mTOR attenuation delays or improves AD-like cerebrovascular dysfunction, we measured the abundance of proteins in the cerebrovascular proteome of AD model mice expressing human amyloid precursor protein with two FAD-associated mutations (hAPP J20). Expression of hAPP led to widespread changes in the brain microvascular proteome and some of these changes are reversed by rapamycin treatment. Among these are proteins relevant to Alzheimer’s disease and neurodegenerative disorders, which will be the focus of future studies.

Materials and Methods

Animals: Four groups of mice for mass spectrometry: hAPP J20 fed chow containing Eudragit (control diet; n=8) or rapamycin (2.24 mg/kg/day; n=5) and wild-type non-transgenic littermates on Eudragit (n=5) or rapamycin (n=7). Diets began at 6 months old (when cerebrovascular and cognitive deficits are already present) and ended 6 months later when brains were collected (12 months old). For Western blot analysis, some genotypes but treatment started at 10 months old and rapamycin treatment lasted 2 months before brain collection (12 months old).

Microvascular Isolation and Analysis: Brain microvascular fractions were isolated by dextran gradient centrifugation. Protein fractions were collected by colloid-form-methanol precipitation and analyzed by LC/MS-MS. Western blot analysis was performed using the Protein Simple Wes system.

Data Analysis: Mean abundance for individual proteins in brain microvascular fractions were compared between transgenic and non-transgenic control and rapamycin treated groups using GraphPad Prism. Ingenuity Pathway Analysis was performed only on proteins with a significant difference between non-transgenic and transgenic control treated mice (p<0.05).

References/Acknowledgements


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This study was conducted with approval of the Institutional Animal Care and Use Committee of UTSA/SCSA.

Results

Figure 1: Selected canonical pathways that were significantly changed in transgenic (Tg) mice. Proteins from these pathways were generally decreased in control treated Tg mice relative to control treated NTg mice. Rapamycin treatment generally increased proteins in the Tight Junction and Synaptic LTP signaling pathways but did not normalize them when compared to control treated NTg mice. Red and blue indicate increase and decrease in protein abundance, respectively.

Figure 2: Percent of proteins out of a total of 3,361 identified by mass spectrometry that are increased, decreased, or not significantly different (unchanged) in the comparison between transgenic mice treated with control diet (A) or rapamycin (B) and control treated non-transgenic mice. Proteins were considered significantly changed when p<0.05 in these comparisons.

Figure 3: Heterogeneous nuclear ribonucleoprotein A/B (hnRNPA), heterogeneous nuclear ribonucleoprotein D (hnRNPD), and nucleoporin 54 (Nup54) are decreased in the control treated hAPP/J20 mouse cerebrovascular proteome and restored to levels indistinguishable from those of NTg mice by rapamycin treatment in mass spectometry data. These trends were validated by Western blot analysis. Vacular ATPase assembly factor (VMA21) was increased by rapamycin treatment in mass spectometry data. Western blot analysis showed that VMA21 was decreased in control treated Tg mice and restored by rapamycin treatment. NTg, non-transgenic WT littermate mice; Tg, transgenic hAPP/J20 mice; *p<0.05 from one way ANOVA with multiple comparisons and Bonferroni correction applied to mean comparisons between NTg Control, Tg Control, and Tg Rapamycin.

Conclusion and Future Directions

Cerebrovascular dysfunction in hAPP/J20 mice3,4 is accompanied by profound changes in the brain microvascular proteome. Chronic mTOR attenuation by rapamycin specifically reverses some of these changes. Among these are several mRNA binding proteins that regulate mRNA stability and localization. Specifically, hnRNP D is known to promote degradation mRNA of cell cycle arrest and senescence associated secretory phenotype (SASP) genes. This is highly relevant to studies in our own lab and others showing the development of cellular senescence/SASP due to AD pathology. Nup54 is a member of the family of nucleoporins, proteins, some of which are altered in aging and neurodegeneration5. VMA21 is a chaperone essential to the assembly of the lysosomal vacuolar ATPase complex, which activates mTORC1 and plays a role in several neurodegenerative disorders. The validation of these findings by Western blot analysis in a separate cohort of mice with shorter treatment initiated at an older age shows that mTOR inhibition robustly reverses these changes both early and late after the onset of cerebrovascular and cognitive deficits. Subsequent genetic or pharmacological studies will determine the role of these proteins in the reversal of AD-like cerebrovascular deficits by mTOR attenuation.