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Analyses of the link between amyloid and tau pathology in an AD mouse model (3xTg-AD): Disease progression with increased levels of Abeta and tau peptides

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Introduction:

Alzheimer's disease (AD) is the most common age related form of dementia worldwide, and in the United States AD is the sixth leading cause of death. Pathological features of AD include the accumulation of extracellular amyloid plaques composed of aggregated amyloid-β (Aβ) peptide and intracellular neurofibrillary tangles consisting of phosphorylated tau protein. Mutations in the genes that encode precursor protein (APP), and presenilin 1 and 2 (PS1/PS2) have been shown to cause familial AD in humans. Studies provided evidence that Aβ accumulation may initiate phosphorylation of tau protein, via Ras/MEK/Extracellular Signal-regulated Kinase (Erk) signaling cascade, activation of the mitogen-activated protein kinase (p38 MAPK), Cyclin dependent kinase (CDK5) and/or glycogen synthase kinase-3β (GSK3β). We studied distribution of Aβ and tau oligomers, and Erk activity in different brain lysates fractions from different age groups of a triple transgenic mouse model (3xTg-AD)[1,2,3] and wild-type mice.

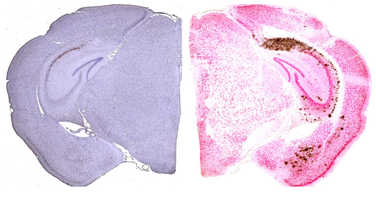

Figure 1: Tau fibrils (left) and Aβ plaques (right) in the hippocampus of a 24-month-old 3xTg-AD female.

Hypothesis:

A reduction of Aβ peptide leads to a reduction of tau peptide.

Methods:

Brain lysates of 4-, 6-, 12-, and 20-month-old 3xTg-AD and wild-type mice were prepared via a 4-step extraction protocol in TBS (soluble), TBS-T, SDS, and formic acid[4,5] (Figure 2). DNA Aβ42 peptide vaccine was administered via gene gun. Aβ and Tau concentrations and Tau phosphorylation levels were monitored by Dot Blot, Semidenaturing detergent agarose gel electrophoresis (SDD-AGE), and ELISA using anti-Abeta and anti-Tau antibodies. Erk1/2 levels were monitored by Western blot using monoclonal antibodies

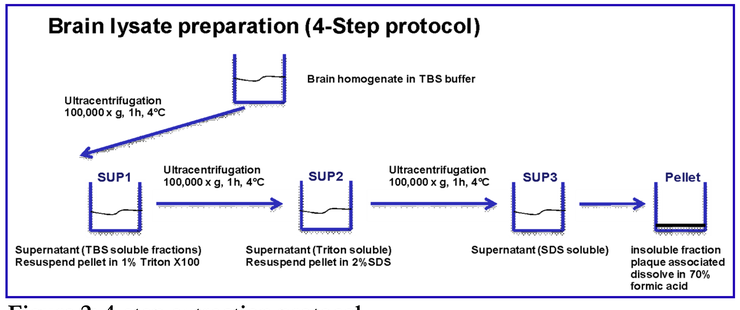

Figure 2: 4-step extraction protocol

Results:

As an initial screening to visualize the proteins in each fraction, a Coomassie brilliant blue stain was preformed. Each fraction showed the presence of high and similar amounts of total protein except the insoluble formic acid fractions in which we could not recover the proteins.

formic acid fraction (Figure 3, left). Quantitative data was gathered by performing ELISA assays to determine the difference in Tau and Aβ peptides in each fraction. A trend of increasing Tau protein and Aβ was found as a function of decreasing solubility.

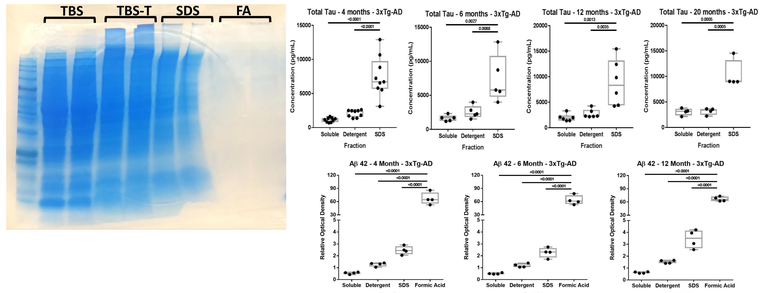

Figure 3: Coomassie brilliant blue dye (left) used to see total protein applied on the gels. ELISA showing increasing amounts of tau (upper panel) and Aβ (lower panel) in brain lysate fractions.

Quantitative data was gathered by performing ELISA assays to determine the increasing amount of tau and Aβ peptides due to age progression. Overall, a trend of increasing Tau protein and Aβ was found.

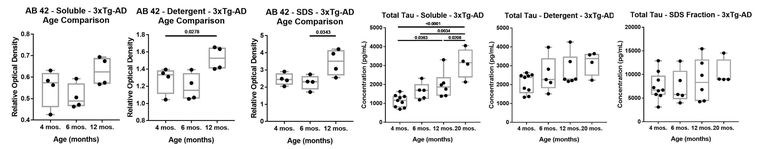

Figure 4: ELISA data showing Tau protein and Aβ levels due to age

To compare total tau levels in different ages of 3xTg-AD mice, a Dot Blot was performed on the soluble fraction and an increase trend was observed due to age. In addition, an SDD-AGE revealed difference when comparing AD transgenic and wild-type mice.

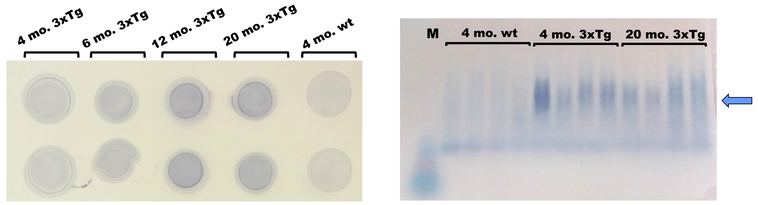

Figure 5: Dot Blot of 4-, 6-, 12- and 20-month-old 3xTg-AD mice using biotinylated-HT7 antibody showing total tau (left). Agarose gel electrophoresis comparing wild-type and 3xTg-AD mice. Tau aggregation indicated by the arrow.

To compare total Erk expression and activity in different aged 3xTg-AD mice, a Western Blot was performed on the soluble fraction and gray values were subsequently produced via ImageJ. Interestingly, the gel showed a positive trend with a drop in Erk activity in 12-month-old 3xTg-Ad mice despite relatively similar total Erk expression. This finding may indicate the shunting of Aβ peptide to plaque formation instead of kinase activation.

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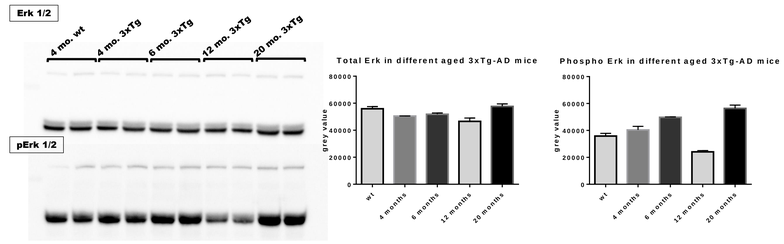

Figure 6: Western Blot of 4-, 6, 12-, and 20-month-old 3xTg-AD detecting total Erk 1/2 (top, left) and active phosphorylated Erk 1/2 (bottom, left). Mean gray values of bands on Western blots (right) obtained via ImageJ.

To visual the possible effects of increased Erk activity, Dot Blot was performed using an antibody specific to phosphorylated tau.

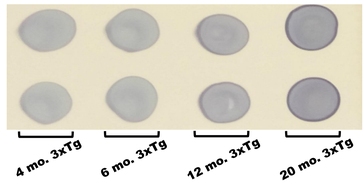

Figure 8: Phosphorylated tau detected in 4-, 6-, 12- and 20-month-old 3xTg-AD mice using antibody T231 specific for tau phoshorylated at residue T231

DNA Aβ42 immunized mice, which produce antibodies specific for oligomeric Aβ, were assessed for total Erk 1/2 and phospho Erk 1/2 (activated) using a Western Blot.

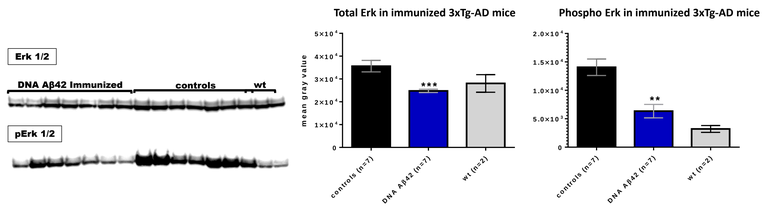

Figure 7: Western Blot of DNA Aβ42 immunized and nonimmunized 3xTg-AD mice detecting Erk 1/2 (top, left) and activate phosphorylated Erk 1/2 (bottom, left). Mean gray values of bands on Western blots (right) obtained via ImageJ.

Conclusions & Future Directions:

The 3xTg-AD mouse model provides a good model to study pathologies and possible treatments for human Alzheimer's disease. Abeta 42 and tau peptide increase due to age in this model. A link between the amyloid is likely found in the wide spectrum of cellular kinases which are upregulated due to Aβ in Alzheimer's disease. Therefore immunization against Aβ and generation of anti-Aβ42 antibody will indirectly reduce tau pathology[6].

References: [1]Oddo et al Neuron 39(3):409-421, 2003, [2]Oddo et al J Biol Chem. 281(3):1599-1604, 2006, [3]Oddo et al J Biol Chem. 281(51):39413-39423, 2006, [4]Kawarabayashi et al J Neurosci.21(2): 372- 381, 2001, [5]Sherman & Lesné Methods Mol Biol. 670:45-56, 2011, [6]Rosenberg, Fu, & Lambracht-Washington submitted 2017,

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