Designing a Model to Explore Tau's Unfolded Protein Response

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Terms to Know

Alzheimer's Disease (AD): a progressive neurodegenerative disease common in elderly patients that destroys important mental functions, including memory, largely due to neuronal loss.

Tau: a microtubule-associated protein that builds up in the brains of AD patients and create neurofibrillary tangles that lead to the neurotoxicity associated with AD.

Tau Pathology: The accumulation of tau is due to it being abnormally hyperphosphorylated leading to its dysfunction and causing toxicity in cells leading to neuronal loss.

ER Stress: a cell becomes stressed in response to pathophysiological conditions that lead to accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER).

Unfolded Protein Response (UPR): a stress response by the ER in response to unfolded or misfolded proteins to attempt to salvage these proteins by degrading misfolded proteins and by upregulating chaperones involved in protein folding, If the protein cannot be salvaged then the cell triggers apoptosis using a variety of different pathways.

Neurotoxicity: Damage to the brain or peripheral nervous system caused by toxic substances (i.e. tau tangles) that alter the activity of the nervous system leading to disrupted cells or cell death.

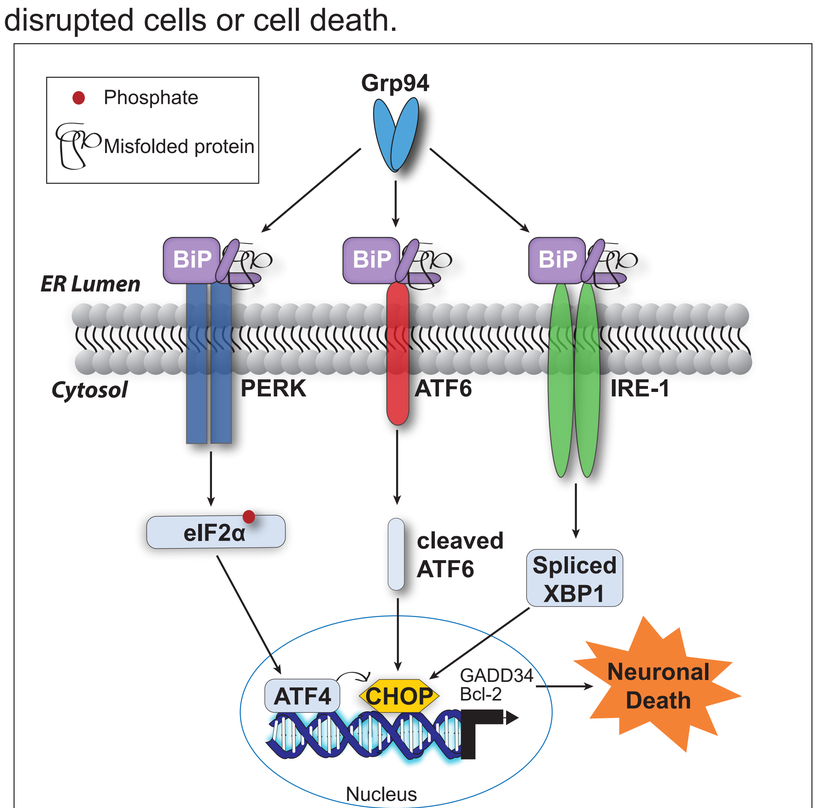

Figure 1. Different ER stress pathways can lead to cell death. UPR is regulated by three master regulators, PERK, ATF6, and IRE-1. Upon sensing unfolded proteins, BiP dissociates and one of these master regulators are activated and each pathway ultimately leads to neuronal cell death if the protein cannot be salvaged.

Background

The microtubule-associated protein tau pathogenically accumulates in Alzheimer's disease (AD) causing neuronal loss and cognitive dysfunction. The number of dead neurons in the AD brain exceeds the number of tau tangles. This suggests that there is likely a secondary route of cell death brought on by the simultaneous accumulation of soluble tau species. Recent work suggests that chronic unfolded protein response (UPR) activation could be a pathway by which these soluble tau species contribute to neuronal death (Figure 1). In younger tissues, the UPR is largely cyto-protective, promoting protein re-folding, anti-oxidant production, and other beneficial effects. However, with age, the components of UPR necessary for these helpful activities are reduced (**Figure 2**), giving way to more harmful UPR pathways that promote apoptosis and cell death. In this way, the aging cell becomes primed for death when there is a problem with the ER protein quality control machinery. Triggering UPR in aged tissue, especially chronic activation, may be the cause of cell death in tauopathies like AD. The purpose of this research is to design a cell model in which to study tau-mediated toxicity to better understand the role of ER stress in this process.

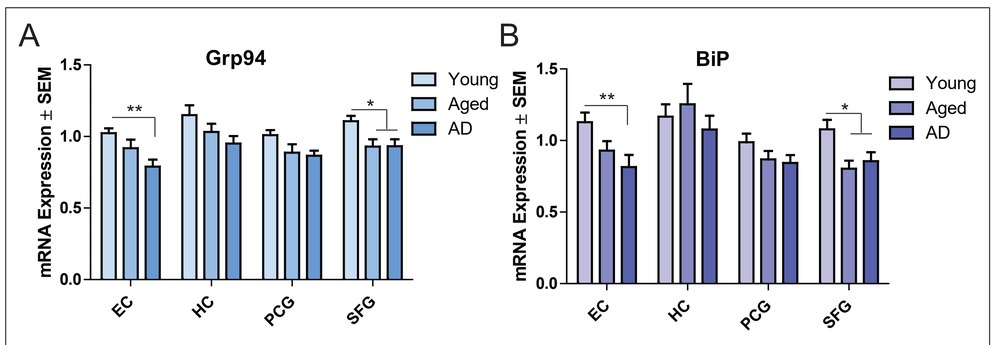

Figure 2. Grp94 and Bip expression decreases with age and AD. (**A**) Grp94 and (**B**) Bip mRNA expression levels in the entorhinal cortex (EC), hippocampus (HC), posterior central gyrus (PCG), and superior frontal gyrus (SFG) of young (20-59yrs), aged (69-99yrs) and AD diagnosed (69-99yrs) samples.

Methods

P301L iHek cells (a cell line that expression tet-regulatable P301L tau) were split into two flasks. One flask was induced with 1µg/mL tetracycline (tet), the other remained as a control. The cell cultures grew until the tet-induced cells died while the control cells remained alive. Marking the length of time it took for the cells to die, the cells were lysed 2 days prior to the determined death date and evaluated via Western blot analysis checking levels of ER stress markers including, CHOP, cleaved PARP, BIP, p62, IRE1α. A visualization of this cell model can be seen in **Figure 3.**

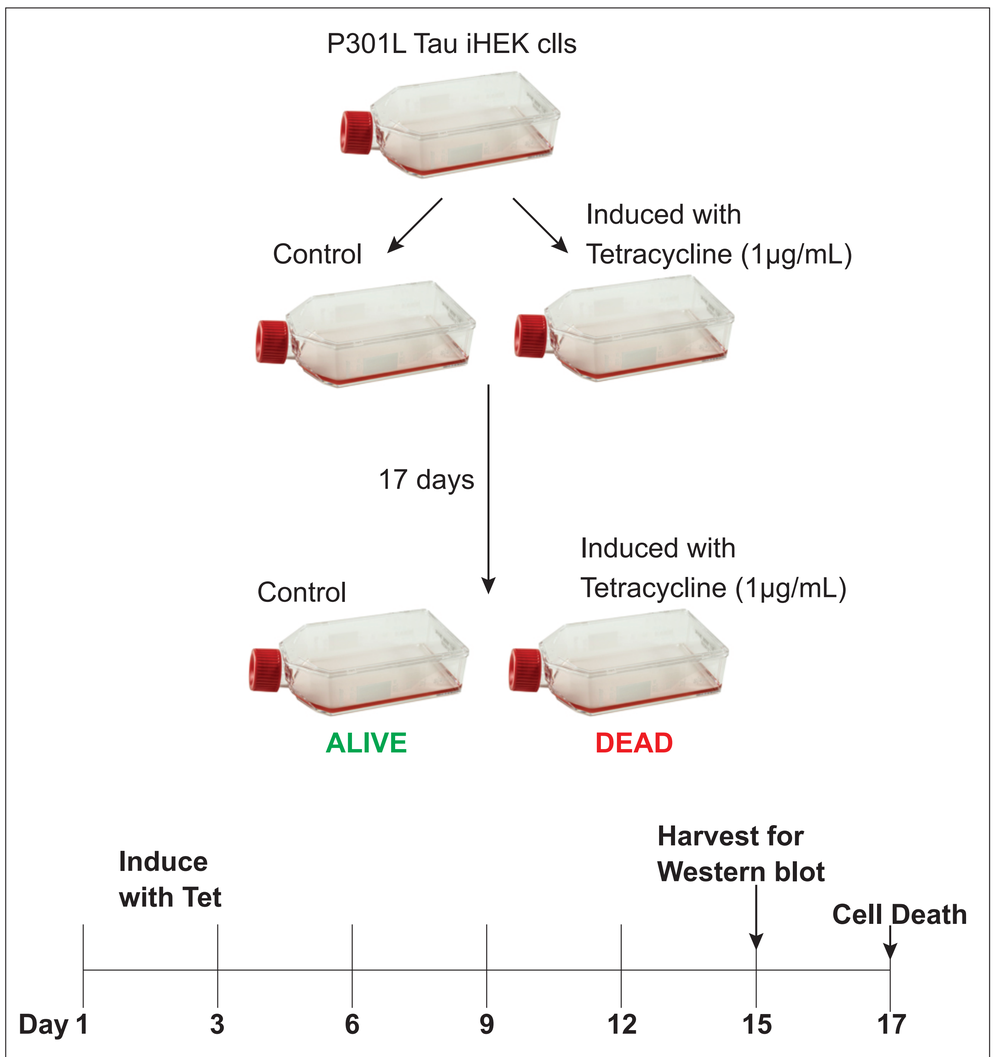

Figure 3. Visualization of tau-induced cell death model.

Results

Cell death occurred after 17 days of tetracycline induction and tau accumulation. Analysis of ER stress markers via western blot demonstrated that the cells induced for 15 days had much higher levels ER stress-associated proteins, such as CHOP, IRE1α, PERK and Hsp70, and a decreased amount of BiP in comparison to the control cells. The comparison of these protein levels in the control and tet-induced cells can be visualized in **Figure 4**.

Conclusion

The increased levels of ER stress chaperones and protein-folding regulators in the tet-induced cells indicate that tau accumulation does increase ER stress leading to neuronal cell death via the three UPR pathways shown in Figure 3. Therefore, a successful working model to explore the role of tau in UPR was successfully generated.

Acknowledgements

This work was funded by the VA, NIMH, NINDS, and the Alzheimer's Association.

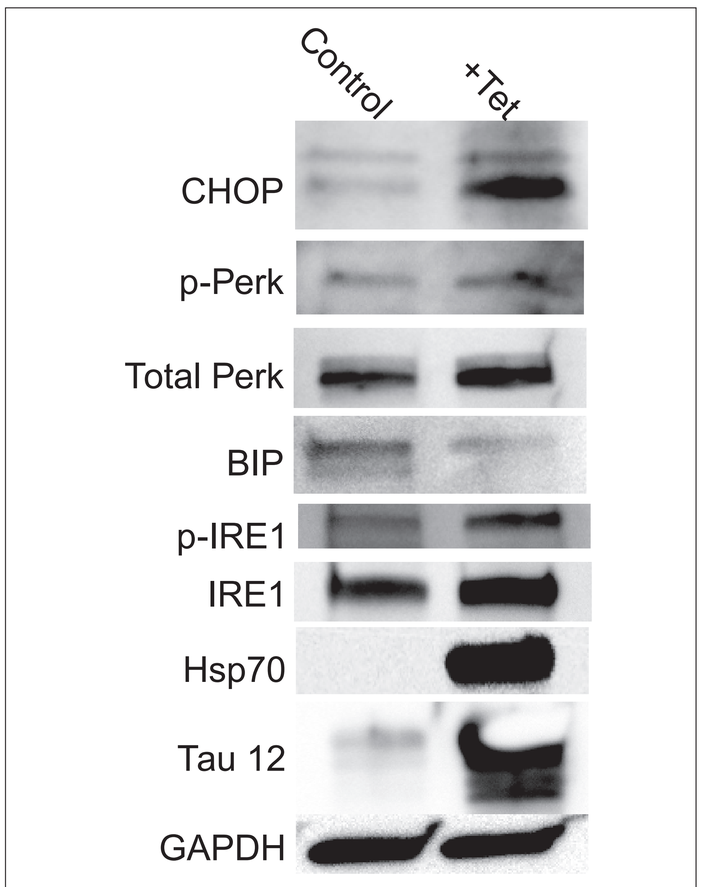

Figure 4. Tau induces UPR right before cell death. Markers of ER stress are induced by tau expression (15 days) in iHekP301L cells.

Continued Research

Further mouse studies were performed with wild type (WT) mice and tau-induced transgenic mice were compared. **Figure 5** and **Figure 6** show how the aged transgenic mice, expressed increased levels of UPR genes and ER stress-associated proteins.

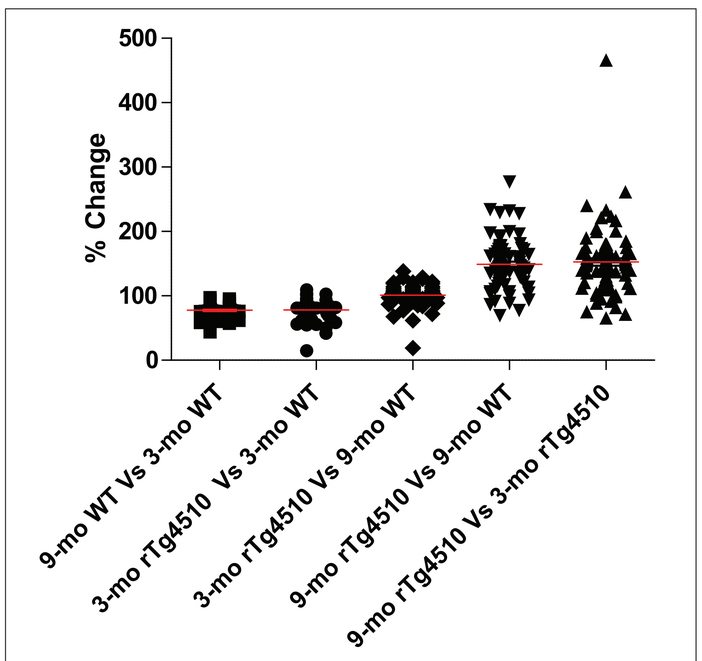

Figure 5. Net change in expression of 84 UPR and UPR related genes in young (3-month) and aged (9-month) rTg4510 tau transgenic (Tau) and wild-type (WT) mice.

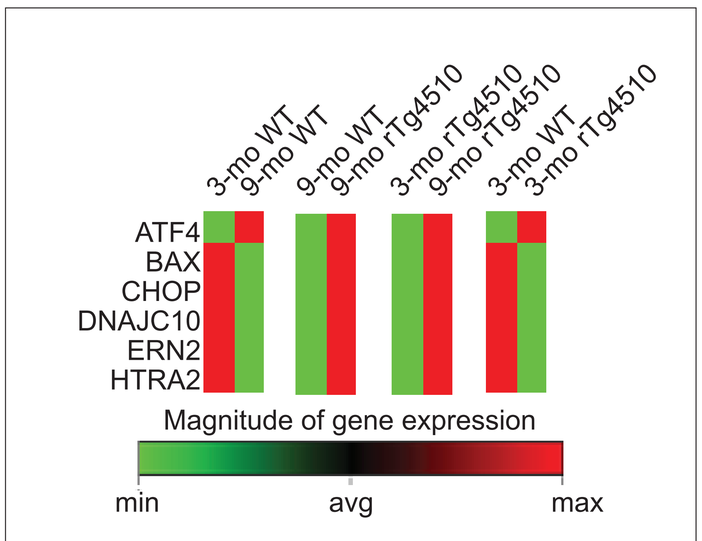

Figure 6. Magnitude of gene expression change in ATF4, BAX, CHOP, DNAJC10, Ern2, and Htra2 in 3-month (young) and 9-month (aged) wild-type (WT) and rTg4510 Tau mice.