Mouse Expression of ANKHD1 and Its Protective Role in Human Tauopathies

Chunlai Wu[1], Nathan Le[1], Elizabeth Reilly Helm[2], Lauren E. Meyer[2] and Xiaolin Tian[1*]

[1]LSUHSC Neuroscience Center of Excellence, Department of Cell Biology and Anatomy

[2]LSUHSC School of Medicine

*Contact info: xtian@lsuhsc.edu

Introduction

- **Tauopathies are neurodegenerative diseases characterized by abnormal filamentous tau protein accumulation**
- **Neurofibrillary tangles (NFT) composed of tau filaments and Aβ deposits are hallmarks of Alzheimer's Disease (AD).**
- **The mutated tau proteins are hyperphosphorylated and aggregate in the hippocampus.**
- **The PS19 transgenic mouse model that expresses the P301S mutant Tau protein in neurons show increased gliosis and neuronal dysfunction and NFT formation at 6 months of age.**
- **In *Drosophila*, the expression of Mask, an Ankyrin repeat and KH domain containing protein, suppresses the neuronal degeneration induced by Tau.**
- **This study examines the ability of ANKHD1, the human homolog of Mask, to modulate neuropathology and cognitive functions in the PS19 mouse model.**

Figure 1

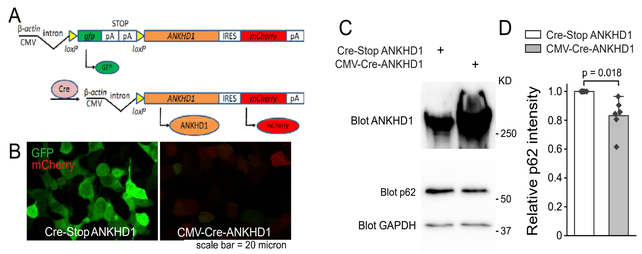

Figure 1. Generating Cre-inducible ANKHD1 transgenic mouse. (**A**) Schematics of the DNA construct for generating Cre-inducible ANKHD1 mouse. (**B**) Representative confocal images of GFP and mCherry auto-fluorescence from HEK293 cells transfect with Cre-Stop ANKHD1 with (right) or without (left) CMV-Cre plasmid. (**C**) Western blots of cell lysates from HEK293 cells shown in (B) with anti-ANKHD1, p62 and GAPDH (loading control). (**D**) Quantification of p62 intensity normalized to GAPDH.

Figure 2

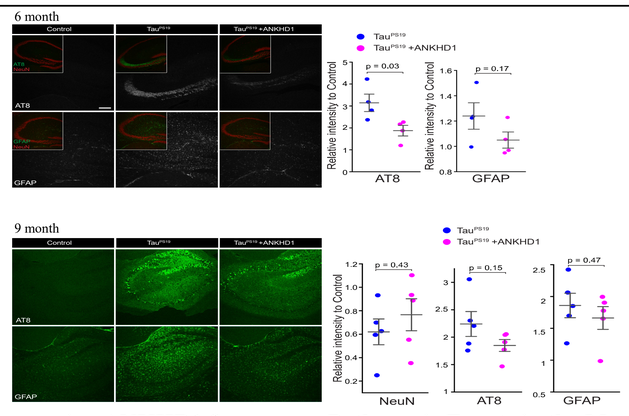

Figure 2. Expressing ANKHD1 Suppresses Pathogenic Events in the Mouse Brain. Comparison of Tau hyperphosphorylation (AT8) and gliosis (GFAP) at 6 months and 9 months of age in TauPS19 and TauPS19 + ANKHD1 mouse lines. At 6 months, there was a significant decrease in intensity of the AT8 signal in hippocampus in the TauPS19 + ANKHD1 mouse brain.

Figure 3

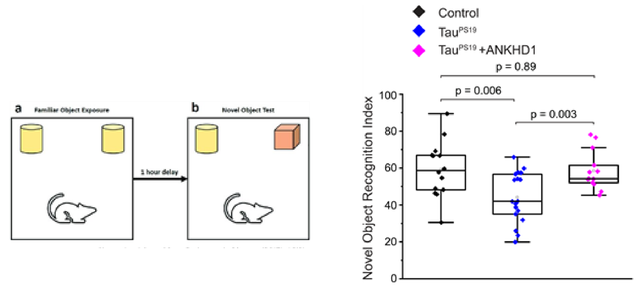

Figure 3. Expressing ANKHD1 Partially Restores Cognitive Functions in PS19 mice. Mouse lines expressing Cre-inducible ANKHD1 and TauPS19 showed increased novel object recognition index at 9 months of age, indicating improved learning and memory.

Results

- **Quantifications and comparisons of markers for neuropathology between the control (PS19) mice and the PS19-ANKHD1 mice showed a statistically significant ($p<0.05$) reduction of hyperphosphorylated Tau at 6 months of age in the PS19-ANKHD1 mice.**
- **At 9 months, there is a statistically significant ($p<0.05$) increase in the novel object recognition index in the female PS19-ANKHD1 mice compared to the control mice.**

Conclusion

- **ANKDH1, a human homolog of the *Drosophila* Mask gene, is indicated to suppress pathogenic Tau protein hyperphosphorylation and accumulation seen in the mouse model for the Tau related Alzheimer's dementia.**
- **The expression of ANKHD1 in transgenic mouse lines suppresses the hyperphosphorylation of Tau and the gliosis associated with tauopathies in the mouse brain.**
- **There is evidence that expression of ANKDH1 can partially restore cognitive functions.**

Methods

- **A Cre-inducible ANKHD1-expressing transgenic mouse line was generated, allowing neuronal specific expression of ANKHD1 in a Cre-dependent manner.**
- **This transgenic line was then combined with the PS19 and the CamK-Cre transgenic mice to achieve the co-expression of ANKHD1 and TauP301S mutant protein in the mouse brain.**
- **Imaging, molecular and histopathological analysis was performed in cultured cells and mouse brain slices.**
- **Whole animal behavior tests were performed to determine the ability of ANKHD1 to modify neurodegeneration in the mouse brain.**

References

- Hovakimyan, A., Antonyan, T., Shabestari, S. K., Svystun, O., Chailyan, G., Coburn, M. A., Carlen-Jones, W., Petrushina, I., Chadarevian, J. P., Zagorski, K., Petrovsky, N., Cribbs, D. H., Agadjanyan, M. G., Ghochikyan, A., & Davtyan, H. (2019). A MultiTEP platform-based epitope vaccine targeting the phosphatase activating domain (PAD) of tau: Therapeutic efficacy in PS19 mice. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-51809-2
- Takeuchi, H., Iba, M., Inoue, H., Higuchi, M., Takao, K., Tsukita, K., Karatsu, Y., Iwamoto, Y., Miyakawa, T., Suhara, T., Trojanowski, J. Q., Lee, V. M., & Takahashi, R. (2011). P301s mutant human tau transgenic mice manifest early symptoms of human tauopathies with dementia and altered sensorimotor gating. *PLoS ONE*, *6*(6). https://doi.org/10.1371/journal.pone.0021050
- Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S.-M., Iwata, N., Saido, T. C., Maeda, J., Suhara, T., Trojanowski, J. Q., & Lee, V. M.-Y. (2007). Synapse loss and microglial activation precede tangles in a p301s tauopathy mouse model. *Neuron*, *54*(2), 343–344. https://doi.org/10.1016/j.neuron.2007.03.022