Assessing The Contribution Of Individual Binding Domains In ScFv-Tau Interactions

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Tauopathies are neurodegenerative disorders defined by the accumulation of misfolded tau protein in the brain, such as Alzheimer's disease, which have no official treatment. Intrabodies, or scFvs (single chain variable fragments), are antibody fragments able to be engineered and delivered as genes to target antigens. ScFvs contain variable heavy (VH) and variable light (VL) chains, but it is unknown which one contributes more to binding. The aim of this research is to find out which, if either, domain of the scFvs dominates in tau binding, and use that to improve a weaker intrabody (scFv-2) by introducing a mutation. Pilot studies will be performed to see how the whole intrabodies degrade tau, and then the single-domain anti-Tau intrabodies will be cloned to create the 6 2-chain fragments of intrabodies 1, 2, and 4. Cells will go through Tau degradation with their separate chains, and data will be collected with imaging and western blots. To improve the function of scFv-2, the procedure will be run again with a mutation. The results will be compared in their ability to degrade tau against prior trials and controls to see if there is any decrease in fluorescent Tau, which corresponds to improvement in binding function.

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