

Final Report

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Title of Project: Generation of phosphorylated tau-specific intrabodies for the treatment of tauopathies

Tauopathies are a group of diseases caused by an aggregation of phosphosphorylated protein tau within neurons, and includes diseases such as Alzheimer's disease (AD) and frontotemporal dementia (FTD), two of the most common forms of dementia. Molecules demonstrated to prevent tau aggregation and promote its clearance from the brain are being actively pursued as a treatment for this group of diseases. Of such molecules, antibodies are common therapeutic agents that can specifically bind to proteins involved in disease and help neutralize them. This project aims to generate novel tau-specific antibodies and engineer these antibodies into functional intrabodies (intracellular antibodies) to target tau intracellularly, where it is predominantly localized. This process requires cloning the variable heavy and variable light chain of the antibody (the regions involved in antigen binding) and joining these domains with a flexible linker to form a single chain variable fragment (scFv). Delivery of the scFv gene to a cell then allows the scFv to be expressed as an intrabody within the cell.

Since beginning this project multiple novel tau-specific antibodies against different tau species implicated in disease have been generated. The original anti-tau antibodies isolated from an antibody library were demonstrated to bind with high affinity to the tau peptide they were selected against but their binding to full-length tau, however, was below optimal for a successful immunotherapeutic. The antibody libraries were therefore re-panned against full-length tau and a new scFv, RNT, was identified and demonstrated to bind with high affinity to both phosphorylated and non-phosphorylated tau. In addition, mice were also immunized with tau peptides encompassing regions of the protein redemonstrated to be critical in disease, which yielded two novel monoclonal antibodies, RN2N and RN235. RN2N is specific for an isoform of tau (2N tau), which has been shown by our lab to interact with proteins implicated in neurodegeneration, and therefore may be an isoform which enhances the disease progression. RN235 on the other hand, binds specifically to tau phosphorylated at Serine235, a phospho-epitope that is identified early in human disease. To our knowledge, we are the only lab to have monoclonal antibodies against these two tau epitopes.

The scFvs were cloned from their hybridoma's and expressed recombinantly for *in vitro* and *in vivo* characterization. Incubation of the scFvs with human tau *in vitro* demonstrated that the scFvs, through their binding to tau, were successfully inhibited the aggregation of tau into a potentially neurotoxic species. To investigate this *in vivo*, transgenic mice, which express mutant human tau and recapitulate the tau pathology observed in AD and FTD were immunized passively once a week with RN2N scFv in the absence and presence of scanning ultrasound (SUS). Scanning ultrasound is a non-invasive technique, which temporarily opens the blood brain barrier to allow therapeutic molecules, which cannot normally cross the blood barrier, such as antibodies, into the brain. We observed that SUS not only enhanced delivery of RN2N to the brain, but it also facilitated neuronal uptake of the scFv (Figure 1). After a month of treatment, the mice that received both the RN2N scFv and SUS demonstrated a significant decrease in the level of disease-associated tau in the brain compared to the RN2N scFv only group (Figure 2). Furthermore, these mice demonstrate an improvement in cognition, suggesting that the prevention of abnormal tau accumulation improves cognition in the transgenic mice. This work is currently ongoing and is expected be completed and submitted for publication in October of this year.

A limitation of passive immunization is that it requires regular immunizations of the antibody for the duration of patient's life and is estimated to cost \$25,000US per patient per annum. Gene therapy delivery of an scFv gene, however, would require a single immunization, thereby cutting the cost of treatment significantly. To investigate whether the scFvs are capable of being delivered as genes, neuronal cells were transfected *in vitro* with scFv plasmid DNA, and intrabody expression was investigated. The expression of the original anti-tau scFvs was very low suggesting that the antibodies required optimization to increase their stability within the cell. Using antibody engineering, stable intracellular expression was achieved by increasing the negative charge of the scFvs (Figure 3). Furthermore, the anti-tau scFvs were shown to localize with tau and enhance the clearance of tau from the cell. For *in vivo* testing, viral gene therapy is the most common method of gene delivery for widespread and long-term gene delivery. In collaboration with Assoc. Prof. Matthias Klugmann from the School of Medical Sciences at the University of New South Wales, the anti-tau scFvs are currently being cloned and packaged into adeno-associated virus (AAV). AAV has no etiologic association with any known disease and has been successfully used to establish efficient and long-term gene expression *in vivo* in a variety of tissues without significant cellular immune responses or toxicity. Furthermore, gene therapy using AAV has been used in over 100 clinical trials. The recently identified AAVRh20 serotype isolated from non-human primates will be used as it demonstrates wide transduction of the brain, long-term neuronal gene expression (years in mice) and can efficiently cross the blood brain barrier. Tau transgenic mice that express mutated human tau and recapitulate the tau pathology observed in human disease will be immunized with the scFvs_AAV genes and tau aggregation and mouse cognition will be assessed.

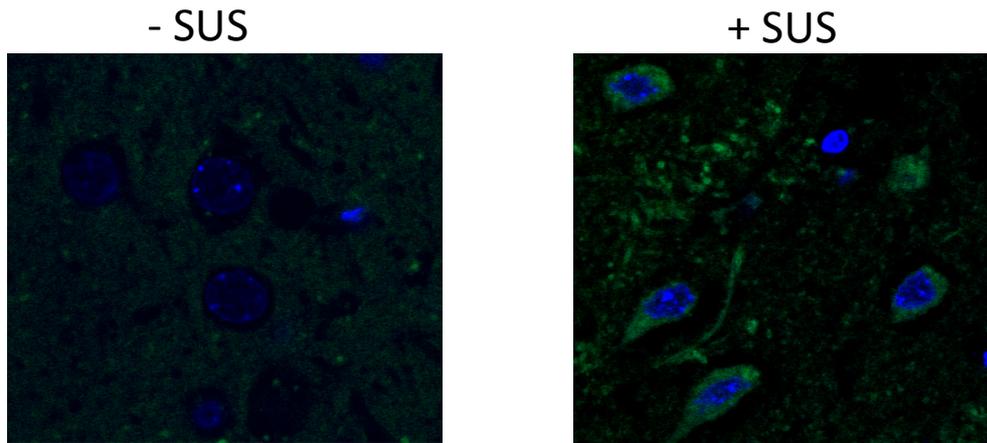


Figure 1. RN2N scFv delivery in tau transgenic mice without scanning ultrasound (left) and with scanning ultrasound (SUS) (right). RN2N scFv (green) is observed in the neurons only after SUS treatment.

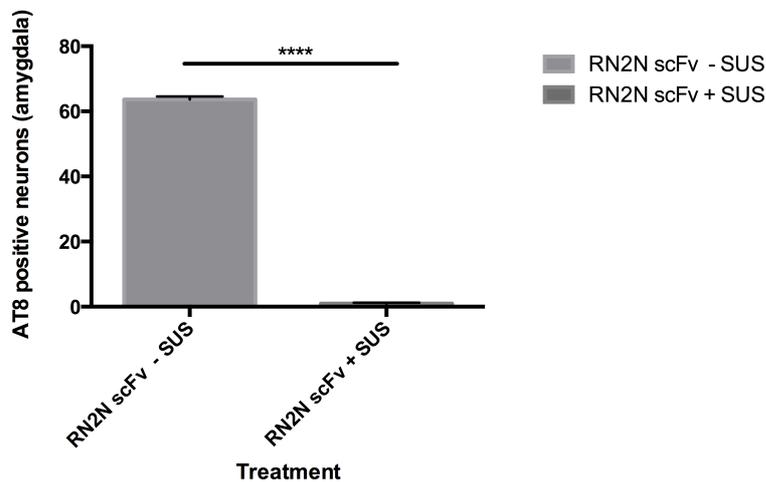


Figure 2. scanning ultrasound delivery of RN2N scFv once a week for 4 weeks significantly reduces the number of cells positive for disease-associated tau

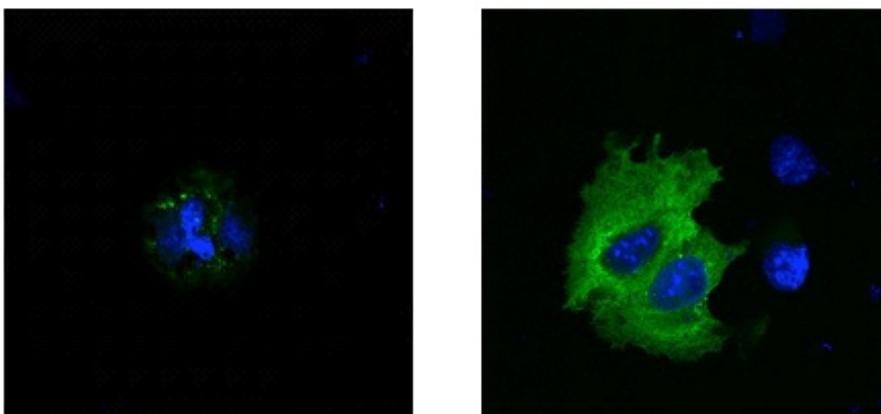


Figure 3. Increasing the solubility of the scFvs by decreasing their charge improves expression within the cell (green). (Left) non-optimised scFv (right) optimised scFv

Hypothesis vs Findings

The results from both our *in vitro* and *in vivo* work are in line with our overall hypothesis, which states that tau-specific intrabodies (intracellular antibodies) will be effective in clearing tau, reducing its aggregation and preventing the progression of disease pathology in transgenic mice.

Unanswered Questions

We look forward to analysing the results generated from the AAV delivered scFv genes in order to determine if tau-specific antibody gene therapy is a successful therapeutic strategy for the treatment of diseases caused by tau aggregation.

What these research outcomes mean

This project has generated three novel anti-tau antibodies, all of which have commercial applicability for both research and therapeutic use. Furthermore, we have demonstrated for the first time intracellular expression of an anti-tau antibody and antibody mediated clearance of intracellular tau. Further testing in mice will determine if these antibodies, delivered as genes or proteins, can progress to clinical trials.