Tau Research Tools

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Introduction

Tau is a target of interest in Alzheimer's Disease, particularly since amyloid-beta-focused therapeutics have repeatedly failed in clinical trials. New research tools, such as pre-formed fibrils (PFFs) allow for the study of tau aggregation and pathology in vitro and in vivo. Here we list and examine fibrils, filaments, and other useful tools for tau research.

Types of Tau Fibrils and Filaments

There are six isoforms of tau expressed in the human brain. The longest isoform of tau consists of four repeat domains (R) and two amino acid inserts (N), and is 441 amino acids long (Tau441, 2N4R). K18 is a truncated fragment that consists of only the four repeat domains (4R). dGAE is a truncated fragment that consists of amino acids 297-391 and fibrillizes in the absence of templates or additives.

P301S, P301L, C322A and ΔK280 are all mutations that enhance aggregation. Tau can aggregate into large fibrils and filaments that can seed further aggregation of monomers in vitro and in vivo. 2N4R P301S PFFs (SPR-329) and K18 P301L PFFs (SPR-330) were fibrillized using heparin (Figure 1). dGAE PFFs (SPR-461 and SPR-462) and K18 ΔK280 PFFs (SPR-477) (Figure 2) were fibrillized without scaffolds and K18 ΔK280 PFFs (SPR-477) can form paired helical filaments (PHFs) independently. 2N4R P301S soluble paired helical filaments (SPR-463) (Figure 3) were made using a linear anionic scaffold which is then removed.

Figure 1. TEM of 2N4R P301S PFFs (SPR-329) (L) and K18 P301L PFFs (SPR-330) (R)

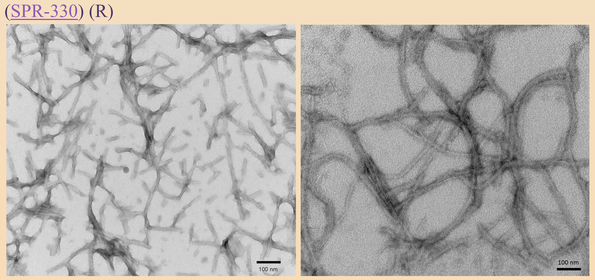


Figure 2. TEM of dGAE WT PFFs (SPR-461) (L), K18 ΔK280 PFFs (SPR-477) (R)

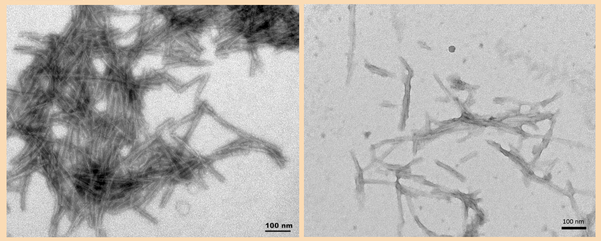
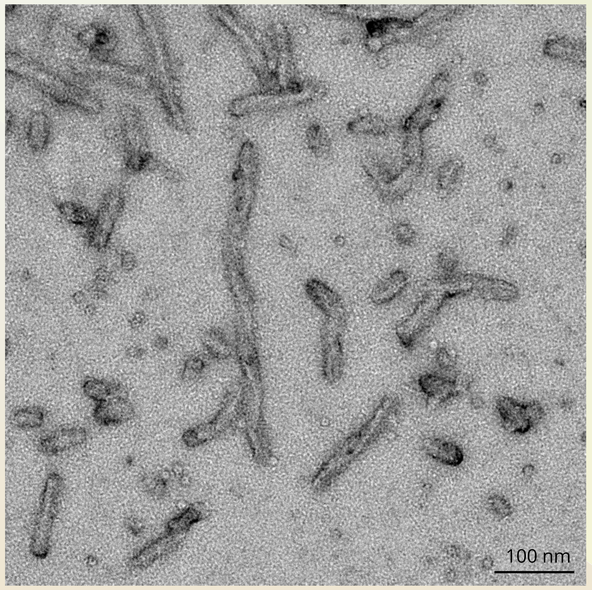


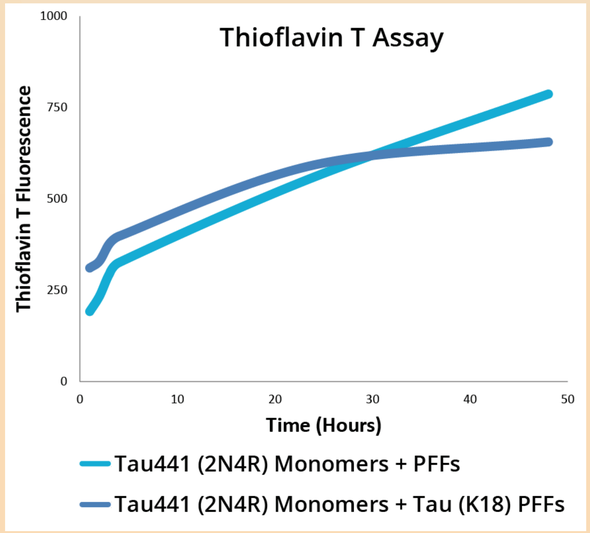
Figure 3. TEM of 2N4R P301S Paired Helical Filaments (SPR-463)



Tau Aggregation

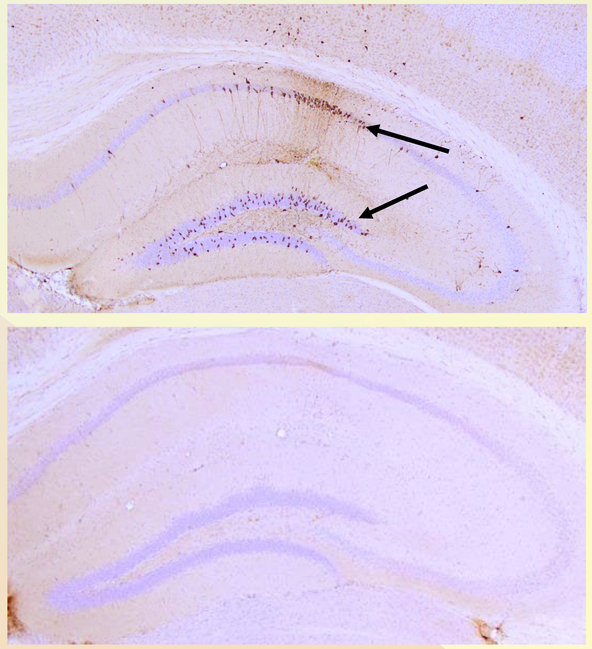
Tau aggregation into paired helical filaments and neurofibrillary tangles is a hallmark of tauopathies such as Alzheimer's Disease. Tau PFFs can seed the aggregation of tau monomers as seen in a thioflavin T fluorescence assay. Thioflavin T is a fluorescent dye that binds to beta-sheet-rich structures, such as those in tau fibrils.

Figure 4.Thioflavin T Fluorescence Assay for 2N4R P301S PFFs (SPR-329) and K18 P301L PFFs (SPR-330) combined with 2N4R P301S monomers (SPR-327)



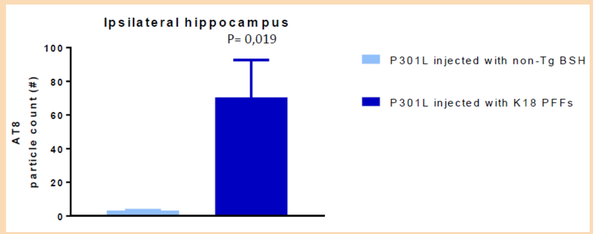
Tau PFFs can not only induce aggregation of tau monomers in solution, but they can also induce aggregation of tau in vivo. K18 P301L PFFs (SPR-330) were injected into P301L mouse brains where they seeded tau aggregation in the hippocampus within nine weeks. This was detected using pSer202/pThr205 tau antibody AT8 (Figure 5).

Figure 5. IHC analysis with AT8 of P301L mouse hippocampus after injection with P301L K18 PFFs (SPR-330) (top) and brainstem homogenate (BSH) of non-Tg mice (bottom). AT8 (pSer202/pThr205) tau antibody shows tangle-like inclusions in PFF-injected mice (top) but not in negative control (bottom)



The number of AT8-positive inclusions is significantly higher in mice injected with K18 PFFs than in mice injected with BSH of non-Tg mice (Figure 6).

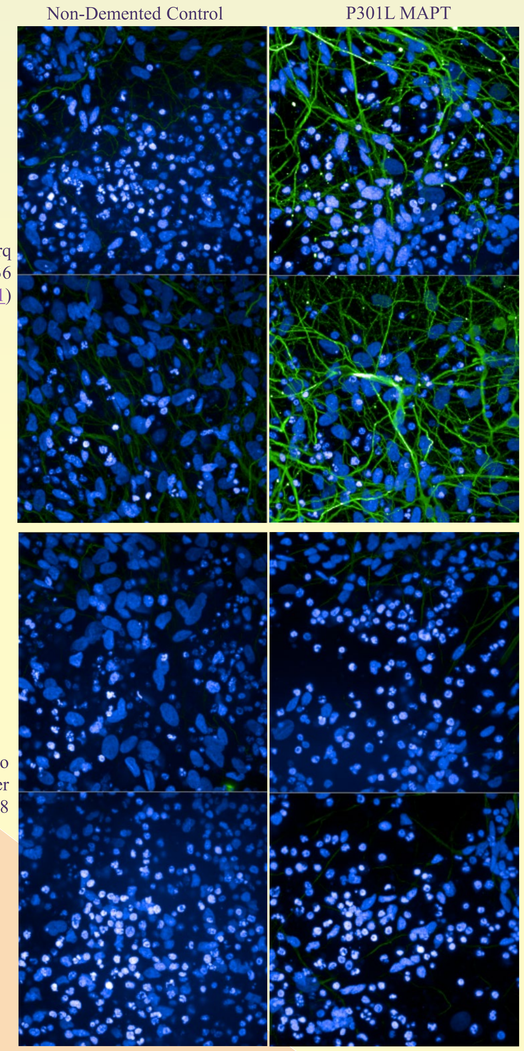
Figure 6. AT8 particle count in P301L mice injected with non-Tg brainstem homogenate (BSH) (negative control) and K18 P301L PFFs (SPR-330)



Tau Antibodies

Hyperphosphorylated tau is found in paired helical filaments. Phosphorylation at certain sites, such as serine 202 and threonine 205, correlates with tau aggregation and is therefore indicative of pathology. AH36 (SMC-601) is a rabbit monoclonal antibody that detects Ser202/Thr205 phosphorylation. It has been tested in iPSC-derived cortical excitatory neurons with and without the MAPT P301L mutation (Figure 7).

Figure 7.ICC/IF analysis of iPSC-derived cortical neurons from non-demented control sample (L) and individual with a P301L MAPT mutation (R) using StressMarq Clone AH36 pSer202/pThr205 tau antibody (SMC-601) and Thermo Fisher Clone AT8 pSer202/pThr205 tau antibody.



Acknowledgments

In vivo experiments and imaging (Figures 5 and 6) were completed at reMYND N.V. with StressMarq-provided PFFs.