Spatial and temporal mapping of 3R and 4R Tau isoforms during mouse brain development using BaseScope™ in situ hybridization technology.

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Background

Microtubule-associated protein Tau (MAPT) is involved in the stability of microtubules, which maintain the cell shape. Tau protein is predominantly expressed in neurons, where it has a role in microtubule assembly and stability, axonal transport, and neurite outgrowth. In neurodegenerative diseases known as tauopathies, such as Alzheimer's disease (AD), Tau function is compromised. Tau expression is developmentally regulated by alternative splicing, giving rise to 6 different isoforms in the human adult brain. Alternative splicing of exon 10 gives rise to protein isoforms with either three or four microtubule-binding repeats in the 3R Tau (exon 10 exclusion) and 4R Tau (exon 10 inclusion) splice variants, respectively. Expression of these isoforms is developmentally regulated and characterized by region-specific neuroanatomic distribution. The high homology of these variants makes presents a challenge in distinguishing between these variants. In this study, we developed specialized probes that detect these variants with high specificity and sensitivity and further identify the spatio-temporal pattern of Tau variant expression during postnatal mouse brain development using our BaseScope technology.

Design

BaseScope™ ISH assay: The expression profiles of 3R and 4R Tau variants were evaluated in formalin-fixed paraffin embedded (FFPE) mouse brains by the BaseScope in situ hybridization (ISH) assay (Figure 1). BaseScope probes were designed and validated to uniquely identify and distinguish between the exon junctions of E9/E11 (3R Tau) and E9/E10 (4R Tau) or E6/E7 (all Tau variants) (Figure 2). Four postnatal developmental time points were selected for analysis: postnatal day (P) 1, 10, 30, and 56 (Figure 3). All scans were analyzed by BaseScope ISH/IHC using the NeuN (neuronal marker) antibody.

Imaging and quantification: Images were acquired using a Case Viewer 3DHISTECH Slide Scanner. Chromogenic BaseScope signals were quantified by QuPath 2.3.0 Software.

Figure 1. BaseScope technology and workflow.

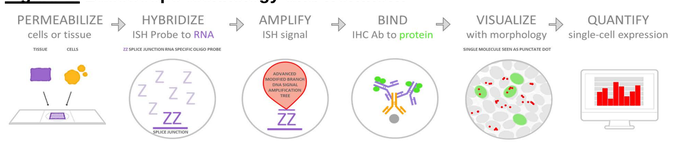
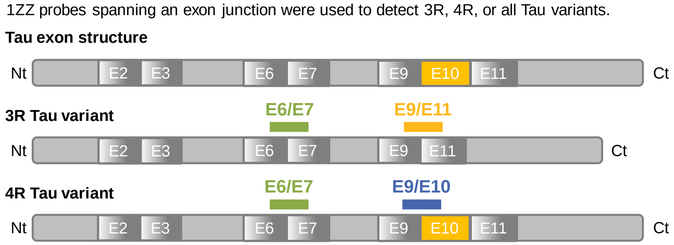


Figure 2. BaseScope probe design to detect 3R Tau and 4R *Tau* variants.

1ZZ probes spanning an exon junction were used to detect 3R, 4R, or all Tau variants.



Results

Figure 2. Validation of MAPT 3R and MAPT 4R mouse probes in untransfected vs transfected cell pellets.

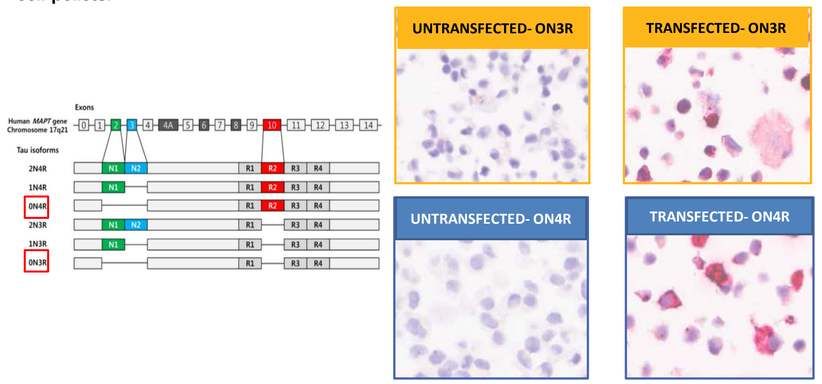


Figure 3. Highly sensitive and specific *in situ* detection of 3R and 4R *Tau* in C57Bl/6J developing mouse brain with focus on cortex, hippocampus, and cerebellum.

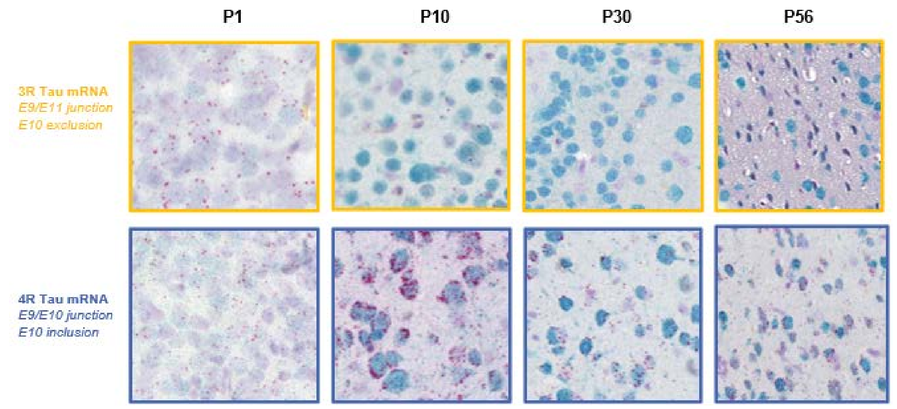


Figure 4. Quantitative isoform expression analysis to generate 3R:4R *Tau* ratio for each ROI per developmental age.

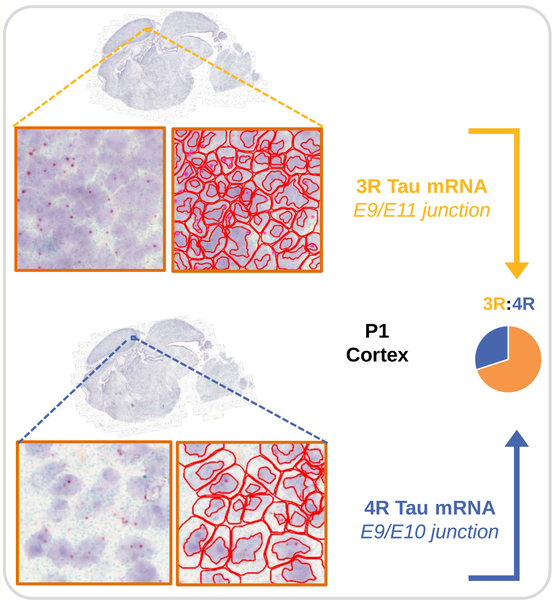
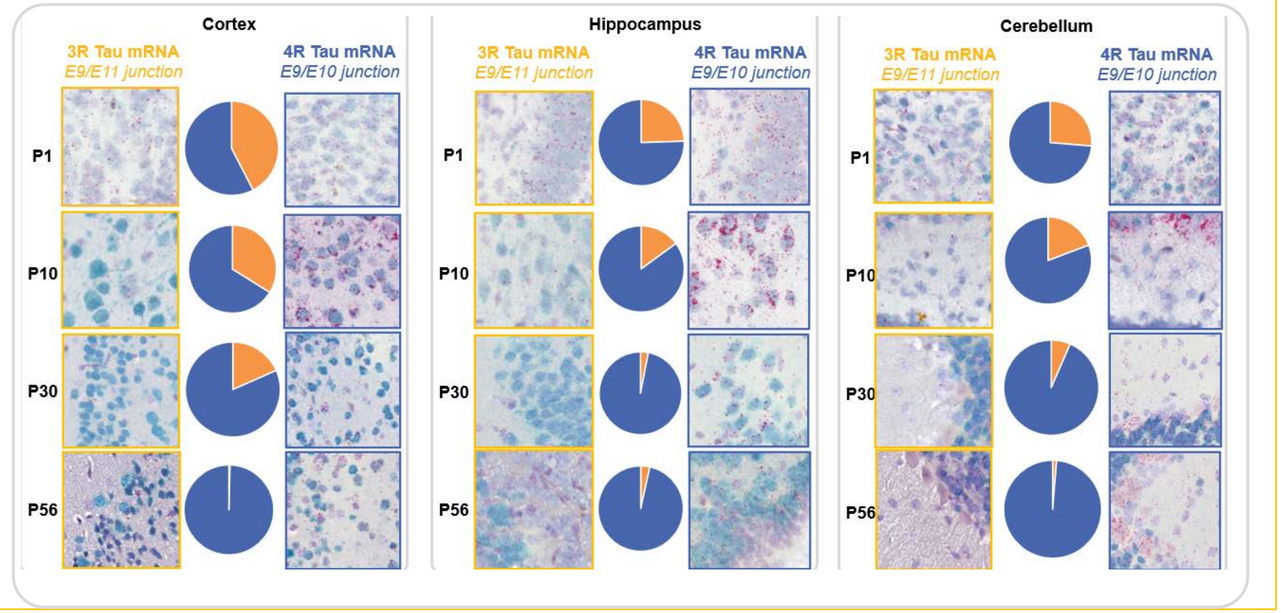


Figure 5. Developmentally-regulated changes in 3R:4R *Tau* isoform expression ratio for ROIs in cortex, hippocampus and cerebellum



Conclusions

Taken together, these results demonstrate the ability of the BaseScope assay to characterize the specific spatio-temporal expression pattern of 3R and 4R Tau variants during mouse brain development at the single cell level. Distinct region-specific differences in the expression patterns of both the 3R and 4R isoforms were observed, which may provide novel insight into how the two Tau isoforms differ biologically. This is the first study of MAPT exon 10 alternative splicing by in situ detection, which enables expression mapping of these highly homologous Tau variants in the morphological and spatial context. A similar assay strategy can also be used to study Tau isoforms in relation to AD and other tauopathies.