Spatio-temporal mapping of 3R and 4R Tau isoforms during mouse brain development using BaseScopeTM in situ hybridization technology

Genomics

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The microtubule-associated protein tau (MAPT) gene encodes a multifunctional protein that is predominantly expressed in neurons where it has a role in microtubule assembly and stability, axonal transport and neurite outgrowth. Neurodegenerative diseases such as Alzheimer's disease are a result of toxic tau aggregates often referred to as tauopathies. Alternative splicing of exon 10 that encodes for the microtubule-binding repeat sequence gives rise to two protein isoforms with either three or four microtubule-binding repeats in the 3R Tau (exon 10 exclusion) and 4R Tau (exon 10 inclusion) splice variant, respectively. The expression of both isoforms is developmentally regulated with each of these isoforms likely to have distinct physiological roles. While both isoforms are balanced in the normal adult brain, this balance or 3R:4R ratio can be skewed in diseased brains like Frontotemporal dementia (FTD). Also, the isoform content of Tau aggregates differs amongst various tauopathies.

In order to elucidate the regulatory mechanisms and functional intricacies of Tau isoforms and develop effective Tau-based therapies, there is a critical need to map the spatial and temporal expression patterns of Tau isoforms at a single-cell resolution with the morphological context during brain development and disease progression. Here we report the development of an in situ hybridization (ISH) assay for the isoform-specific detection of 3R and 4R Tau mRNA isoforms and their profiles during mouse brain development.

The BaseScope ISH technology was employed in this study to detect the alternative splicing of MAPT exon 10 at the single cell level. BaseScope probes were designed to target the exon 9 and exon 10 junction and the exon 9 and exon 11 junction to specifically detect 4R and 3R Tau, respectively. We used transfected cells as a model system to optimize the probe designs to achieve highly specific detection of these highly homologous isoform-specific junctions. These probes were then used to evaluate the neuroanatomic expression of 3R and 4R Tau variants during mouse brain development in postnatal ages P1, P10, P30, and P56 of adult C57Bl/6J mouse. Combining these probes with an antibody for NeuN in an ISH/IHC dual assay, our results revealed a clear opposing dynamic between these two isoforms over the course of development in the cortex and hippocampus regions. To conclude, the unique BaseScope probe designs used in this study provide a powerful approach to study the spatial and temporal expression patterns of various Tau splice variants in various cell-based and animal models