A Tauopathy hiPSC model for MAPT alternative splicing investigation

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Exon Specific Isoform Expression Reporter System (EXSISERS)

Aim

Establishment of a novel human MAPT<sup>EXSISERS</sup>, holding the potential for haplotype specific 4R:Total Tau ratio investigation with high throughput screening application in hiPSCs and derivatives.

Methods

A novel method of alternative spliced isoforms analysis, the Exon Specific Isoform Expression Reporter System (EXSISERS), with its noninvasive, scarless post-translational excision of an exon present reporter domain, circumvents limits of established methods for alternative splicing investigation. By inclusion of a reporter in an alternative spliced exon of interest and a second reporter in a universally expressed exon, present in any protein isoform, the EXSISERS enables non-invasive quantification of exon usage on a protein level without disruption of the endogenous protein sequence nor structure. The reporters are co-translated and rapidly released by spliced intein events, preserving the original isoform ratios.
**MAPT**<sup>EXSISERS</sup> hiPSC model for pharmacological highthroughput screening

### Results

In order to establish a human induced pluripotent stem cell (hiPSC) based MAPT alternative splicing model, we exploit the novel EXSISERS by generating an MAPT<sup>EXSISERS</sup> hiPSC model. Focusing on the regulation of the alternatively spliced exon 10, we adapted the novel EXSISERS to report on the 4R isoforms and total Tau expression. We quantified the precision of the EXSISERS for accurate 4R isoform reporting by the DYRK1A/GSK-3 inhibitor 5-Iodotubercidin (5-ITU) in hiPSC MAPT<sup>EXSISERS</sup> derived small molecule neuronal progenitor cells (smNPCs) and MAPT<sup>EXSISERS</sup> derived forebrain organoids (FBO). In case of MAPT<sup>EXSISERS</sup> hiPSC derived smNPCs a treatment with 1 µM 5-ITU over the time course of 48 h showed a significant increase in 4R/Total Tau ratio compared to vehicle control. Treatment over a period of 72 h increased the 4R/Total Tau ratio even more. Similar results were shown when MAPT<sup>EXSISERS</sup> hiPSC derived FBOs with 0.25, 0.5 or 1.0 µM 5-ITU over the time course of 10 days.
Conclusion

Our novel human EXSISERS hiPSC model closes the gap of lacking disease models enabling the investigation of 4R/Total Tau ration regulation in relation to haplotype specificity, holding the potential for discovery of pharmacological tauopathy therapy development and isoform studies spanning several developmental stages with close patient association in a high throughput screening scale.

Results

To demonstrate non-invasive monitoring of the exon of interest (MAPT exon 10) under endogenous Tau expression, we differentiated MAPTEXSISERS and the tauopathy associated IVS10+16 c>t point mutation carrying MAPTV10+16EXSISERS hiPSC models into hiPSC derived neurons while measuring the 4R/Total Tau expression through Nano Luciferase and Firefly Luciferase over a period of 3 months. Intriguingly, MAPTV10+16EXSISERS hiPSC derived neurons showed an elevated 4R/Total Tau ratio already in the undifferentiated hiPSC compared to MAPTEXSISERS, despite an overall low 4R isoform expression in this state, not detected by immunoblotting.