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HORIZON DISCOVERY & AXOL BIOSCIENCE

MODELLING NEUROLOGICAL DISEASE: *IN VITRO* GENE EDITING AND IPSC DIFFERENTIATION COMBINE TO CREATE POWERFUL NEW TOOLS

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INTRODUCTION

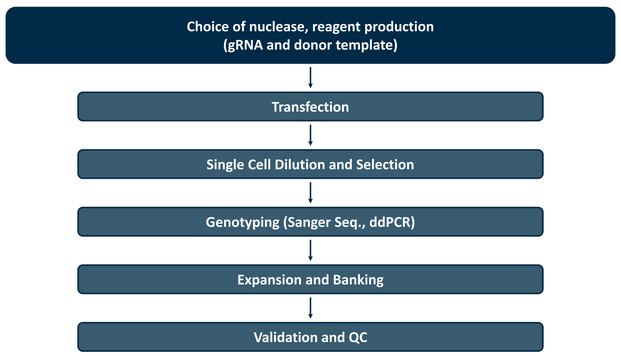
Neurodegenerative diseases, such as Parkinson's disease, Huntington's disease, Alzheimer's disease and other age-related dementias are incurable and debilitating conditions, with Alzheimer's disease alone accounting for ~60-70% of cases. Induced pluripotent stem cells (iPSCs) and gene-editing technology, offer unprecedented biomedical potential for disease modelling, high-throughput drug screening and development of therapeutic strategies for such diseases. We have generated stable human iPSC lines from healthy human dermal fibroblasts and patient-derived fibroblasts. The fibroblasts were reprogrammed using a non-integrating episomal method coding for Yamanaka factors (license agreement with iPS Academia Japan) and then differentiated into neural stem cells (NSCs) to provide a complete modelling solution in a dish.

Using the CRISPR-Cas9 genome-editing technology, we generated patient-relevant disease models carrying microtubule-associated protein TAU (MAPT) mutations. Microtubules are critical for neuron function and facilitate the growth and integrity of axons and dendrites, and transport between the cell body and distant dendrites. Clinically identified missense mutations reduce the ability of TAU to promote microtubule assembly, resulting in neuronal cell death and subsequent disease phenotype.

These renewable and biologically relevant resources will further enable investigation of the mechanisms of disease progression, with additional models relevant to Alzheimer's disease, Parkinson's disease, Huntingdon's disease and epilepsy being generated to aid in the identification of novel drug discovery targets.

iPSC GENE-EDITING USING CRISPR

(A)



(B)

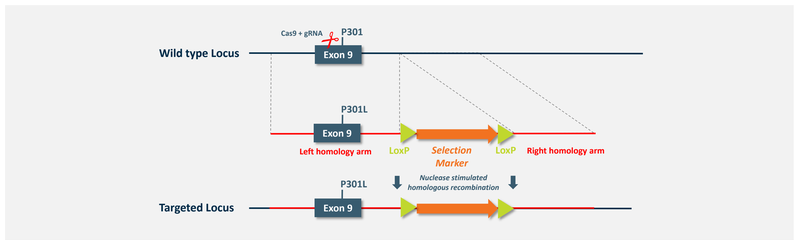
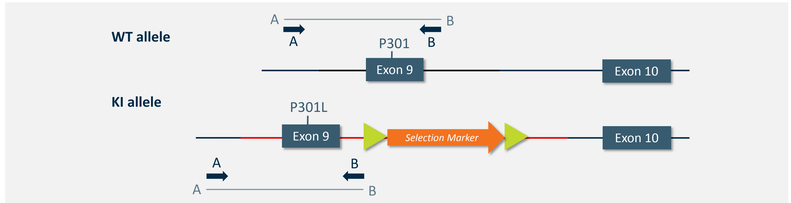


Figure 1: Approach to engineer the three MAPT mutations; MAPT P301L, MAPT V337M and MAPT R406W. (A) An iPSC line known to differentiate well to NSCs was transfected with plasmids expressing Cas9 nuclease (either wild-type or nickase), a validated gRNA and a donor template containing a selection cassette. Single cell clones were derived and screened for the incorporation of the mutation of interest. The genotype of the resultant iPSC clone was validated by Sanger sequencing and ddPCR. (**B)** Schematic representation of a donor template vector used for engineering the different MAPT mutations (e.g. MAPT P301L). The protospacer adjacent motif (PAM) site was disrupted in all plasmid donor templates to prevent re-cutting by the gRNA.

GENOTYPING VALIDATION AND TARGETING EFFICIENCY

(A)



(B)

Mutation	% modified clones*	% modified clones sequenced	% KI/KI$	% KI/WT$	% KI/KO$
MAPT P301L	87.5	63	33	4	43
MAPT V337M	50	64	16	29	0
MAPT R406W	83	43	29	0	44

** Modified clones refers to clones identified by PCR that have incorporated a selection cassette at the right locus*

$ Calculated relative to the number of modified clones sequenced

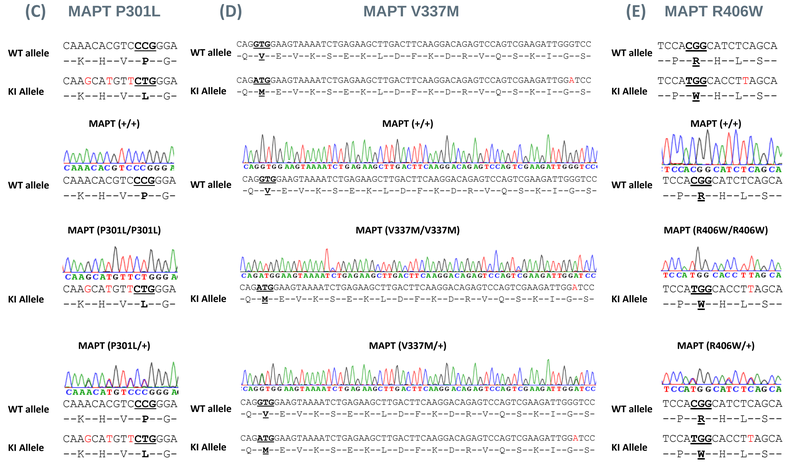


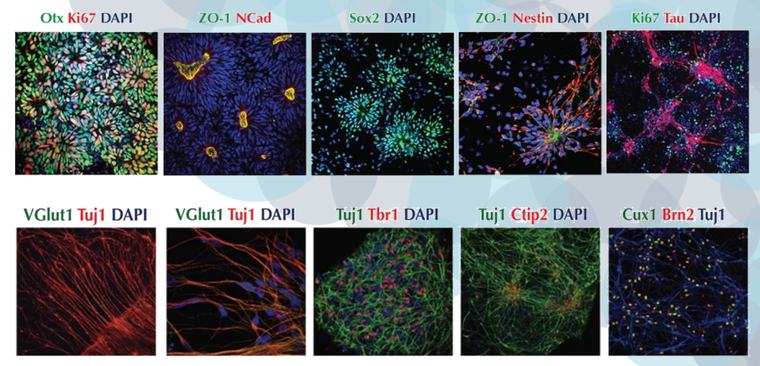
Figure 2: Genetic validation of generated mutant lines. (A) Schematic representation of genotyping primers used. **B)** Table indicates the targeting efficiencies of all desired genotypes. (**B-D)** Sanger sequencing data indicated that six genotypes were achieved, including **C)** MAPT (P301L/P301L) and MAPT (P301L/+), **(D)** MAPT (V337M/V337M) and MAPT (V337M/+), and **(E)** MAPT (R406W/R406W) and MAPT (R406W/+). Silent mutations added to disrupt binding of the gRNA to the donor sequences are highlighted in red. Copy number variation assessment by digital droplet PCR revealed no off-target integration of the plasmid donor template in any of the validated clones (data not shown).

AXOL iPSC-DERIVED NEURAL STEM CELLS

The foundational platform for the CRISPR-Cas9 based MAPT isogenic lines lies with Axol's robust and reliable human iPSC-derived NSCs and cerebral cortical neurons (CCNs) technology and protocols. The **axolGEMs** (Axol Genetically Edited Models) iPSC-Derived NSCs express typical markers such as PAX6 and FOXG1, and spontaneously form polarized neural tube-like rosette structures when plated as a monolayer culture or in 3D (Figure 3). Additionally, Axol iPSC-Derived NSCs are capable of generating a spectrum of cortical neurons that typically express TBR1, CTIP2, BRN2 and CUX1 (Figure 3), as well as being electrically active and having the ability to form functional synapses and circuits *in vitro* (Figure 4).

DIFFERENTIATION AND VALIDATION OF iPSC-DERIVED NEURAL STEM CELLS

(A)



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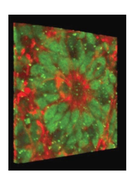


(C)

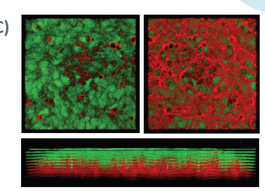
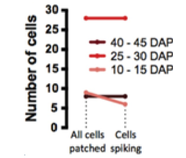


Figure 3: Differentiation of iPSC-Derived Neural Stem Cells. (A) Neural stem cell and cerebral cortical neuron marker expression. (**B)** On culturing human iPSC-derived NSCs on top of the RAFT™ collagen matrix (TAP Biosystems/Lonza), cell migration into the matrix was observed. The cells form the 3D structure of commonly seen neural rosettes. Outside of the rosette, there is a matrix of cells ordered in a non-uniform manner (FOXG1, red; nestin, green). (**C)** On culturing human iPSC-derived CCNs on top of the collagen gel, they formed a uniform static layer of cell bodies. Neurites projected out of these cells and grew downwards creating a network of interconnected neurites (TBR1, red; TUJ1, green).

(A)



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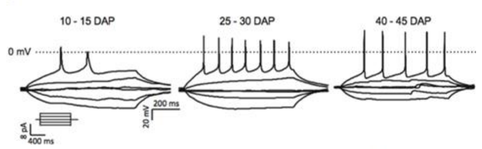
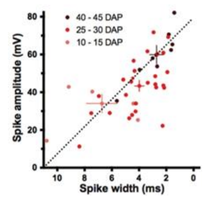
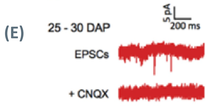


(C)



(D)

(E)



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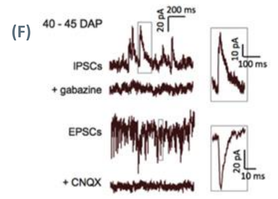

Figure 4: Whole cell patch clamp recordings. (A) Number of cells recorded that showed evoked action potentials compared to the number of total cells recorded. Three different development stages were analyzed: 10-15 days after plating (DAP) on coverslips, 25-30 DAP and 40-45 DAP. **(B)** Representative traces of evoked action potentials. **(C)** Developmental profile of the spike properties of neurons derived from human NSCs. **(D)** Voltage clamp recording at -70 mV from NSCs at 10-15 DAP - no synaptic currents were detected. **(E)** 25-30 DAP, some synaptic currents were excitatory postsynaptic currents (EPSCs) and were blocked by the AMPA and kainate receptors blocker CNQX (10 μM). **(F)** Fully mature neurons at 40-45 DAP showed both EPSCs and inhibitory post-synaptic currents (iPSCs), which could be blocked using a GABAA receptor blocker Gabazine (2 μM).
(*Whole cell patch clamp recordings were carried out in collaboration with Ana González Rueda, Ole Paulsen Lab, University of Cambridge*)

SUMMARY

The combined technology platforms of both Horizon Discovery and Axol Bioscience offer a unique opportunity to derive an unlimited amount of patient-specific, disease-relevant neural stem cells, which can then generate functional brain tissue for neurological research, as well as drug discovery and screening.

- ✓ Matched set of wild-type and MAPT mutation in the same genetic background allows accurate analysis of gene function and the underlying role in disease establishment or progression
- ✓ Can be used as controls for disease modelling along with patient-specific iPSC-derived neural stem cells & cerebral cortical neurons

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