Characterisation & Potential Applications of Human iPS Cell Derived Neural Progenitor Cells

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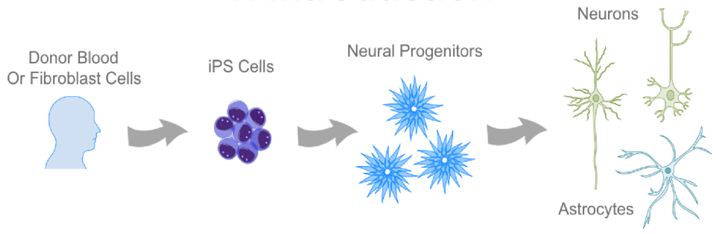
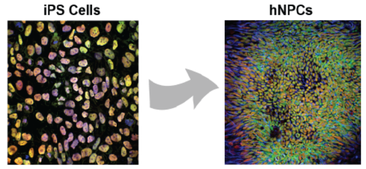
1. Introduction



- In 2012, Shinya Yamanaka was awarded a Nobel Prize for demonstrating that adult cells can be reprogrammed into induced pluripotent stem cells (iPS cells) using defined factors- Oct3/4, KLF4, Sox2 and c-Myc[1] in mouse in 2006[2] and human in 2007[3].
- iPS cells can be differentiated into human neural progenitor cells (hNPCs) and cerebral cortical neurons (hCCNs) from healthy donors and patients[4-6].
- **We characterised iPS cell-derived human neural progenitor cells (hNPCs) and their progeny produced using optimised methods to examine their suitability for neurobiology research.**

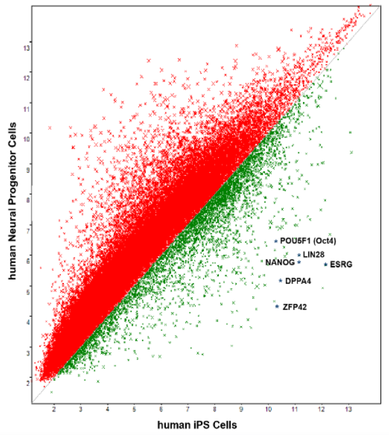
2. Transcriptome Analysis: Axol hNPCs vs iPS Cells

- Integration free iPSCs were generated using an episomal vector.
- hNPCs were generated from iPSCs using Axol's proprietary method for comparative transcriptome analysis.

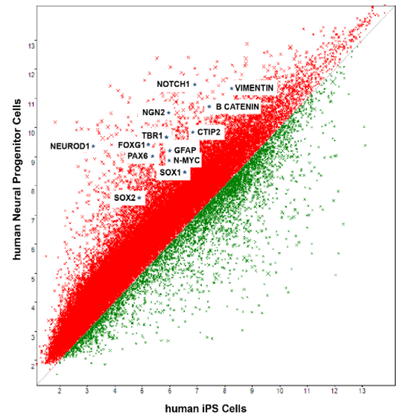


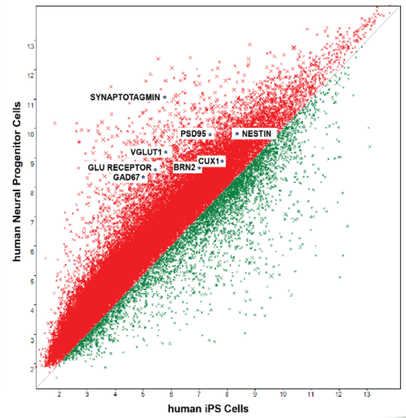
Experiments were performed using the Affymetrix GeneChip® Human Transcriptome Array 2.0 platform. Results were analysed using Affymetrix® Expression Console™ and Affymetrix® Transcriptome Analysis Console (TAC) 2.0 Software.

Expression of pluripotency markers e.g.Oct4 and Nanog is down-regulated in hNPCs vs iPS Cells



Expression of neuronal markers e.g. FOXG1,Vimentin, Nestin and Synaptoagmin is up-regulated in hNPCs in comparison to iPS Cells

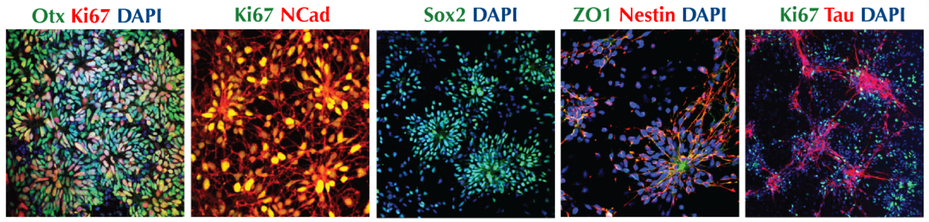




3. hNPC and hCCN Morphology

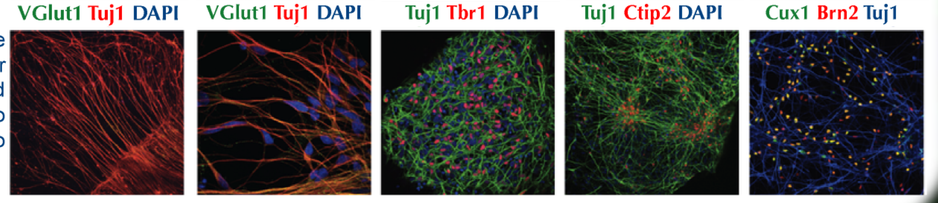
Axol hNPCs

Using immunocytochemistry (ICC), hNPCs were stained for markers known to be expressed in this cell type. Neural rosette formation can also be enforcing the evidence that these cells are indeed neural progenitors.



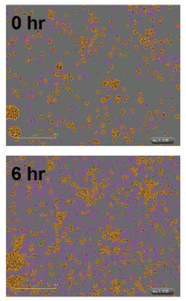
Axol hCCNs

To examine the potential of hNPCs, we induced their differentiation using our standard protocol. The differentiated progeny of hNPCs- hCCNs- were also stained using ICC and can be seen to express typical neuronal markers.



4. Neurite Outgrowth

Re-Plating Differentiated hNPCs



- Cells were thawed into a 35-mm dish and differentiated for 4 days.
- Cells were lifted with Axol Unlock™ and seeded into 1x 96-well TPP plate in Axol Sure Boost™.
- **Establishment of neurites occurred within 6 hrs post-plating.**
- Passaging the cells post-differentiation could be used as a model to study, neurite outgrowth as well as neurodegeneration and regeneration.

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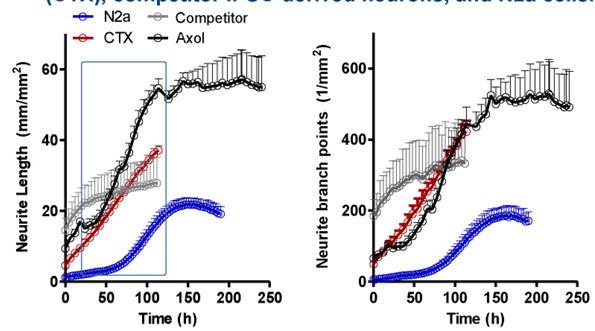
Axol hNPCs yielded the highest Neurite length and branch point values in comparison to rat primary cortical neurons (CTX), competitor iPSC-derived neurons, and N2a cells.



Axol hNPC neurite outgrowth was assessed by S. Lopez Alacantra and T. Dale at Essen Bioscience Ltd. using the IncuCyte NeuroTrack platform

5. 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 developmental stages were analysed: 10 to 15 days after plating (DAP) in coverslips, 25 to 30 DAP and 40 to 45 DAP.

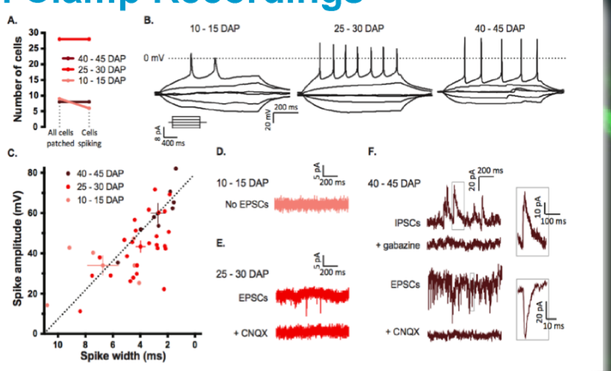
B. Representative traces of evoked action potentials.

C. Developmental profile of the spike properties of neurons derived from hNPCs.

D. Voltage clamp recording at -70 mV from hNPCs at 10 to 15 DAP. No synaptic currents were detected.

E. 25 to 30 DAP, some synaptic currents were observed. These currents were excitatory postsynaptic currents (EPSCs) and were blocked by CNQX (10 µM), an AMPA and kainate receptor blocker.

F. Fully mature neurons at 40 to 45 days post-plating showed both EPSCs and inhibitory postsynaptic currents (IPSCs), which could be blocked using a GABAA receptor blocker (gabazine, 2 µM). Inhibitory postsynaptic currents (IPSCs) were recorded at 0 mV.



§ Whole Cell Patch Clamp Recordings were carried out by Ana González Rueda of the Ole Paulsen Lab at the University of Cambridge

6. Summary

- Axol hNPCs express neural markers at both the gene and protein level
- The progeny of Axol hNPCs-hCCNs express markers typically observed in cerebral cortical neurons.
- Live imaging of Axol hNPCs reveals that these cells can form neural networks with increased neurite length and branching in comparison to competitor products.
- Whole Cell Patch Clamp Recordings demonstrated that hNPCs can produce electrically active cells.
- These properties make Axol hNPCs suitable for neurobiology research including disease modelling, drug screening, toxicology studies and more.

7. References

1. **iPS cell technologies: significance and applications to CNS regeneration and disease.** Okano & Yamanaka. Mol Brain, 2014.
2. **Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors.** Takahashi & Yamanaka. Cell, 2006.
3. **Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors.** Takahashi et al. Cell, 2007.
4. **Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses.** Shi et al. Nature Neuroscience, 2012.
5. **A human stem cell model of early Alzheimer's disease pathology in Down syndrome.** Shi et al. Science Translational Medicine, 2012.
6. **Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks.** Shi et al. Nature Protocols, 2012.

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