Characterization and Potential Applications of Human iPSC-Derived Neural Stem Cells

Overview
We conducted a series of experimental procedures to examine the characteristics and potential application of iPSC-derived neural stem cells for neurobiological research.

Introduction

- Adult cells can be reprogrammed into iPSCs using defined factors - Oct3/4, KLF4, Sox2 and c-Myc\(^1\-3\).
- iPSCs can be differentiated into many cell types including human neural stem cells (hNSCs) and cerebral cortical neurons (hCCNs) from healthy donors and patients suffering from disease\(^4\-6\).
- We characterized iPSC-derived hNSCs and their progeny to examine their suitability for neurobiology research.
- We have demonstrated that these cells can be used as a model for the study of human neuronal development.

Results

Transcriptome Analysis
Integration-free iPSCs were generated using an episomal vector and subsequently differentiated into hNSCs using Axol’s proprietary method.

| Pluripotency markers are down-regulated in hNSCs vs. iPSCs | Neuronal markers are up-regulated in hNSCs vs. iPSCs |

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.

Neural Cell Morphology

Human Neural Stem Cells
Axol human neural stem cells (hNSCs) form neural rosettes and express markers typically observed in neural precursor cells as seen by immunocytochemistry.

Human Cerebral Cortical Neurons
Differentiation of hNSCs generates human cerebral cortical neurons (hCCNs) that express neuronal markers observed using immunocytochemistry. These neurons increase in maturity over time in culture.

3D Culture

Foxg1 - Nestin
On culturing hNSCs 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.

Tbr1 - Tuj1
On culturing hCCNs 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.

Conclusion

- Axol hNSCs and their progeny express neural markers at both the gene and protein level and can be cultured in 2D & 3D systems.
- Live imaging of these cells shows that they form neural networks while whole cell patch clamp and MEA recordings show the cells are electrically active.
- The properties of Axol hNSCs and hCCNs make them ideal for numerous applications including disease modeling, drug screening, toxicology studies and more.

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

Whole Cell Patch Clamp

Multi-electrode Array
Drug effect of spontaneous firings at 100 and 240 culture days in vitro

Epilepsy phenomenon was evoked by administration of pentylentetrazole (PTZ) and inhibited by anti-epilepsy drug phenytoin and sodium valproate (VPA)

References