Effect of Passage Number on Human Induced Pluripotent Stem Cell Derived Neurons Culture Contaminants with Non-Neuronal Cell Types

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Background: The study of primary human neurons is hindered due to the potential for irreversible damage to a patient during biopsy. However, reprogramming of adult human somatic cells into induced pluripotent stem cells (iPSCs) with subsequent sensory neuronal differentiation has proved to be a reliable, patient-specific method for the study of the pathophysiology of diseases of the human peripheral nervous system. The impact of iPSC culture conditions, such as passage number, on the generation of pure, mature neuronal cultures has not been definitively established. Therefore, we set out to determine the effect of iPSC passage number on maturity and presence of contaminating cell types in iPSC-derived sensory neuron (iPSC-dSN) cultures.

Methods: Peripheral blood mononuclear cells were isolated from whole blood of three individual donors and reprogrammed into iPSCs. The three iPSC lines were passaged until each of three target passage numbers were reached: low (5-10), middle (20-26), and high (30-38). Neuronal differentiation was then induced using an induction medium for eight days. On day nine, cells were passaged and replated in a maintenance medium. On day 33, total RNA was extracted from the cells. Normalized expression values for marker genes of potential contaminating cell types and pluripotency were compared using paired Student's t-tests and ANOVA, with the cell line and passage number as independent variables. P-values less than 0.05 were considered significant.

Results: Lower passage number was associated with decreased expression of pluripotency and astrocyte markers, and increased expression of myelinating glial cell markers. No significant differences in the expression of markers for other common contaminating cell types were observed.

Conclusion: Lower passage numbers optimally replicated a pure, mature peripheral nervous system (PNS) phenotype, with lower expression of marker genes for common contaminating cell types, and higher expression of marker genes for key components of the PNS. Future studies will further identify the impact of these non-neuronal cell types on downstream assays.