Abstract

Human pluripotent stem cells, including embryonic (ES) and induced pluripotent stem (iPS) cells, offer an essentially unlimited source of neural cells that can be used to investigate mechanisms of human neurological disease and neural regeneration. A critical step during the derivation of neurons, astrocytes, or oligodendrocytes from pluripotent stem cells is generating a robust and homogeneous neural progenitor cell population, which ultimately impacts the efficiency of downstream differentiation protocols and helps control experimental reproducibility. Understanding population heterogeneity is an important step in the optimization of differentiation protocols, which is challenging with existing methods. This study demonstrates how Single-Cell Western blot analysis can complement traditional verification approaches by providing insights into protein expression both at the population and single-cell level. In this study, human iPSC lines were differentiated into neural progenitor cells by kit-induced NPCs homogenously express Pax6 and SOX1 while lacking lack Pax6-expressing cells. Using OCT-4 as a marker for pluripotent stem cells and Pax6 as a marker for neural progenitor cells, we observed that the single-cell level, that differentiation the cells shift from an exclusively Oct-3/4 expressing population to one that is robustly Pax6-positive. We were also able to identify subpopulations of neural progenitor cells that express Pax6 at high or low levels. Additionally, single-cell analysis also used to characterize the population distribution of terminally-differentiated neural cells following growth factor withdrawal-induced differentiation of iPS-derived neural progenitor cells.

Single-Cell Western Analysis of Neural Progenitor Cell Differentiation

Conclusions

- The StemVivo® Neural Progenitor Differentiation Kit efficiently differentiates human pluripotent cells into neural progenitors.
- The NPC-derived cells were analyzed using flow cytometry to confirm the purity of the population.
- Single-Cell Western blotting shows NPC population homogeneity following differentiation.
- High and low expressing populations are identified within Oct-3/4 positive NPCs and Pax6-positive neural progenitor cells.
- Single-cell Western and flow cytometry can be used to characterize cell type population distribution in iPS-derived neural cell cultures.