

Purifying Specific Neural Progenitor Populations from Human Embryonic Stem Cells

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Introduction and Objective: Human embryonic stem cells (hESCs) offer an ideal source for deriving neural progenitor cell (NPC) grafts to treat brain disorders. However, the NPC stage(s) optimal for neural repair is unclear. The aim of this study was to enrich for different developmental stages of NPCs from hESCs using promoter- or cell surface antigen-based sorting strategies. **Methods:** H7 and H9 hESCs (Wicell) were differentiated into NPCs in defined media. To enrich multipotent NPCs (mpNPCs), stable hESC reporter lines (hSox3-GFP) were generated in which a partial human Sox3 promoter, specific for forebrain NPCs, drives GFP. GFP⁺ cells were enriched by flow sorting and their potential for proliferation and differentiation were assayed. Gene expression profiling was performed to characterize the molecular signature of enriched putative mpNPCs, and results were validated by quantitative RT-PCR (Q-RT-PCR). Neuronal restricted precursors (NRPs) were identified by polysialylated neural cell adhesion molecule (PSA-NCAM) immunoreactivity and flow-sorted with anti-PSA-NCAM antibody. **Results:** Sox3/Nestin/MAP2⁺ NPCs derived from hESCs in defined media formed neural tube-like rosettes. Stably transfected hSox3-GFP lines expressed GFP only after neural differentiation. GFP⁺ cells formed Sox3⁺ rosettes and generated more neurospheres than the GFP⁻ fraction. GFP⁺ cells gave rise to neurons and glia after further differentiation. Gene expression profiling and Q-RT-PCR showed NPC- and forebrain-specific transcripts were highly enriched in GFP-sorted cells after 17 day neural differentiation vs. unsorted or undifferentiated populations, while GFP-sorted cells showed lower expression of transcripts specific for pluripotent, non-ectodermal or posterior CNS cell types. FACs for PSA-NCAM⁺ NRPs after 3-week differentiation yielded over 44%. The enriched NRPs formed neurospheres and differentiated into neurons. **Conclusions:** Promoter- or cell surface marker-based purification strategies enable the enrichment of specific NPC populations from hESCs. These different populations will be tested for restorative efficacy in brain injury models to determine the ideal NPC stage for neuroregenerative therapeutic transplantation.