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Properties of a Fetal Multipotent Neural Stem Cell (NEP Cell)

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Abstract

Multipotent neural stem cells (NSCs) present in the developing neural tube (E10.5, neuroepithelial cells; NEP) were examined for the expression of candidate stem cell markers, and the expression of these markers was compared with later appearing precursor cells (E14.5) that can be distinguished by the expression of embryonic neural cell adhesion molecule (E-NCAM) and A2B5. NEP cells possess gap junctions, express connexins, and appear to lack long cilia. Most candidate markers, including Nestin, Presenilin, Notch, and Numb, were expressed by both NEP cells as well as other cell populations. Fibroblast growth factor receptor 4 (FGFR4), Frizzled 9 (Fz9), and SRY box-containing gene 2 (Sox2) as assessed by immunocytochemistry and *in situ* hybridization are markers that appear to distinguish NSCs from other precursor cells. Neither Hoechst 33342 nor rhodamine-123 staining, telomerase (Tert) expression, telomerase activity, or breakpoint cluster region protein 1 (Bcrp1) transporter expression could be used to distinguish NEP stem cells from other dividing cells. NEP cells, however, lacked expression of several lineage markers that are expressed by later appearing cells. These included absence of expression of CD44, E-NCAM, A2B5, epidermal growth factor receptor (EGFR), and platelet-derived growth factor receptor-alpha (PDGFRα), suggesting that negative selection using cell surface epitopes could be used to isolate stem cell populations from mixed cultures of cells. Using mixed cultures of cells isolated from E14.5 stage embryos, we show that NEP cells can be enriched by depleting differentiating cells that express E-NCAM or A2B5 immunoreactivity. Overall, our results show that a spectrum of markers used in combination can reliably distinguish multipotent NSCs from other precursor cells as well as differentiated cells present in the CNS.

Keywords

NSC; NEP; NRP; GRP; differentiation; precursor

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