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# Neural-Competent Cells of Adult Human Dermis Belong to the Schwann Lineage

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## SUMMARY

Resident neural precursor cells (NPCs) have been reported for a number of adult tissues. Understanding their physiological function or, alternatively, their activation after tissue damage or in vitro manipulation remains an unsolved issue. Here, we investigated the source of human dermal NPCs in adult tissue. By following an unbiased, comprehensive approach employing cell-surface marker screening, cell separation, transcriptomic characterization, and in vivo fate analyses, we found that p75NTR$^+$ precursors of human foreskin can be ascribed to the Schwann (CD56$^+$) and perivascular (CD56$^-$) cell lineages. Moreover, neural differentiation potential was restricted to the p75NTR$^+$CD56$^+$ Schwann cells and mediated by *SOX2* expression levels. Double-positive NPCs were similarly obtained from human cardiospheres, indicating that this phenomenon might be widespread.

## INTRODUCTION

The search for adult neural precursor cells (NPCs) outside of the CNS has been the focus of extensive research due to the accessibility and envisioned use of these cells in the treatment of neurodegenerative disease. Tissue-specific multipotent cells with the capacity to generate neural (i.e., neuronal and glial) progeny have now been isolated from a number of adult tissues, including bone marrow, fat, heart, intestine, palate, pancreas, skeletal muscle, skin, and uterus. Somewhat less surprisingly, NPCs can also be derived from peripheral nerves, ganglia, enteric glia, and the carotid body. However, even though NPCs can be obtained from diverse tissues, in most instances their origin remains unknown (Joseph and Morrison, 2005).

Among the above-mentioned examples, the skin is arguably the tissue that is the most easily accessible and requires less invasive extraction procedures. Sphere-forming neural-crest-derived adult precursors reside in the dermis (Toma et al., 2001; Wong et al., 2006), and subpopulations of these appear to present stem cell properties (Biernaskie et al., 2009). Using antibodies against the low-affinity neurotrophin receptor p75NTR, previous studies isolated postmigratory neural crest stem cells (NCSCs) from both embryonic and adult tissues (Kruger et al., 2002; Morrison et al., 1999). In this report, we explore the source of NPCs in adult human dermis and find that they originate from p75NTR$^+$CD56$^+$ cells belonging to the Schwann lineage. Further, the expression levels of *SOX* genes seem to be tightly regulated in p75NTR$^+$ cells of human skin, and we show that *SOX2* expression levels correlate with the neural competence of dermal precursors, as described for other systems (Hutton and Pevny, 2011; Kim et al., 2003; Taranova et al., 2006).

## RESULTS

We investigated the identity of human NPCs extracted from the foreskin dermis through fluorescence-activated cell sorting (FACS)-based isolation of cell subpopulations and subsequent characterization of the sorted cells (Figure 1). Immunofluorescent and flow-cytometry analyses of sphere cultures showed the existence of a rare p75NTR (also known as Gp80-LNGFR, CD271, and TNFRSF16)-positive cell subpopulation (2.9% ± 1.7% of total; n = 24) in primary dermal sphere cultures that was Nestin$^+$ and CD34$^-$ and presented a characteristic morphology (i.e., a single

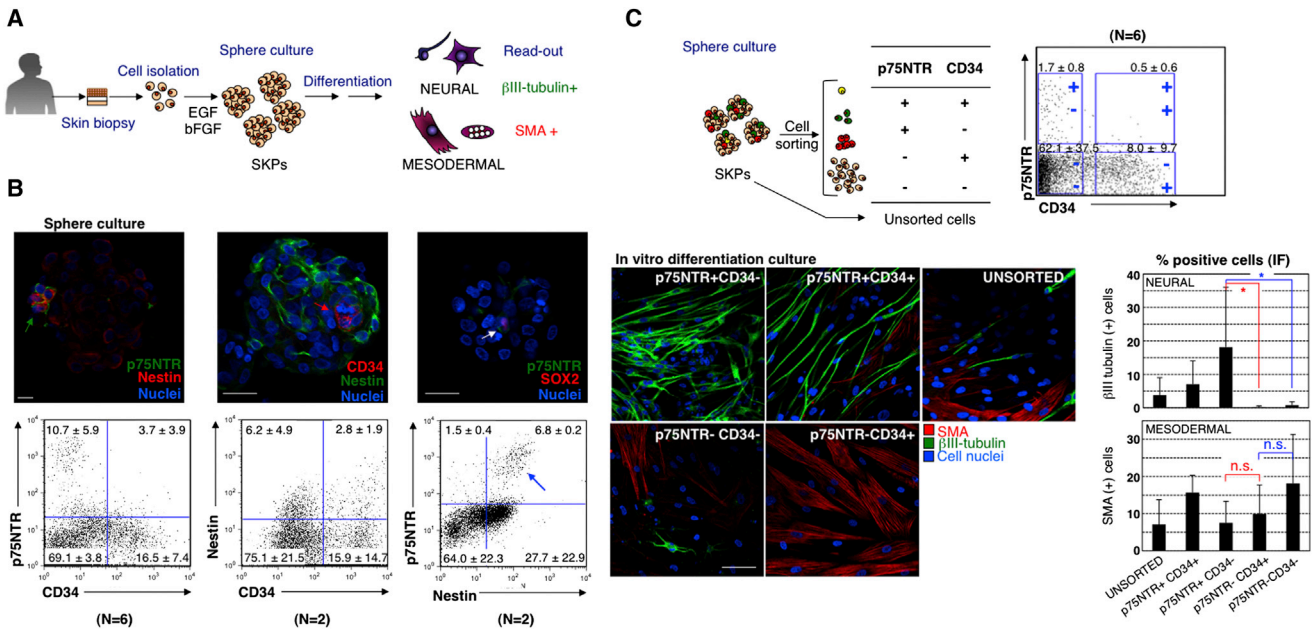


**Figure 1. Human Dermospheres Contain p75NTR$^+$ Nestin$^+$ SOX2$^+$ NPCs**

(A) Cell isolation and characterization procedure.

(B) Characterization of primary dermospheres by immunofluorescence and confocal microscopy. A subgroup of cells (green arrow) expressed p75NTR and Nestin. In contrast, CD34$^+$ cells (red arrow) were Nestin$^-$. SOX2 colocalized with p75NTR$^+$ cells (white arrow). Nuclei were counterstained with Hoechst 33258 (blue). Scale bars, 10 μm. Flow-cytometry analyses confirmed separate cell subpopulations that were positive for p75NTR and CD34, and a distinct p75NTR$^+$Nestin$^+$ population (blue arrow).

(C) Primary dermospheres were dissociated, double sorted with anti-p75NTR and anti-CD34 antibodies, and put into differentiation culture. Representative stainings for neural (βIII tubulin, green) and mesodermal (SMA, red) progeny are shown. Nuclei were counterstained with Hoechst 33258 (blue). Scale bar, 10 μm. Quantifications of the percentages of neural (upper panel) and mesodermal (lower panel) cells are shown in relation to the total cell number. Statistical significance (one-way ANOVA) values were p = 0.034 (*, n = 6) for the comparison of p75NTR$^+$CD34$^-$ versus p75NTR-CD34$^+$ (neural), p = 0.039 (*, n = 6) for p75NTR$^+$CD34$^-$ versus p75NTR-CD34$^-$ (neural), p = 0.147 (n.s., n = 6) for the comparison p75NTR$^+$CD34$^-$ versus p75NTR-CD34$^+$ (mesodermal), and p = 0.674 (n.s., n = 6) for p75NTR$^+$CD34$^-$ versus p75NTR$^-$ CD34$^-$ (mesodermal). Error bars indicate SD.

See also Table S2 and Movies S1 and S2.

process extending from the soma; Figure 1B; Movie S1 available online). Intriguingly, 74.6% ± 2.9% of p75NTR$^+$ cells coexpressed SOX2 by immunofluorescence analyses (Movie S2), indicating that p75NTR$^+$ cells might be more precursor-like. Isolation of p75NTR$^+$CD34$^-$ cells enabled a significant enrichment of precursors committed to the neural lineage in vitro, as assessed by an average 22.7-fold increase (18.2% versus 0.8% of total cells; n = 6) in their neural differentiation capacity when compared with p75NTR-CD34$^-$ cells (Figure 1C).

To determine whether p75NTR$^+$ cells exhibit the same differentiation potential in ovo, we sorted, expanded, and injected human cells into the neural crest migratory stream (Hamburger-Hamilton stage 17 [HH17] chicken embryos, hindlimb-level somites; Figure 2A). Cross-sections of HH26 embryos, immunolabeled with anti-human nuclei (anti-HuNu) for the detection of transplanted cells, showed that the human p75NTR$^+$ cells survived; migrated to the neural crest, dorsal root ganglia (DRG), and skin; and differentiated into βIII tubulin$^+$ cells (Figures 2B–2F). Since βIII tubulin was recently reported to mark human melanocyte lineage cells, as well as peripheral neurons in the dermis (Locher et al., 2013), we confirmed the peripheral neuronal phenotype of HuNu$^+$ cells by showing coexpression of neurofilament 200 (NF200; Figures 2G–2I). These markers were not present in the original dermal cultures (Figure S1). In contrast, p75NTR$^-$ cells were hardly detected at the HH26 stage, if at all, and most of the surviving cells did not express any of the aforementioned markers (Figures 2J and 2K; data not shown). A quantification of transplant-derived HuNu$^+$ cells showed that, as expected, p75NTR$^+$ NPCs showed increased survival, migration to peripheral tissue, and neural differentiation when compared with both unsorted and p75NTR$^-$ cell fractions. On average, 10.8-fold more p75NTR$^+$ cells survived, migrated to peripheral tissue, and differentiated into peripheral neurons than p75NTR$^-$