

**Isolation and characterization of neural crest stem cells derived from in vitro-differentiated human embryonic stem cells.**

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**Public Summary:**

**Scientific Abstract:**

The neural crest is a transient structure of vertebrate embryos that initially generates neural crest stem cells (NCSCs) which then migrate throughout the body to produce a diverse array of mature tissue types. Due to the rarity of adult NCSCs as well as ethical and technical issues surrounding isolation of early embryonic tissues, biologic studies of human NCSCs are extremely challenging. Thus, much of what is known about human neural crest development has been inferred from model organisms. In this study, we report that functional NCSCs can be rapidly generated and isolated from in vitro-differentiated human embryonic stem cells (hESCs). Using the stromal-derived inducing activity (SDIA) of PA6 fibroblast co-culture we have induced hESCs to differentiate into neural crest. Within 1 week, migrating cells that express the early neural crest markers p75 and HNK1 as well as numerous other genes associated with neural crest induction such as SNAIL, SLUG, and SOX10 are detectable. Fluorescence-activated cell sorting (FACS)-based isolation of the p75-positive population enriches for cells with genetic, phenotypic, and functional characteristics of NCSCs. These p75-enriched cells readily form neurospheres in suspension culture, self-renew to form secondary spheres, and give rise under differentiation conditions to multiple neural crest lineages including peripheral nerves, glial, and myofibroblastic cells. Importantly, these cells differentiate into neural crest derivatives when transplanted into developing chick embryos in vivo. Thus, this SDIA protocol can be used to successfully and efficiently isolate early human NCSCs from hESCs in vitro. This renewable source of NCSCs provides an invaluable source of cells for studies of both normal and disordered human neural crest development.

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