COMMENTARY

Human Hair Follicles: "Bulging" with Neural Crest–Like Stem Cells

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Several studies have reported the existence of precursor cells residing within various adult tissues that appear to either retain or recapitulate features of neural crest stem cells (NCSCs). In rodents, unique populations of both epidermal and dermal cells, resident within hair follicles, exhibit such characteristics, although the existence of equivalent NCSC-like cells in human tissues has remained uncertain. In this issue, Yu *et al.* show that NCSC-like cells also reside within the bulge region of adult human hair follicles.

The neural crest is a transient embryonic tissue that is composed of neural crest stem cells (NCSCs) and originates at the dorsal tip of the neural tube as a result of inductive interaction between the neuroectoderm and the overlying ectoderm (primitive epidermis) (Le Douarin and Kalcheim, 1999). Interestingly, as neural crest cells migrate away from the neural tube, interaction with environmental factors specifies a diverse array of cell fates, including peripheral neurons (autonomic and sensory neurons), Schwann cells, melanocytes, and vascular smooth muscle, as well as connective tissue, adipocytes, and craniofacial skeletal structures. It is this multilineage differentiation capacity that makes NCSCs so fascinating, particularly in light of several studies suggesting that adult tissues, including hair follicles, harbor precursor cells endowed with NCSC properties.

NCSC-like precursors in hair follicles

Dermal precursors. In their attempt to identify an accessible source of neural progenitors, Toma *et al.* (2001) isolated a population of self-renewing cells capable of clonal, multilineage differentiation, which they termed skin-derived precursors (SKPs). Like NCSCs, the diverse progeny derived from bulk or clonally derived SKPs *in vitro* are reminiscent of the NCSC repertoire, and indeed SKPs express many neural crest–related genes (e.g., *Slug*, *Snail*, *Twist*, *Pax3*, *Sox9*, *p75*) (Biernaskie *et al.*, 2009; Fernandes *et al.*, 2004b). Recently, it was shown that SKPs originate from Sox2+ dermal precursors residing within the dermal sheath and dermal papilla of hair follicles and that these cells exhibit properties of dermal stem cells (Biernaskie *et al.*, 2009). Moreover, a recent report argues that SKPs (or their derivatives) are thought to be the cell of origin for dermal tumors found in patients with neurofibromatosis type 1, a neural crest–related disorder in which patients develop malignant skin tumors composed of Schwann cells, melanocytes, and dermal fibroblasts (Le *et al.*, 2009), providing further support for their similarity to NCSCs. Several studies have also isolated multipotent dermal precursors from human dermis, demonstrating both neural and mesodermal potential (Belicchi *et al.*, 2004; Hunt *et al.*, 2008; Joannides *et al.*, 2004; Shih *et al.*, 2005; Toma *et al.*, 2005); these, too, appear enriched within the human hair follicle dermal papilla (Hunt *et al.*, 2008).

Epithelial precursors. Two different NCSC-like populations have been derived from the outer root sheath bulge region of rodent hair follicles (Amoh *et al.*, 2005; Sieber-Blum *et al.*, 2004). Epidermal NCSCs (EPI-NCSCs) are derived from rodent whisker follicles and *in vitro* generate all major neural crest derivatives (Sieber-Blum *et al.*, 2004). Lineage-tracing experiments confirm that EPI-NCSCs are derived from the neural crest and exhibit sustained expression of many neural crest–related genes but also possess a unique genetic signature that distinguishes them from other hair follicle stem cells (Hu *et al.*, 2006). Others have identified a population of nestin+K15− cells in the bulge region of rodent hair follicles that are multipotent and can generate neural cell types, keratinocytes, and adipose and smooth muscle cells *in vitro* and following transplant *in vivo* (Amoh *et al.*, 2005; Mignone *et al.*, 2007).

Yu et al (2006) have reported that a similar self-renewing cell could be isolated from human hair follicles and *in vitro* could give rise to cells exhibiting features of melanocytes, neural cells, and smooth muscle cells. From coincidental staining patterns of K15 and Oct4 in isolated stem cells and in the hair follicle bulge region, they inferred that these cells might originate from within the bulge.

In situ hair follicle sphere formation

In this issue, Yu *et al.* (2010) extend these findings. Because the use of human tissues precludes the use of genetic markers, they devised an alternative method to determine where these cells might reside within hair follicles. First, they removed the mesenchymal compartment of the follicle to eliminate any potential contribution by dermal precursors. Then, by culturing intact hair follicles in the presence of basic fibroblast growth factor (bFGF) and embryonic stem cell medium, the authors cleverly coaxed these hair follicle stem cell/neural crest cells (HFSC/NCCs) to proliferate *in situ* within their niche, forming large, spherical colonies

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that erupted from the outer root sheath beneath the sebaceous gland, thereby identifying the bulge region as a putative niche within human follicles.

Interestingly, Yu *et al*. (2010) observed hair follicle sphere formation in only 20% of cultured hair follicles. Is this inefficiency inherent to the hair follicle isolation procedure, or can it be attributed to intrinsic differences among hair follicles? Does the activation status of epidermal hair follicle stem cells (Zhang *et al*., 2009) influence their responsiveness to exogenous mitogens?

> The therapeutic potential of an accessible neural crest–like adult stem cell in human hair follicles is enormous.

Hair follicle spheres express NCSC markers and are composed of label-retaining cells

Yu *et al*. (2010) show that within hair follicle spheres, many cells express the neural crest–associated markers Twist, Slug, and Sox10 but do not express markers of more differentiated cell types (i.e., s100, ck20, neurofilament, MITF, c-Kit, or collagen type VII). Although a more thorough examination of known epithelial markers would be informative in narrowing the identity of HFSC/NCCs within the bulge, these results certainly confirm the presence of a progenitor phenotype within the hair spheres. Furthermore, HFSC/NCC colonies also contained cells expressing Bmi1 and Notch1, which the authors infer as an additional link to NCSCs because both signaling pathways have been shown to influence the self-renewal of NCSCs (Molofsky *et al*., 2003; Wakamatsu *et al*., 2000).

Yu *et al*. (2010) show that approximately 30% of the cells within the hair spheres were dividing. Following BrdU pulse and a 9-day chase period, 12% of HFSC/NCC cells retained the label. HFSC/NCC also divided asymmetrically, as shown by asymmetric segregation of BrdU-labeled chromosomes. Unfortunately, Yu *et al*. (2010) did not further characterize the identity of these label-retaining cells for expression of NCSC markers (or for more specific keratinocyte markers). Without double labeling, the identity of these HFSC/NCCs within the bulge remains unclear. It would be interesting to examine neural crest–related markers as well as known epithelial bulge markers (such as K15, K14, K5, CD200, CD34, and p75) in order to determine which markers are expressed in the putative label-retaining HFSC/NCC population versus "transit amplifying" cells within follicle spheres. This may provide hints as to the identity of these HFSC/NCCs and perhaps some insight into their endogenous function within the hair follicle. Because Yu *et al*. (2010) identify both Notch1 and Bmi-1 within the HFSC/NCCs, it would also be interesting to know whether inactivation of either of these signaling pathways impacts self-renewal of HFSC/NCCs, perhaps resulting in their eventual depletion, as has been reported for NCSCs (Molofsky *et al*., 2003; Wakamatsu *et al*., 2000).

The authors go on to ask how similar human HSFC/NCCs are to other neural crest–like precursors derived from rodent skin. A comparison of transcriptional profiles of HFSC/NCCs to those published for rodent EPI-NCSCs and SKPs showed that all the genes composing the EPI-NCSC signature are similarly activated in HFSC/NCCs, suggesting that these two populations are equivalent. This is a reasonable conclusion considering their coincident residence within the bulge and their capacity to generate multiple neural crest derivatives. In comparing dermal precursors, Yu and colleagues (2010) found that 18/19 human orthologs of the SKP signature were also expressed by HFSC/NCCs. Moreover, HFSC/NCCs showed a profound enrichment (114-fold) for the dermal marker gene *dermo-1*, as well as dermal papilla–associated genes *Shox2*, *twist*, *alkaline phosphatase*, *versican*, and *fibronectin* (Rendl *et al*., 2005), all of which are also expressed in SKPs, but not typically in the outer root sheath.

The surprising similarities observed among various populations of hair follicle NCSC-like cells highlight an important point. Perhaps it is residence within the hair follicle microenvironment rather than developmental origin (i.e., neural crest origin versus non–neural crest origin) that is key to inducing or recapitulating this neural crest–like multipotency. In removing cells from the repressive niche signaling, or, alternatively, by overwhelming tissue-derived restrictions by applying exogenous signals (as exemplified by bFGF-induced formation of *in situ* follicle spheres in this study), this dormant plasticity is liberated. On the other hand, for remnants of the neural crest (such as EPI-NCSCs or facial SKPs) the hair follicle niche may promote retention of embryonic traits (Fernandes *et al*., 2004a; Sieber-Blum *et al*., 2004). A side-by-side comparison of these precursor populations would help to determine whether they are indeed as similar as they appear to be phenotypically.

HFSC/NCCs are multipotent

Yu and colleagues (2010) performed single-cell clonal differentiation experiments to confirm the multipotency of HFSC/NCC cells. Under specific differentiation conditions, HFSC/NCCs generated s100[+] melanocytes, neurofilament[+] neuron-like cells, SMA[+] smooth muscle cells, adipocytes, osteocytes, and chondrocytes. The investigators also note that, despite repeated attempts, HFSC/NCCs did not give rise to keratinocyte lineages—a surprising result, considering their location within the bulge and the presumption that they originate from Oct4^{+}K15^{+} cells (Yu *et al*., 2006). This raises the question of what the endogenous function of these cells is within the hair follicle/skin.

As Yu and colleagues explain, the therapeutic potential of an accessible neural crest–like adult stem cell is enormous. Before we can contemplate their therapeutic utility, however, it is imperative to provide definitive evidence that their derivatives are truly functional. In this regard, glial cells have been successfully derived from SKPs (Biernaskie *et al*., 2007; McKenzie

et al., 2006) and from nestin-expressing bulge cells (Amoh *et al.*, 2005), as evidenced by the formation of compact myelin following transplant to injured sciatic nerve or into damaged spinal cords. Unfortunately, bona fide neuronal differentiation from nonneural adult tissues has proven much more difficult to obtain, and none of these cells has demonstrated functional competency beyond an immature neuronal phenotype (Fernandes *et al.*, 2006; Joannides *et al.*, 2004). That is, both groups have confirmed the presence of voltage-dependent $Ca2^+$ channels, but neither has provided evidence for $Na2^+$ channel–dependent depolarization and generation of an action potential. In this regard, Yu *et al.* attempted to characterize HFSC/NCC-derived neurons further. First, they have shown that exposure of HFSC/NCCs to neuronal differentiation conditions induces robust expression of a host of genes associated with nervous system development and synaptic transmission, indicating that these cells are committing to the neural lineage. The authors then asked whether HFSC/NCC-derived "neuronal" cells generated *in vitro* are functional *in vivo*. Differentiated HFSC/NCCs were transplanted into the forebrain of immunocompromised mice and examined 4 weeks later. Interestingly, many of the cells survived and retained their neurofilament-positive phenotype. This is an exciting first step, although it does not address the question of whether NFSC/NCCs are capable of generating mature neurons. Future studies of neuronal differentiation from somatic stem cells should include more definitive measures of mature neuronal function such as coincident expression of specific neurotransmitter phenotypes, evidence of synaptic connectivity and integration within pre-existing neural circuitry, and electrophysiological competency.

Summary

The work by Yu and colleagues imparts important insights into hair follicle biology and adult somatic stem cell biology. The investigators provide further evidence that human hair follicles are a repository of neural crest–like stem cells and that these cells persist in adult human skin. Their work also generates further impetus for studies to address the endogenous function and functional viability for therapeutic use, not only of NCSC-like stem cells within the hair follicle, but also of those potentially residing within other niches, such as bone marrow (Nagoshi *et al.*, 2008), gut (Kruger *et al.*, 2002), cornea (Yoshida *et al.*, 2006), and heart (Tomita *et al.*, 2005).

CONFLICT OF INTEREST
The author states no conflict of interest.

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