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Previews

There and Back Again: Hair Follicle Stem Cell Dynamics

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Recently in *Cell*, Hsu et al. (2011) defined the relationship between stem cells and differentiated progeny within a hair follicle lineage. Their work reveals that stem cell descendants that have migrated out of the bulge can return to this niche and actively contribute to its function.

Stem cells are defined by self-renewal and multipotency and participate in homeostasis and injury repair in numerous tissues within the adult organism. They are often characterized by their relative quiescence, as well as residence in specialized niches throughout the body. While differentiated stem cell progeny have been described for multiple lineages, the circumstances under which a daughter cell, or descendant, adopts a permanently committed state remain unclear. Recently in *Cell*, Hsu et al. (2011) used the murine hair follicle (HF) as a model system to address questions of fate commitment and function for multiple cell types in a stem cell lineage, both within and outside of the niche. Their findings demonstrate that recent HF stem cell derivatives return to the bulge niche to serve as future stem cells, while more committed progeny home back to a distinct layer of the niche to maintain stem cell quiescence.

Throughout the postnatal hair cycle, the follicle undergoes phases of regression (catagen), rest (telogen), and regeneration (anagen), producing a new hair fiber during each cycle. Over 20 years ago, a reservoir of slow-cycling, label-retaining cells was identified by nucleotide pulse-chase experiments in the permanent, upper portion of the murine follicle, continuous with the outer root sheath (ORS), in a compartment known as the "bulge" (Cotsarelis et al., 1990). While this local expansion of the ORS is not visible in murine pelage (coat) follicles until approximately 3 weeks after birth, recent findings have established that slow-cycling bulge progenitors exist much earlier and are specified during embryonic development (Nowak et al., 2008). Clonal and in vivo lineage analyses of bulge cells, coupled with reconstitution assays, revealed that these undifferentiated cells are able to self-renew and contribute to all epithelial lineages in the skin, including the HF, sebaceous gland, and interfollicular epidermis (Blanpain et al., 2004; Morris et al., 2004).

During periods of HF growth, previous transplantation and genetic marking studies have demonstrated that stem cells from the bulge migrate downward along the ORS to the base of the HF, giving rise to transit-amplifying matrix cells, which in turn proliferate and differentiate to generate the various layers of the inner root sheath and hair shaft (Oshima et al., 2001; Nowak et al., 2008). The characteristics of these migratory cells upon exiting the bulge have not previously been defined, though several lines of evidence point to retained stem cell properties. For example, portions of the vibrissa (whisker) follicle ORS located below the bulge are able to generate clonogenic keratinocytes and form skin epithelial lineages upon embryo transplantation in a hair-cycle-dependent manner (Oshima et al., 2001). Moreover, ORS cells express numerous bulge stem cell markers that are not found in the more differentiated epithelial cells at the base of the follicle (Fuchs, 2009), lending further support to the notion that early bulge descendants may retain some properties of their stem cell precursors. However, the in vivo dynamics of these cells beyond follicle growth and their particular relationship to the bulge stem cell niche have remained elusive.

Hsu and colleagues (2011) have used a sophisticated combination of lineage tracing and nucleotide pulse-chase experiments at various time points to monitor the activity of ORS cells throughout the HF cycle and precisely determine the timing and nature of their lineage commitment. The authors first employed a Tet-Off system whereby administration of doxycycline repressed expression of a histone *H2B-GFP* transgene throughout the skin epithelium. A long doxycycline chase that began before the first postnatal growth phase revealed that ORS cells along the length of the follicle display a range of proliferative activity during anagen, with the cells closest to their bulge predecessors cycling the slowest and, further, that these upper ORS cells survive the destructive phase of the cycle. By preferentially labeling upper ORS cells during midanagen utilizing a tamoxifen-inducible *LacZ* transgene driven by the *Lgr5* promoter or a short BrdU pulse in combination with the Tet-Off *H2B-GFP* model, the authors demonstrated that upper ORS cells are the main contributors to the new bulge and hair germ during telogen.

Postponing the BrdU pulses until late anagen using the Tet-Off *H2B-GFP* system revealed that cells in the mid-zone of the ORS supply additional cells to the telogen hair germ. The authors then employed a Tet-On *H2B-GFP* lineage tracing model under the control of the *keratin 14* (*K14*) promoter to induce GFP expression in the ORS upon application of doxycycline during midanagen. Coupling this system with a BrdU pulse in late anagen, the authors demonstrated that lower ORS cells are also able to home back to the stem cell niche, giving rise to

cells in the CD34$^-$K6$^+$ inner layer of the new bulge.

The cells in this unique inner bulge population expressed numerous HF stem cell transcription factors and were shown to remain quiescent and stationary during the following hair cycle through further nucleotide pulse-chase experiments. Additional lineage tracing analysis in the Tet-Off *H2B-GFP* system with a chase throughout multiple hair cycles revealed that, importantly, CD34$^+$ new bulge and hair germ cells are the sole contributors to newly developing hair follicles, effectively ruling out a role for the inner bulge layer in HF homeostasis.

The authors next explored functional differences between the bulge layers using wounding and cell ablation experiments, together with BrdU pulses applied at the time of injury. Upon introduction of punch wounds to the skin or ablation of CD34$^+$ bulge cells by means of an inducible *K15-DTR* (diphtheria toxin receptor) model, CD34$^+$ new and old bulge cells briefly proliferated during wound repair, whereas K6$^+$ inner bulge cells remained quiescent. Alternatively, targeted ablation of K6$^+$ bulge cells through an inducible *Sox9-DTR* model led to hair loss and rapid re-entry into anagen, marked by a prolonged increase in CD34$^+$ bulge cell proliferation. In examining the mechanism by which K6$^+$ bulge cells might contribute to HF quiescence, the authors revealed high expression of *Fgf18* and *Bmp6* in these cells and demonstrated that injection of each factor was capable of inhibiting activation of CD34$^+$ bulge cells at the time of K6$^+$ cell ablation.

Several novel findings of broad importance to both HF and stem cell biology are introduced in this study. First, slow-cycling stem cell descendants persist outside of the niche during hair growth. These cells survive the widespread apoptosis of the lower follicle during catagen and, furthermore, serve as functional stem cells during the next cycle of follicle regeneration. Hsu and colleagues (2011) thus provide direct evidence to support the hypothesis foreshadowed by previous studies (Oshima et al., 2001; Jaks et al., 2008) that HF stemness is not wholly maintained by the bulge niche but is an intrinsic characteristic of the cell itself, consistent with evidence from the hematopoietic stem cell field.

Second, rapidly cycling ORS cells are also able survive catagen and return to the bulge, albeit in a distinct layer. This observation puts into context the prior finding that actively cycling Lgr5$^+$ bulge and hair germ descendants in the mature follicle return to these structures by the following telogen (Jaks et al., 2008). While these lower ORS cells are permanently committed and no longer possess proliferative potential, they serve two vital roles in the stem cell niche, namely, anchoring the club hair and maintaining stem cell quiescence during telogen. The cellular dynamics demonstrated here lend support to key aspects of the HF predetermination hypothesis proposed by Panteleyev et al. (2001), in that lower ORS cells are spared from apoptosis during catagen and retain a memory of the previous hair cycle that shapes their future function in the follicle.

Finally, the authors contribute significant functional data to substantiate the heterogeneity of cell types in the bulge described by Blanpain et al. (2004). They clearly demonstrate that cells in the CD34$^+$ outer bulge layer function as bona fide stem cells capable of follicle regeneration and wound repair, consistent with previous genetic lineage tracing results (Morris et al., 2004; Ito et al., 2005), while CD34$^-$K6$^+$ inner bulge cells, though quiescent, actively contribute to the niche environment. Future studies in the field must now take into account that HF stem cells beyond the first postnatal cycle are not naive and immobile residents of their niche, but that their movements during previous cycles may have exposed them to various signaling climates along the length of the follicle that may have imparted these cells with as yet unrecognized attributes.

Having established a range of properties and fates for HF stem cell descendants, it will now be interesting to address how these characteristics are acquired and maintained outside of the bulge niche. In particular, the question of whether HF stemness is directly correlated with the number of cell divisions or influenced by additional signaling and architectural cues in the local environment. The unique combination of lineage tracing and labeling techniques employed in this study provide a robust model with which to explore these questions.

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