Have Hair Follicle Stem Cells Shed Their Tranquil Image?

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In a recent issue of *Nature Genetics*, Jaks et al. (2008) demonstrate that hair follicle cells expressing the intestinal stem cell marker Lgr5 are hair follicle epithelial stem cells. In contrast to the established bulge stem cell population, Lrg5$^+$ cells are actively cycling and reside in part outside the bulge.

Reflecting on the limits of knowledge, Friedrich Nietzsche claimed that "there are no facts, only interpretations." Nietzsche's aphorism is brought to mind by a recent study (Jaks et al., 2008) that challenges some of the prevailing ideas about the nature of hair follicle epithelial stem cells (HFeSCs).

Hair follicles undergo continuous cycling of growth (anagen), involution (catagen), and quiescence (telogen). The growth phase requires a large number of keratinocytes that are thought to be derived from a pool of long-lived HFeSCs giving rise to all epithelial layers of the hair follicle during each hair growth cycle (Cotsarelis, 2006; Fuchs, 2007). Studies over the last two decades have provided support that these stem cells reside in the hair follicle bulge (Figure 1A), a region of the outer root sheath that is adjacent to the site of the arrector pili muscle attachment. The bulge region is thought to provide a unique niche for stem cell maintenance. Epithelial cells migrate from the bulge region downwards along the outer root sheath and into the hair follicle bulb during anagen. Keratinocytes of the bulge are quiescent and long-lived (as measured by BrdU label retention), multipotent (can give rise to all cell lineages of the hair follicle), and have a high proliferative potential, thus fulfilling many of the traditional criteria for true stem cells. In addition to BrdU label retention, bulge cells are characterized by CD34 expression and high transgenic keratin 15 (K15) promoter activity. On the basis of these markers, investigators have isolated putative HFeSCs to study their molecular characteristics and to follow their fate in vivo (Morris et al., 2004; Tumbar et al., 2004). While there has been strong consensus that the bulge harbors quiescent stem cells, it remains unclear what portion of epithelial cells in this location are true HFeSCs.

Enter the aforementioned study by Jaks et al. that centers on the Leucine-rich G protein-coupled Receptor 5 (Lgr5), previously identified as a marker of intestinal stem cells (Barker et al., 2007). In telogen hair follicles, Lgr5 is expressed in the lower bulge and the adjacent secondary germ, an aggregation of epithelial cells located at the base of the telogen follicle between the bulge and the dermal papilla (Figure 1A). Keratinocytes of the secondary germ are thought to be derived from the lower bulge and initiate construction of the hair follicle (Ito et al., 2004). Active proliferation was observed in Lgr5$^+$ cells of the secondary germ during early anagen and in the lower outer root sheath during mid anagen; interestingly, Lgr5$^+$ cells and their progeny are rarely BrdU label retaining. Jaks et al. used elegant in vivo lineage tracing experiments to show that Lgr5$^+$ cells give rise to all cell lineages of the hair follicle below the sebaceous gland; contribution to the sebaceous gland was only found in transplantation experiments, which may not reflect the physiological role of these cells. Assuming there was no leakiness in the lineage-tracing experiments, Lgr5-derived cells persist for at least 14 months, indicating that Lgr5$^+$ cells are long lived, consistent with their function as true stem cells. Lgr5$^+$ cells are actively cycling, yet long living and multipotent, and thus, the authors challenge the prevailing idea that HFeSCs are quiescent. And since Lgr5$^+$ cells are found outside the bulge—partially during telogen and completely during late anagen—the authors also question whether a specific niche within the bulge is required for maintaining "stemness."

These intriguing findings raise the question of whether we are seeing the beginning of a new paradigm for HFeSCs or whether the results of Jaks et al. somehow fit within the existing paradigm of quiescent bulge stem cells. Previously, Morris et al. used the K15 promoter to mark bulge cells, thus demonstrating that these cells give rise to all cell lineages of the hair follicle (Morris et al., 2004). These results are similar to the Lgr5 cell-fate experiment, with the exception that Lgr5$^+$ cells do not contribute to the sebaceous gland under normal hair follicle cycling conditions. A caveat of both these studies is that while K15 promoter activity and Lgr5 expression seem to mark HFeSCs, populations labeled with these molecular markers may be heterogeneous; only a subset of both the K15$^+$ and Lgr5$^+$ cell pools is likely to be true stem cells. Since there is overlap between K15 and Lgr5 expression in the lower bulge region during telogen, it is formally possible that the two studies marked the same cell population. However, the fact that cells traced with the K15 promoter gave rise to sebocytes in addition to hair follicle lineages suggests the possibility that a subset of K15$^+$ cells, presumably quiescent, are the most proximal stem cells giving rise to both sebocytes and the Lgr5 lineage, which in turn gives rise to cell lineages of the hair shaft proper (Figure 1B). It should be noted that under this model, the Lgr5$^+$ cells are also multipotent stem cells, albeit with a slightly more limited lineage potential than the Lgr5$^-$K15$^+$ stem cells, because at least a subset of Lgr5$^+$ cells are long lived. It is also possible that there are two independent populations of distinct stem cells, both marked by K15 promoter activity: one (Lgr5$^+$) giving rise to the lower hair follicle and the other (Lgr5$^-$)

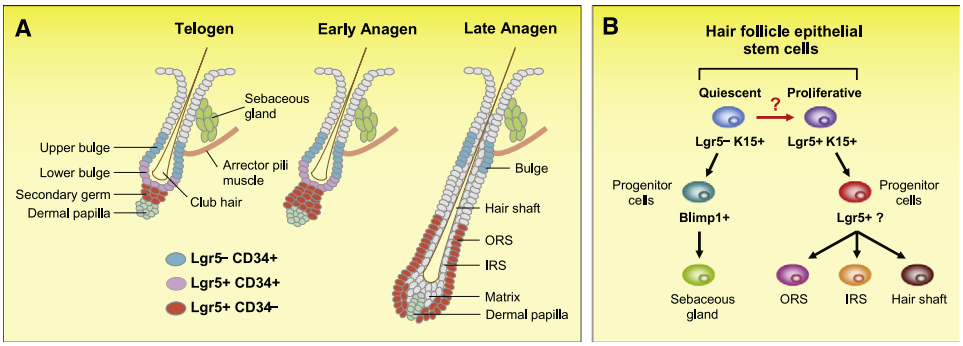

Figure 1. Lgr5$^+$ Cells Contain Hair Follicle Epithelial Stem Cells

(A) Schematic representation of the expression of Lgr5 and CD34 in telogen and anagen hair follicles. BrdU label-retaining cells are only found within the bulge. (B) A possible model for hair follicle epithelial stem cell (HFeSC) hierarchy based on lineage-tracing studies. The model proposes that quiescent Lgr5$^-$K15$^+$ HFeSCs give rise (indicated by red arrow) to more proliferative Lgr5$^+$K15$^+$ HFeSCs, which in turn give rise to cell lineages of the hair shaft as well as the outer and inner root sheaths. In addition, the Lgr5$^-$K15$^+$ HFeSCs give rise to the sebaceous gland cells. It is also possible that the Lgr5$^-$K15$^+$ and Lgr5$^+$K15$^+$ HFeSC populations are lineage independent (indicated by the question mark above the red arrow). It is still unknown whether shorter-lived progenitor cells derived from Lgr5$^+$ HFeSCs also express Lgr5. Note that K15$^+$ indicates high keratin 15 promoter activity in transgenic mice; endogenous K15 expression is more widespread. ORS, outer root sheath; IRS, inner root sheath.

giving rise to the sebaceous gland and most of the upper part of the hair follicle (Figure 1B). Finally, it is possible that hair follicle epithelial cells are highly plastic and multiple distinct stem cells exist in several locations, including the bulge, secondary germ, bulb, and sebaceous gland (Ghazizadeh and Taichman, 2001; Kopan et al., 2002; Legue and Nicolas, 2005).

One of the important contributions of the Jaks paper is that it has provided a more detailed molecular definition of the bulge region. While all bulge cells appear to be CD34$^+$, only the lower bulge is Lgr5$^+$ (Figure 1A), suggesting that these two regions of the bulge may have distinct roles in hair follicle homeostasis. In fact, the Lgr5$^+$CD34$^+$ cells have the highest colony-forming ability in vitro, suggesting that this subpopulation is particularly enriched for stem cells. An unbiased and systematic comparison of global gene expression in these two populations of the bulge may lead to the identification of novel markers allowing the isolation of purer populations of HFeSCs.

The Lgr5$^+$ cell populations at telogen also consist of two different subpopulations: Lgr5$^+$CD34$^+$ cells in the lower bulge and Lgr5$^+$CD34$^-$ cells in the secondary germ (Figure 1A). Since most of the experiments in the Jaks paper were done with the total Lgr5$^+$ cell population, the possibility of heterogeneity remains to be tested. The in vitro colony-forming ability was found in the Lgr5$^+$CD34$^+$ FACS isolated cells, but no data were provided testing whether Lgr5$^+$CD34$^-$ cells have this ability. The hair follicle reconstitution assays, as well as lineage tracing experiments, were performed using Lgr5$^+$ cells, consisting of both CD34$^+$ and CD34$^-$ cells. Thus, while the authors demonstrated stem cell growth characteristics for Lgr5$^+$ cells of the lower bulge, it is unclear whether Lgr5$^+$ cells of the secondary germ are equally enriched in stem cell activity. It will be important in future experiments to further define and test the function of subpopulations of the total Lgr5$^+$ cell population within hair follicles.

The novel findings of Jaks et al. highlight the power of in vivo cell lineage tracing studies and the importance of discovering new cell surface markers for the isolation of HFeSCs. Further studies will be required to determine whether the existing model of quiescent bulge stem cells will survive the challenge of the identification of actively cycling HFeSCs.

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