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Niche Crosstalk: Intercellular Signals at the Hair Follicle

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A recent series of papers, including Festa et al. (2011) in this issue, has revealed unexpected interdependent relationships among cell populations residing in and around the hair follicle. These interactions between different lineages of stem cells are crucial for hair follicle growth and cycling and point to a complex crosstalk in stem cell niches.

Adult stem cells that contribute to sustained tissue homeostasis maintain their ability to act as self-renewing progenitor cells by virtue of their cellular microenvironment, referred to as the stem cell niche. One of the most widely studied stem cell compartments is the hair follicle, which is characterized by a remarkable diversity and complexity of stem cell repositories. The epithelial bulge, located in the permanent portion of the upper follicle, is the most-well studied of these. However, with increasingly refined molecular dissection and lineage tracing techniques, epithelial cells outside this bulge have also been shown to display stem cell characteristics. The bulge itself recently was subdivided conceptually into a number of mininiches, or compartments, according to functional capabilities or specific expression profiles (Woo and Oro, 2011).

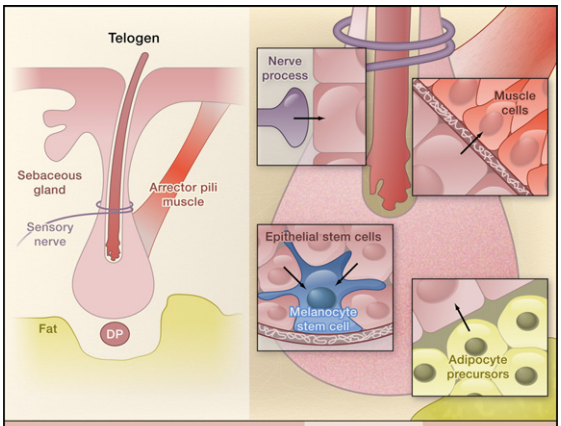
The hair follicle is unique among mammalian biological systems because of its repetitive cyclic activity. Periods of fiber growth involve intense mitotic activity (anagen) and are followed by a short apoptotic phase (catagen), during which the follicle regresses, and then a "dormant" stage (telogen) before the resumption of another growth phase. A series of recent papers has begun to elucidate how neighboring cells, both within and outside the niche, influence hair follicle growth and cycling in fascinating new ways. Some of these studies echo a hypothesis that emerged some 50 years ago and was championed by William Bullough and colleagues, who described an antimitotic substance in skin that stimulated epidermal mitosis when it was removed and whose effects could be reversed. Bullough coined the term "chalones" for these molecules, which act to inhibit epidermal proliferation via a negative feedback mechanism (Bullough 1962). Chalones are tissue specific, and their presence in the skin, as in other tissues, has remained elusive. Nonetheless, in recent years, evidence has begun to emerge identifying a source of inhibitory signals that reside outside of the hair follicle itself. In particular, the long recognized synchrony between hair follicle lengthening and shortening and the oscillations in thickness of the underlying adipocyte layer in the skin have been linked to inhibitor and activator signals acting in tandem (Plikus et al., 2008). Likewise, other components of the skin oscillate with the hair cycle; for example, previous work has demonstrated considerable plasticity in skin and follicle innervation, including loss of nerve fibers at the end of the cycle and an increase during anagen (Botchkarev et al., 1997).

Here, we will highlight new findings on cells and structures both within and outside of the follicle, including melanocytes, muscle, nerves, and fat, as well as their unexpected contributions to hair follicle maintenance and cycling. These recent studies highlight the rich complexity of stem cell-niche interactions (Figure 1) and suggest the potential for alternative mechanisms and new directions in studying skin disorders.

The Epidermal-Melanin Unit: Dependence of Melanocytes on Neighboring Keratinocytes

Clinical observations have hinted at the fact that melanocytes and keratinocyte stem cells must have synchronized activities because there are disease states in which these two properties have become uncoupled. For example, from studies in the depigmenting disease vitiligo, it is known that that keratinocytes retain their function even as melanocytes are destroyed by autoimmune processes, resulting in loss of epidermal pigment. Likewise, when the hair regrows in alopecia areata, the first hairs to grow back are often colorless or white, and melanin pigment returns when the immune response against hair follicle melanocytes has subsided. In both instances, restoration of melanin pigmentation depends on new melanocytes that emanate from the hair follicle bulge, suggesting an intimate functional relationship between these co-inhabitants of the niche (Figure 1).

In two recent papers, Tanimura et al. (2011) and Rabbani et al. (2011) provide molecular evidence for a dependence of melanocytes upon keratinocytes—two cell types of distinct embryonic origin, neural crest, and ectoderm that both reside in the hair follicle bulge. First, Tanimura et al. show that the hair follicle keratinocyte stem cells themselves play a key role in establishing the melanocyte stem cell niche environment in the bulge. By knocking out *Col17a1*, a basement membrane collagen gene exclusively expressed in hair follicle keratinocytes, but not in melanocytes, it was found that *Col17a1* was essential for maintaining the quiescence of the bulge stem cells that form a



Epithelial stem cells maintain melanocyte stem cells	Col17a1, TGFβ	Tanimura et al., 2011
Epithelial stem cells promote melanocyte differentiation	Wnt ligands	Rabbani et al., 2011
Arrector pili muscle attaches to bulge cells which promotes muscle differentiation	Nephronectin and alpha8 Integrin	Fujiwara et al., 2011
Signals from peripheral nerves alter the properties of the epithelial cells in the upper bulge	Shh	Brownell et al., 2011
Adipocyte precursors stimulate follicle cycling	PDGF	Festa et al., 2011

Figure 1. Intercellular Interactions in the Hair Follicle Niche
(Top) Schematic illustration representing the reported interplay between the follicle and four different associated cell types. (Far-left) The overall anatomy of the hair follicle in telogen. Insets on right focus on pairwise cell-cell interactions. Signaling via nerves (purple) produces a direct influence on local epithelial stem cells, and the development and attachment of the arrector pili muscle (red) is indirectly dependent on epithelial cells. Fat cell precursors (yellow) induce progression of the follicle from telogen to anagen and thus affect follicular cycling, and melanocyte stem cell (blue) activation during follicle regeneration is influenced by neighboring epithelial stem cells. DP, dermal papilla.
(Bottom) Summary of intercellular interactions and the molecules implicated in the cross-cell signaling.

network of supporting cells around the melanocyte stem cells. Intrinsic signaling within the keratinocyte niche is altered, and this in turn leads to a secondary disruption of the melanocyte niche. Moreover, these surrounding keratinocytes are a source of TGFß, which is in turn required to maintain melanocyte stem cells by ensuring their immaturity and quiescence, demonstrating that keratinocyte-derived TGFß signaling promotes niche properties of hair follicle keratinocyte stem cells required for melanocyte stem cell maintenance.

The second paper by Rabbani et al. (2011) sought to identify factors that control coordinated repigmentation and hair growth in the context of organ regeneration that defines hair follicle cycling. By focusing on the Wnt pathway, the authors elegantly manipulated Wnt signaling in both the keratinocytes and the melanocyte compartments using a number of different mouse models. They report that Wnt signaling was shown to be a key intrinsic pathway for melanocyte stem cells, yet paradoxically, the melanocytes themselves do not express Wnt ligands. Instead, it was found that the neighboring keratinocytes produce Wnt ligands that activate Wnt signaling within the melanocytes. The target of Wnt signaling in the melanocyte may be endothelins, which are known to be potent mitogens for melanocytes. The converse of this observation is, of course, that a Wnt-suppressed environment is required to maintain melanocyte stem cells in an undifferentiated state. One major unexplained finding in hair follicle stem cell activation has been the presence of Wnt inhibitors in the same compartments of the hair follicle as Wnt-activating molecules. The authors suggest that the role of Wnt inhibitors may be to maintain melanocytes in the undifferentiated state.

Both groups draw from intriguing examples in the *Drosophila* literature in which stem cell niches are maintained via similar mechanisms. Tanimura et al. (2011) cite the example of the *Drosophila* testes in which niche function is provided by one type of stem cell (hub cells) for another niche-resident germline cells by governing the cell adhesion environment to establish the niche (Leatherman and Dinardo, 2010). Likewise, Rabbani et al. (2011) raise the example of collaboration between stem cell types in the *Drosophila* ovary, where germline and escort stem cells mutually establish a niche for one another (Kirilly and Xie, 2007). This physical arrangement promotes synchronous cell divisions and comigration of the cells within the ovary, reminiscent of the physical association of melanocytes with keratinocytes as melanosomes are deposited into the keratinocytes by dendritic processes on the melanocyte surface. The discovery of a critical shared regulatory factor for both cell types, yet provided by only one, raises the notion of efficiency of resource utilization in simpler systems in the fly ovary, as well as the generation of complex organs such as the hair follicle.

In mammals, another example of interacting stem cell populations comes from the hematopoietic system. The bone marrow stem cell niche is known to include mesenchymal stem cells that are capable of expanding and differentiating in tandem with hematopoietic stem cells. This provides evidence to support the coregulation of stem cell compartments and how systemic signals can regulate the local microenvironment to promote homeostatic regeneration (Méndez-Ferrer et al., 2010). Finally, the findings from both papers raise the alluring possibility that treatment of pigmentary disorders may be targeted at hair follicle keratinocytes as an indirect entry point, albeit via a neighboring cell, for restoration of melanocyte function.

Local Influences on the Epithelial Stem Cell Niche: Bulging Muscles and a Bundle of Nerves

The skin is the most distant outpost of the nervous system, and the hair follicle itself is home to specialized neural cell types, such as Merkel cells and nestin-positive epithelial cells, as well as a specialized muscle known as the arrector pili muscle (APM), which contracts in response to temperature, touch, and other sensory inputs by making the hairs "stand on end." However, the mechanistic relationship of these cell populations to hair follicle epithelial stem cells has remained elusive.

Two recent studies indicate that muscle and nerve interact with cells in the epithelial bulge in very different ways. In the case of the APM, the work of Fujiwara et al. (2011) shows that, during follicle development, bulge cells specifically expressing a key APM anchoring the protein nephronectin at the basement membrane interact locally with dermal cells expressing its receptor, α8 integrin, promoting muscle differentiation and attachment. When nephronectin, also shown by the authors to be a Wnt target gene, is genetically ablated in mice, the attachment site of the APM to the stem cell compartment is relocated

higher (with compensatory expression by a nephronectin family member EGFL6). Crucially, however, there is no shift in the pattern of expression of the stemness marker K15 displayed by bulge cells. Thus, the APM does not directly affect epithelial stem cell activity, showing that not all elements of the niche environment are implicated in epithelial stem cell regulation. Moreover, the focal perifollicular distribution of basement membrane constituents around the follicle stem cell compartment would suggest an influence on inner-epithelial cells. However, in this case, it defines an external feature, the APM attachment point. Indeed, it could be argued that, compared to dermal sheath cells that have the potential to signal to and interact with epithelial stem cells on the other side of a basement membrane, mature muscle is far less interactive, and as a consequence, epithelial cells immediately adjacent to the APM attachment point could be maintained in a quiescent state. On the other hand, the authors point to some close parallels in molecular expression between the epithelial cells adjacent to the APM and tendons and indeed suggest that the bulge cells could be functioning in this manner. This is interesting given that there is evidence linking mechanical stress in keratinocytes to cell proliferation via the MAP kinase pathway (reviewed in Reichelt, 2007).

The close connection between skin innervation and hair follicles has been the subject of much investigation (Paus et al., 2008). In studying the input of sensory nerves to the hair follicle, Brownell et al. (2011) demonstrate a direct neural influence on bulge stem cells via specific retrograde Shh signaling and describe how this localized interaction can establish a compartment or micro-niche. In telogen, it was possible to show two discrete regions of the follicle that expressed Gli1-LacZ as a readout of Hh signaling. One is located at the top of the bulge, corresponding to the zone where peripheral nerves surround the hair follicle, and the other at the dermal papilla (DP) and the lower bulge and hair germ. Surgical deinnervation of those follicles (as shown by loss of neurofilament staining) also resulted in a loss of Gli1-LacZ staining in adjacent upper-bulge cells. Thus, the consequences of deinnervation were failure of intraneural Shh signaling and then of Gli1 signaling in a compartment of the upper bulge. Although the lack of innervation caused no visible anatomical changes to the follicle, the loss of local signaling around the upper bulge compartment produced changes to the molecular signature of the associated epithelial population, manifested as a shift in expression of the stem cell marker K15. Importantly, this is not just fine-tuning, as although there is no direct influence on follicle epithelial cell homeostasis and cycling, alteration of this micro-niche resulted in subtle but distinctive functional changes to the epithelial subpopulation. In wound healing experiments, the cells were still initially capable of replacing skin epidermis but were no longer able to support long-term skin maintenance.

Unlike the observations by Fujiwara et al., (2011) in which the APM niche is manipulated without corresponding detectable changes to the bulge stem cells, ablation of Hh signaling by deinnervation alters the upper-bulge micro-environment. Neural molecular signaling alters the epithelial niche, and signal loss leads to modifications in the adjacent epithelial cells despite no change in their position. The work of Brownell et al. (2011) shows how subcompartments can be created within a niche through localized and specific external influences, whereas Fujiwara et al. (2011) illustrates how the epithelial niche itself can influence the creation and positioning of an external feature.

Long-Range Influences on Fat and Follicles: Together through Thick and Thin

Unlike the above interactions between follicles and melanocytes, nerves, or arrector pili muscles, in which hair follicle cycling is unaffected, the interaction between fat cells and follicles appears to primarily influence cyclic activities. In a modern twist on the chalone/inhibition concept, Plikus and colleagues (2008) provided a novel molecular explanation for how hair follicles are held in their mitotically quiescent state (telogen). They demonstrated a previously unrecognized wave of bone morphogenetic protein (BMP) expression in the perifollicular rodent dermal environment, which did not directly coincide with the Wnt pathway signaling that locally activates follicle stem cell activation (reviewed in Fuchs, 2007). BMPs are known inhibitors of follicle activity, and when present in the dermis at high levels, the tissue is ''refractive'' to regeneration signals. Reduction in BMP (coincidentally, follicle bulge stem cells express the BMP inhibitor noggin) converts the perifollicular environment to a ''permissive'' state, responsive to positive regenerative Wnt signaling. By experimentally decreasing or increasing levels of BMP, these researchers were able to produce shorter or longer periods of telogen in support of the hypothesis. Intriguingly, the bulk of the BMP expression was found to be not in the adjacent dermis but, rather, in the adipocyte cells underlying the follicles, thereby highlighting the importance of the fat/follicle axis.

In the current issue, Festa et al. (2011) extend this relationship further by uncovering a direct and positive influence of fat precursor cells on hair follicle cycling. Using labeling and FACS studies, they demonstrate that the increase in thickness of the skin fat layer that coincides with follicle regeneration is due to cell proliferation and not just hypertrophy. They show that an increase of a subpopulation of adipocyte precursors, previously identified and isolated in other white fat depots, parallels or perhaps even precedes the onset of follicle activation at the key telogen-to-anagen transition point.

By transplanting these adipocyte precursor cells, follicles transitioned from the quiescent to the active phase of the growth cycle. In one particularly elegant experimental strategy, they were able to isolate preadipocyte cells from the skin of mutant mice (Azip/F1) lacking mature fat cells because the final stages of adipogenesis (involving expression of FABP4) are blocked. These donor cells are injected into the skin of another fat-deficient mouse model (Efb1 knockouts) that has no adipocyte precursors in skin because adipogenesis is inhibited at an earlier developmental phase. The fact that the transplanted preadipocytes were able to locally stimulate follicles in the Efb1 mouse to move out of telogen into anagen is strong evidence that they act to initiate the onset of the new hair cycle in regular mouse skin. In terms of the actual signaling modality, Festa et al. (2011) focus on PDGFα as the stimulatory factor. However, it is important to bear in mind that many other signals, including Shh, have been shown experimentally to accelerate the initiation of anagen (Sato et al., 1999). Indeed, one of the key difficulties in elucidating the molecular control of hair follicle activities is discriminating between the primary endogenous signals and

the many other factors closely linked to the cascade of associated changes within and outside of the follicle. Consequently, some caution should be taken when interpreting genetic mouse models in which perturbation of nonspecifically targeted associated cells and tissues indirectly manifest alterations to follicle growth and cycling. Going forward, it will be interesting to see whether adipocyte precursors secrete other factors that contribute to anagen initiation.

Among the many intriguing observations emerging from the Festa et al. (2011) study is that of differentiating adipocytes located directly beneath follicles coincident with the reinitiation of the hair cycle. It will be important to establish exactly how this localized activity comes about, particularly as one cannot preclude a reciprocal step in which signals from the follicles control, perhaps locally, development of the preadipocytes. There is even the possibility that follicle-derived mesenchymal cells (with known adipogenic potential) could contribute to this population. We suspect that this pioneering study is only scratching the surface of follicle/fat crosstalk, which is likely to turn out to be a two-way conversation.

Conclusions and Perspectives

What insights have these studies provided, beyond what appears, on the surface, to be simple structure-function relationships? Further, to what extent does mouse hair follicle biology have relevance to human hair growth, particularly as the hair cycle is not synchronized across follicles throughout life as it is in rodents, and cyclic events can also be modulated by environmental/systemic factors, such as pregnancy and nutritional status. In this context, a recent paper has expanded the original activator (WNT)/inhibitor (BMP) hypothesis to account for the diversity of growth patterns seen in different animals, including humans (Plikus et al., 2011). The themes brought out in these papers raise the possibility of a much more dynamic dialog between neighboring cells. Further, these properties may apply to complex disorders of skin in which the source of pathology may come from both within and outside of the follicle or from interactions between two sources.

Work from Festa et al. (2011) opens the door to important questions regarding fat-follicle interrelationships, beginning with how to reconcile the stimulatory influence of the preadipocyte subpopulation against the refractive adipocyte background environment encompassing the adipocyte layer shown by Plikus et al. (2008). Indeed, it remains to be seen how or whether the adipocyte precursor PDGFα signaling crosstalks with the Wnt/BMP pathway interrelationships described by Plikus et al. (2008). For example, could the PDGFα be acting on BMP signaling by stimulating adipocyte cells to produce a BMP inhibitor/antagonist, thus abrogating the key inhibitory influence on follicle cycling?

The connection between the hair follicle and the nervous system is also an aspect deserving of additional attention. Because skin homeostasis is so frequently perturbed by physiologic or psychoemotional stress, the work of Brownell et al. (2011) provides intriguing molecular evidence for how and where such stress signals may have a direct impact on hair follicle stem cell behavior. Collectively, these studies also raise the prospect of what other resident cell types may also provide molecular input into the hair cycle. For example, the hair follicle is also home to a dynamic and diverse local immune system that has not been widely studied (Christoph et al., 2000). The autoimmune nature of vitiligo and alopecia areata suggest that, at least in a pathological state, the immune system plays a role in regulation of melanocyte stem cells and cyclical hair growth, respectively. These findings suggest that complex autoimmune skin diseases may originate from crosstalk between the hair follicle and immune cells.

Another unifying theme from these papers is that the authors have traced the origin of perturbations in the hair cycle to an extrinsic source, whether a neighboring cell in the niche itself or a cell type outside of the follicle. Many previous studies have attempted to ascribe the significance of changes in gene expression to the follicle itself. However, the studies discussed here elegantly apply genetic strategies to show that, in some cases, the crucial molecular events can originate from other cell types. These studies should encourage researchers to look beyond the follicle for answers to longstanding enigmas in hair biology.

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