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The Dynamic Duo: Niche/Stem Cell Interdependency

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SUMMARY

Most tissues in our bodies undergo constant cellular turnover. This process requires a dynamic balance between cell production and elimination. Stem cells have been shown in many of these tissues to be the major source of new cells. However, despite the tremendous advances made, it still remains unclear how stem cell behavior and activity are regulated in vivo. Furthermore, we lack basic understanding for the mechanisms that coordinate niche/stem cell interactions to maintain normal tissue homeostasis. Our lab has established a novel imaging approach in live mice using the skin as a model system to investigate these fundamental processes in both physiological and pathological settings such as cancer, with the goal of understanding how tissues successfully orchestrate tissue regeneration throughout the lifetime of an organism.

The Hair Follicle as an Ideal Model System to Study Stem Cells and Their Niche

The hair follicle stands up as a paradigm for stem cell biology given that several of its diverse cellular components, such as mesenchymal and epithelial cell types, as well as utilized signaling pathways are conserved in many other tissues (Cunha and Hom, 1996; Ribatti and Santoiemma, 2014). The advantage of the hair follicle over other tissues lies both in its unique accessibility for investigation as well as its stereotypic and continuous pattern of regeneration. This process relies on a stem cell pool that is maintained through the sequential phases of growth (Anagen), regression (Catagen), and rest (Telogen) of hair regeneration (Figure 1). These key features enable the field to use this model system to study the regulation of stem cell quiescence and activation in the context of a complete mini-organ. Additionally, the epithelial component of the follicle is highly compartmentalized, which allows us to distinguish different cell types, such as distinct stem cell populations as well as their differentiated progeny, on the basis of their location, morphology, as well as molecular markers (Kretzschmar and Watt, 2014; Rogers, 2004; Schepeler et al., 2014). Specifically, within the hair follicle, the stem cell compartment is comprised of two spatially distinct epithelial populations: the bulge, which surrounds the base of the hair proper (called hair shaft), and the hair germ, which is located directly below the bulge stem cells and in direct contact with the mesenchymal dermal papilla (DP) niche (Cotsarelis et al., 1990; Ito et al., 2005; Jahoda et al., 1984; Panteleyev et al., 2001; Rahmani et al., 2014; Sennett and Rendl, 2012; Tumbar et al., 2004) (Figure 1). While previous data supported a bulge stem cell-centric model to initiate hair follicle growth, our work and that of others have opened up a new view that relies on the coexistence of two functionally distinct pools: the activated hair germ cells, which can more quickly respond to the environmental stimuli to engage in a new growth and the quiescent bulge stem cells. This bi-compartmental organization reconciles the need of the tissue for rapid growth while maintaining a long-term stem cell pool and has been found to be utilized by other tissues such as the blood and the brain (Greco and Guo, 2010; Greco et al., 2009; Li and Clevers, 2010).

Capturing Stem Cell Behaviors during Tissue Regeneration In Vivo

At the start of a new cycle of regeneration, the epithelial compartment of the hair follicle begins its downward growth. We set out to test whether this directional growth was achieved through a spatial organization of cell divisions or instead by randomized cell divisions followed by downward migration and reorganization. To capture behaviors such as cell divisions and migrations within an intact organ, my group developed an intravital multiphoton imaging system, which allowed us to noninvasively image the skin of live mice over time. To visualize the hair follicle in vivo, we utilized transgenic mouse lines that were previously made to label epithelial (*K14-H2BGFP*) and mesenchymal (*Lef1-RFP*) hair follicle populations (Rendl et al., 2005; Tumbar et al., 2004) (Figure 1). Using these reporters in combination with our intravital imaging system, we have performed time-lapse recordings by generating 3D-optical stacks of hair follicles at regular time intervals throughout the phases of hair follicle regeneration (Figure 1). These approaches allowed us to directly capture hair follicle growth beginning with spatially confined epithelial cell division, which occurs in the activated hair germ compartment at the interface with the mesenchymal DP niche. Furthermore, the axes of these divisions are oriented perpendicular to the mesenchymal DP and parallel

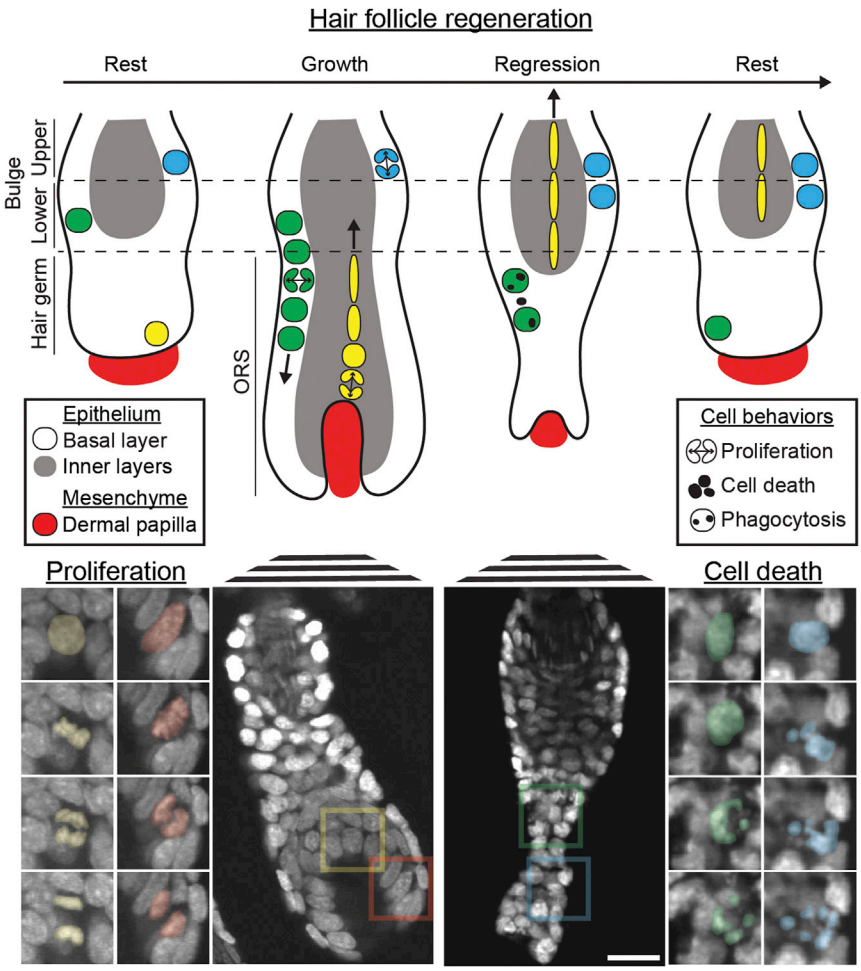

Figure 1. Live Imaging of Hair Follicle Stem Cell Behaviors and Fate during Tissue Regeneration

The hair follicle is comprised of both epithelial and mesenchymal populations. To visualize both cellular compartments in vivo, we utilized the transgenic mouse lines *K14-H2BGFP* (epithelial) and *Lef1-RFP* (mesenchymal). Combining these reporter lines with our multiphoton intravital imaging system, we have performed time-lapse recordings of hair follicles during both growth and regression phases of the hair cycle. We find that cell behaviors, including proliferation, migration, cell death, and phagocytosis, are all spatiotemporally restricted events within subcompartments of the hair follicle epithelium. Coordination of these tissue dynamics results is spatially regulated fate of epithelial stem cells with relation to the mesenchymal DP niche. Scale bar, 25μm.

to the long axis of growth of the hair follicles (Rompolas et al., 2012). These oriented divisions contribute to the newly formed inner differentiated layers, while the expanding basal epithelium (also called outer root sheath or ORS) is generated by a spatially restricted proliferation zone (Rompolas et al., 2013; Sequeira and Nicolas, 2012) (Figure 1). While cell production and loss are often concurrent events in several tissues, the hair follicle provides the advantage of studying them in isolation. To understand how cells are eliminated, we focused on the hair follicle regression phase, which previous work has defined as the destruction phase where the majority of epithelial cells are eliminated. Our imaging approaches showed that (1) cell death targets only the undifferentiated basal cells but spares differentiated inner cells and (2) cell death begins at the bottom of the follicle in contact with the mesenchymal DP niche and then spreads upward in the remaining basal epithelium. Strikingly, we found that epithelial cellular debris was not cleared by professional phagocytes. Rather, basal epithelial cells collectively act as phagocytes to clear dying epithelial neighbors (Mesa et al., 2015) (Figure 1). These findings in the hair follicle, along with work in the mammary gland (Monks et al., 2005), support a new paradigm of physiological epithelial self-clearance.

Our work demonstrated that stem cell behaviors such as proliferation, migrations, cell death, and clearance are spatially organized within compartments and with respect to their proximity to the mesenchymal DP niche. However, we still failed to understand the long-term consequences of these cell behaviors and their spatial regulation toward tissue function during a regeneration cycle. To address whether the stem cell position with respect to their niche impacts their fate, we developed an approach to lineage trace single cells in the stem cell compartment and assess their contribution to the different epithelial layers that are generated during the hair regeneration cycle (~3 weeks). To achieve this, we combined inducible genetic labeling, through inducible Cre-recombinases and fluorescent reporter alleles, with our intravital imaging. This allowed us to revisit hundreds of entire hair follicles (over several millimeters of skin and to depths of over 200 μm