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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

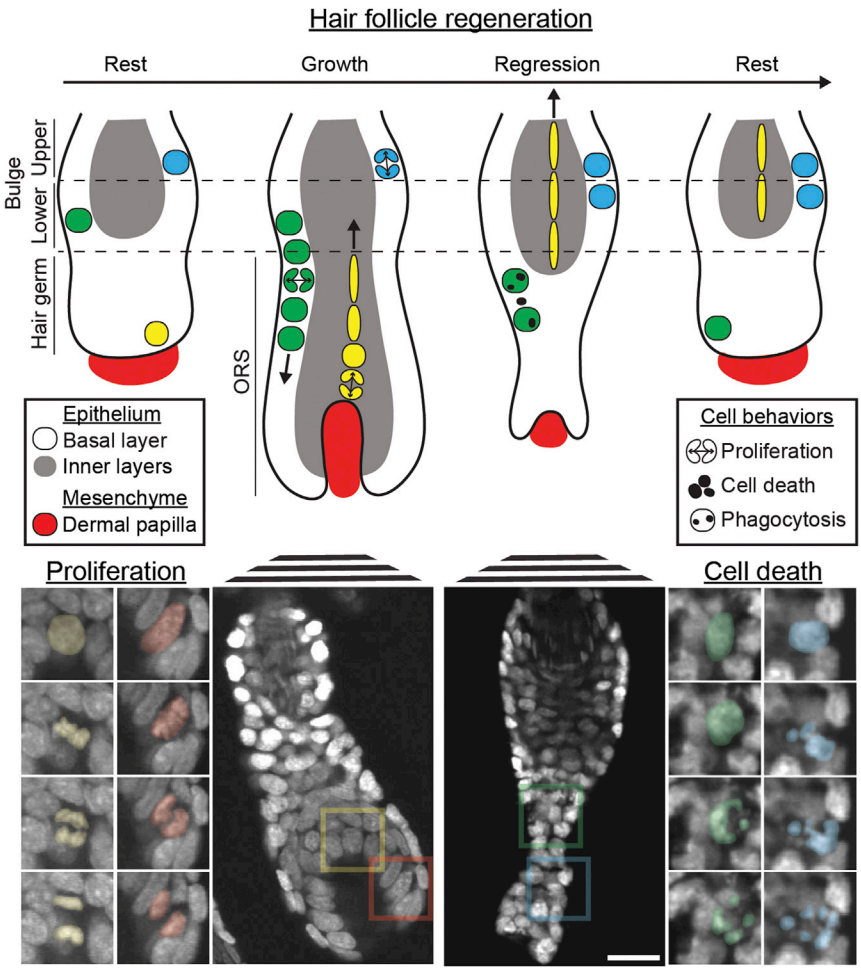

Figure 1. Live Imaging of Hair Follicle Stem Cell Behaviors and Fate during Tissue Regeneration

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

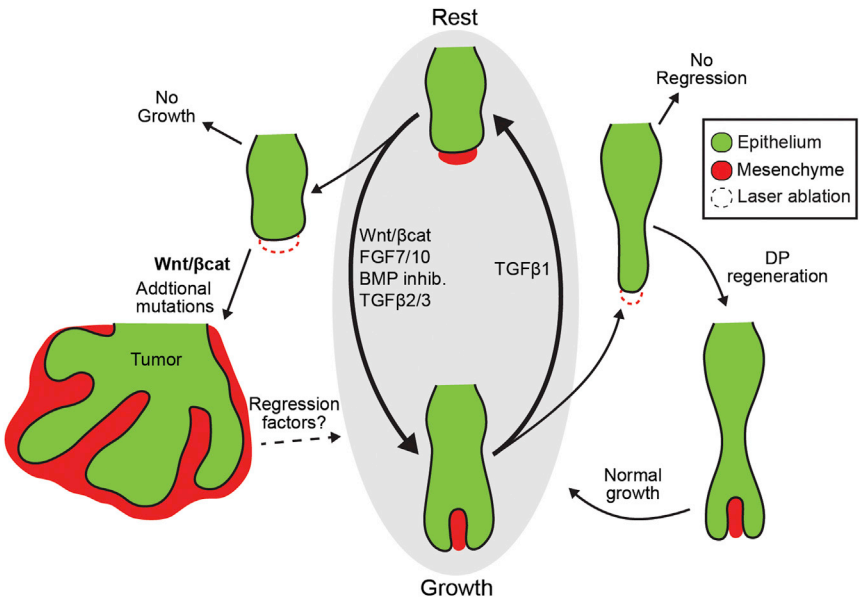

Figure 2. Epithelial/Mesenchymal Crosstalk Coordinates Tissue Regeneration

Laser ablation in combination to our live imaging has allowed us to assess the role of the mesenchymal niche during tissue regeneration. We find that ablation of the niche blocks hair follicle regeneration, both in growth and regression. Epithelial/mesenchymal crosstalk relies on various molecular signaling pathways. Genetic modulation of these signals can lead to aberrant tissue regeneration through either βcat activation or TGF-β inhibition. Reconstitution of a mesenchymal niche can drive tissue growth into both the hair follicle and pathological settings, such as cancer. Conversely, growing tissues can be inducing to regress by niche factors such as retinoic acid (RA) or TGF-β1.

within the skin) to trace the long-term fate of stem cells during hair follicle regeneration (Rompolas et al., 2013). Our revisits of labeled cell lineages revealed that stem cell fate is stereotypic and correlates with initial cell position with respect to the mesenchymal niche at the onset of growth. Specifically, the degree of commitment to differentiation was directly related to the proximity with the mesenchymal DP niche. Cells located at the furthest distance from the DP niche in the upper part of the bulge did not contribute to the next round of hair follicle growth, whereas cells located in the lower bulge engaged during growth to expand the undifferentiated basal ORS layer of the hair follicle. In contrast, cells located in hair germ, therefore in closer proximity to the DP niche at the onset of growth, were consistently found to contribute to the newly forming differentiated layers of the hair follicle, which collectively give rise to the new hair shaft (Rompolas et al., 2013). Conversely, lineage tracing of single basal ORS cells during regression showed that a spatial gradient of cell death emanates from the tip of the follicle. Thus, epithelial cells located in higher proximity of the mesenchymal DP niche have lower chances of surviving than cells located at further distances (Mesa et al., 2015). Finally, revisits over several rounds of hair regeneration cycles revealed the stepwise progression of bulge stem cells in their differentiation path whereby lower bulge stem cells give rise to basal ORS cells and form the hair germ during a complete regeneration cycle and will eventually make differentiated cells during the subsequent regeneration cycle (Rompolas et al., 2013) (Figure 1).

These data collectively demonstrate a direct correlation between stem cell fate and their location with respect to the mesenchymal DP niche and establish a stepwise progression of bulge stem cell fate, with these cells undergoing an ORS/hair germ intermediate before contributing to the differentiated layers of the regenerating hair follicle. Positional correlation with stem cell fate has since been found in additional tissues such as the intestine (Ritsma et al., 2014)

Niche Regulation of Stem Cell Activity

Epithelial-mesenchymal interactions are essential in many developmental processes as well as for the regeneration of several adult tissues (Cunha and Hom, 1996; Ribatti and Santoiemma, 2014). Previous work has demonstrated that the mesenchymal niche of the hair follicle provides molecular signals that are sufficient to induce hair growth (Greco et al., 2009; Jahoda et al., 1984). However, it remained unclear whether the DP is required for hair regeneration and specifically to initiate hair follicle growth and/or regression. To test this, we developed a laser ablation procedure using our multiphoton imaging system to specifically remove the DP prior to the hair regeneration phase under interrogation.

Revisits of hair follicles in which the DP was ablated prior to growth onset showed that DP-ablated hair follicles remained stalled in the rest phase while neighboring unablated hair follicles continued to grow (Figure 2). This finding provides clear evidence that mesenchymal interactions are required to initiate hair growth and is consistent with previous work from invertebrate systems (Byrd and Kimble, 2009; Losick et al., 2011).

When similar experiments were performed at the initiation of regression, we found that DP ablation resulted in a

dramatic reduction in cell death in the hair follicle epithelium, leading to retention of excess basal cells by the end of the regression phase when compared with neighboring follicles. This suggested that the DP promotes basal epithelial cell elimination during the regression phase and raised the question of whether DP-mediated regression serves to eliminate either exhausted basal cells or functional cells from an expanded stem cell pool. To test the functionality of the cells eliminated during regression, we devised an experimental setup to transiently remove the mesenchymal DP (Rahmani et al., 2014) during regression and then track the fate of retained cells. Upon DP regeneration, hair follicles were able to fuel a new round of growth from the pool of cells normally targeted for elimination. This work demonstrates that regression is a regulated process that actively reduces the stem cell pool following expansion during growth (Figure 2) (Mesa et al., 2015). This control of stem cell fate led us to ask whether the niche was sufficient to promote stem cell activity. To test this, we used laser ablation to specifically remove the stem cell compartment at the onset of growth. Interestingly, we found that epithelial cells that do not normally contribute to hair follicle regeneration compensated for bulge stem cell loss and once in contact with the mesenchymal DP niche gave rise to hair follicles that appear indistinguishable from surrounding unablated follicles. These data demonstrate that the niche is capable of conferring a stem cell fate upon injury to neighboring cell populations (Rompolas et al., 2013). Collectively, these data demonstrate that the niche is critical for stem cell activation, elimination, and regulating stem cell fate and behavior.

Signaling Crosstalk between Stem Cells and Their Niche during Hair Regeneration

Growth and regression phases are characterized by different epithelial cell behaviors, yet the mesenchymal DP niche promotes both sets of behaviors. One possible model to account for this regulation is that the mesenchymal DP niche utilizes different signals in each phase of regeneration.

At the onset of hair growth, transcriptional and expression analysis have previously identified signaling pathways that are activate in the mesenchymal DP, including FGF7/10 and BMP inhibitors (Greco et al., 2009; Plikus et al., 2008), as well as in the hair germ, such as activation of Wnt/β-catenin (Choi et al., 2013; Myung et al., 2013; Silva-Vargas et al., 2005). The sufficiency and requirement of several of these pathways for hair growth have been established (Lin and Yang, 2013; Oshimori and Fuchs, 2012). However, it remains unclear whether ectopic activation of downstream regulators of these pathways within the stem cells would overcome the dependence on the endogenous DP for tissue growth. To test this, we combined DP-ablation experiments with genetic β-catenin activation and showed that β-catenin activated hair stem cells generated new hair outgrowths in the absence of their physiological mesenchymal DP niche. This is in contrast with the stall in growth observed in WT DP-ablated follicles. Intriguingly, surrounding mesenchymal cells were observed to broadly contact the newly formed hair growths, although they failed to spatially self-organize like DP clusters. This induction of a new mesenchyme around the growth is consistent with previous work (Collins et al., 2011; Gat et al., 1998; Silva-Vargas et al., 2005). Therefore, ectopic stem cell activation independent from the endogenous niche overrides normal tissue homeostasis signals to promote tissue growth. These results highlight that signals downstream of DP niche activation can drive aberrant growth within the stem cells independently of their endogenous physiological niche and possibly dependent on the induction of a new aberrant niche (Deschene et al., 2014) (Figure 2).

Mesenchymal/epithelial crosstalk has been largely investigated during tissue growth; however, the signals that trigger tissue regression remain elusive. To understand the molecular signaling that facilitate epithelial cell death during regression, we investigated the transforming growth factor-β (TGF-β) signaling pathway, as exogenous delivery of TGF-β1 ligand induces precocious hair follicle regression (Foitzik et al., 2000). We found that TGF-β ligands are expressed in the DP during regression. Additionally, TGF-β signaling was found to be active in the hair follicle epithelium located near the DP niche. This suggested to us that epithelial/mesenchymal crosstalk during regression may be facilitated by TGF-β signaling and prompted us to functionally test the role of TGF-β signaling on epithelial cell death. Conditional ablation of TGF-β receptor I (TGF-βRI) in the hair follicle epithelium during regression led to a stall in hair follicles regression and inhibition of epithelial cell death. This demonstrated that hair follicle regression is regulated through an extrinsic regulation mediated via TGF-β signaling to induce epithelial cell death and spatially restrict the pool of surviving stem cells (Mesa et al., 2015).

All together, these data show that niche/stem cell interactions and their molecular signals are key to regulate tissue growth but also to properly maintain tissue morphology and function.

Niche and Stem Cells in Cancer

The interplay between stem cells and their niche is not only central to tissue regeneration but has also been shown to be critical for cancer. Consistent with this, mutations affecting critical pathways such as Wnt/β-catenin and TGF-β have been found to fuel several cancers, including squamous

cell carcinoma (SCC) in the skin (Guasch et al., 2007; Malanchi et al., 2008; Massagué, 2008). In order to understand how growth is regulated in a cancer setting, we investigated the effects of β-catenin activation on hair follicle stem cell behaviors as ectopic hair growths were forming. Strikingly, our data showed that β-catenin-activated stem cells recruited WT neighboring follicles into the growths. These WT cells contributed to fueling the new ectopic growth by actively proliferating. Transcriptional and genetic approaches demonstrated that β-catenin-activated stem cells created a field of Wnt activation around them by upregulating Wnt ligand production (Deschene et al., 2014). This finding expands our understanding of tumoral growth and expands the focus from the mutated stem cells to the changes in their environment in contribution to this aberrant growth.

The skin regeneration cycle primed us to investigate potential mechanisms of tumor regression that could overcome tumoral growth. To this end, we utilized a peculiar skin tumor known to self-regress, called keratoacanthoma (KA) (Ko, 2010). This tumor has provided us with a powerful platform for understanding physiological principles of tumor regression. Our parallel approaches of SCC with KA revealed that while both tumors share Wnt activation during growth, regressing KAs displayed an upregulation of Wnt inhibitors in their surrounding mesenchyme and an overall reduction of Wnt signaling in the basal proliferative epithelium. Transcriptional approaches identified RA upregulation as an early event during KA regression and prior to Wnt downregulation. To test the function of RA signaling on tumor regression, we ectopically delivered RA to growing KAs and SCCs, which resulted in Wnt downregulation and massive regression of both forms of skin cancer (Zito et al., 2014).

Epithelial cancers such as SCC have mutations that dysregulate epithelial/mesenchymal crosstalk and promote excessive tissue growth through pathways such as Wnt. This works provides a model where distinct behaviors of mesenchymal and epithelial components may shed light on therapeutic approaches to combat tumor growth and re-engage normal tissue regeneration.

Conclusions

Altogether, our innovative live imaging combined with genetic and cellular approaches has provided a new platform for understanding tissue regeneration and cancer in a living mammal. While the emphasis in the field has been centered on the stem cells, our work has shifted the attention to a critical role of the niche, which is consistent with pioneering work in several invertebrate systems. This work has further extended our understanding of tissue regeneration to reveal novel cellular behaviors that contribute to the process, as well as a critical role that the niche plays in orchestrating stem cell activity.

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