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Epithelial Stem Cells and Implications for Wound Repair

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Abstract

Activation of epithelial stem cells and efficient recruitment of their proliferating progeny plays a critical role in cutaneous wound healing. The reepithelialized wound epidermis has a mosaic composition consisting of progeny that can be traced back both to epidermal and several types of hair follicle stem cells. The contribution of hair follicle stem cells to wound epidermis is particularly intriguing as it involves lineage identity change from follicular to epidermal. Studies from our laboratory show that hair follicle-fated bulge stem cells commit only transient amplifying epidermal progeny that participate in the initial wound re-epithelialization, but eventually are outcompeted by other epidermal clones and largely disappear after a few months. Conversely, recently described stem cell populations residing in the isthmus portion of hair follicle contribute long-lasting progeny toward wound epidermis and, arguably, give rise to new inter-follicular epidermal stem cells. The role of epithelial stem cells during wound healing is not limited to regenerating stratified epidermis. By studying regenerative response in large cutaneous wounds, our laboratory uncovered that epithelial cells in the center of the wound can acquire greater morphogenetic plasticity and, together with the underlying wound dermis, can engage in an embryonic-like process of hair follicle neogenesis. Future studies should uncover cellular and signaling basis of this remarkable adult wound regeneration phenomenon.

Keywords

Wound healing; Re-epithelialization; Epithelial stem cells; Hair follicle stem cells; Hair follicle neogenesis

1. Introduction

Skin epithelium is comprised of epidermis and appendages such as the hair follicle. Both epidermis and hair follicles show significant regenerative potential during their physiological renewal. Epidermis regenerates in a steady state via balanced proliferation of basal cells and shedding of cornified squamous cells at its surface. Hair follicles regenerate via a complex cyclical process. This cycle starts with the activation of stem cells, followed

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by proliferation and differentiation of their progeny. It terminates with apoptotic involution, during which most of the epithelial lineages expanded from hair follicle stem cells at the beginning of the cycle are eliminated. Stem cells themselves survive this apoptotic involution, and regenerate the lost portion of the hair follicle in the next new growth cycle.

Unlike physiological renewal, regeneration of the skin after wounding was historically thought to be only partial, limited to the formation of stratified epidermis over the dermal scar. However, recent findings from our laboratory show that more complete skin regeneration during wound healing is possible. New hair follicles regenerate in the center of large cutaneous wounds via a process that resembles their normal embryonic development (Ito M *et al*, 2007). These findings warrant new inquiries into the true morphogenetic potential of adult epithelial stem cells during wound repair. Therefore, this review will focus on what is currently known about the contribution of various epithelial stem cells to wound healing. It will also introduce the emerging field of embryonic-like wound regeneration.

2. Diversity of epithelial stem cells in the skin

Restoration of skin barrier function is the key priority during wound repair. This is accomplished via rapid re-epithelialization, when the wound becomes covered with the new stratified epidermis. Interestingly, numerous distinct stem cell populations become activated during the healing process and are recruited into the wound. To understand the significance of contribution from these various epithelial stem cells, first we will briefly discuss their physiological heterogeneity and anatomical distribution in the skin.

Epithelial stem cells, in general, fit a broader definition of adult somatic stem cells, as they are quiescent but self-renew and differentiate into at least one type of progeny. Historically, scores of epithelial stem cell populations were identified based on various *in vitro* and *in vivo* methods. However, recently, it has become apparent that many of these likely represent only a few distinct stem cell types.

2.1 Inter-follicular epidermal stem cells

Physiological renewal of the epidermis is supported by proliferation of cells in its basal layer, and normally does not require additional support from epithelial appendages, such as hair follicles (Ito M *et al.*, 2005; Levy V *et al.*, 2007; Nowak J *et al.*, 2008). Since epidermal renewal continues throughout one's lifetime, it has been postulated that at least a portion of epidermal basal cells behave like stem cells. Historically, the favored model has been that basal layer stem cells give rise to transiently amplifying progeny that, in turn, undergo a limited number of divisions to generate the upper strata of the epidermis (Mackenzie I, 1970; Potten, 1974). According to this model, each stem cell generates an epidermal clone, termed the Epidermal Proliferative Unit (EPU) (Potten C and Bullock J, 1983; Potten C and Hendry J, 1973; Mackenzie I, 1997). The size of each EPU is thought to be constrained to a limited number of cell divisions prior to terminal differentiation. The entire epidermal sheet is thus maintained by a collection of co-existing steady state EPUs with one stem cell at the center of each of them. Experimental support for the EPU model of epidermal organization came from mouse studies where a replication-deficient retroviral vector was used to genetically mark epidermal cells at low frequency. In these experiments discrete vertical columns of labeled keratinocytes reminiscent of hypothetical EPUs could be seen to arise from the basal layer (Mackenzie I, 1997). Further support for the EPU-based epidermal organization came from the pulse-chase labeling studies that revealed the presence of a small number of quiescent, label-retaining cells scattered throughout the basal layer (Morris R *et al.*, 1985; Kaur P and Potten C, 2011; Ghadially R, 2012).

In recent years, the EPU model has been challenged. Using a low frequency inducible *Cre* genetic model, Clayton E *et al.* (2007) and Doupe D *et al.* (2010) were able to mark and analyze the fate of individual proliferating basal cells after a period of over one year. In contradiction to the canonical EPU model, which predicts the size of each EPU to be finite, it was shown that some epidermal clones continuously expand in size, while others shrink and disappear, and yet others behave like typical EPUs (reviewed in Klein A *et al.*, 2007). Mathematical modeling of these variable clone patterns suggested a stochastic model for epidermal renewal, in which each proliferating basal cell can give rise to two new proliferating basal cells, two differentiated progeny or both (Clayton E *et al.*, 2007). According to this Committed Progenitor (CP) model, epidermis is maintained by a uniform population of basal progenitors that undergo stochastically distributed symmetric divisions to maintain the basal layer and asymmetric divisions to generate more differentiated progeny (Jones P *et al.*, 2007). In support of this model, recent data shows that a single basal epidermal cell can indeed divide both symmetrically to produce two new basal cells and asymmetrically to generate more differentiated progeny (Poulson N and Lechler T, 2010).

Whether epidermal renewal strictly follows either EPU or CP model still remains a subject of debate (Kaur P and Potten C, 2011; Ghadially R, 2012; Doupe D and Jones P, 2012). In an effort to merge these two models, it has been proposed that both committed progenitors and epidermal stem cells co-exist, the first to assure life-long epidermal maintenance and the latter to be marshaled for rapid engagement and proliferation after wounding (Doupe D and Jones P, 2012). Recent study by Mascré G *et al.* (2012) has provided experimental evidence that supports this combined model.

2.2 Heterogeneity of hair follicle stem cells

One of the major challenges to the field of cutaneous epithelial stem cell biology derives from the paucity of specific markers for reliable labeling, isolation and fate mapping of distinct stem cell populations. A very different scenario exists for hair follicle stem cells. The discovery of a label-retaining population of cells within the bulge (Cotsarelis G *et al.*, 1990) led to the first identification of stem cells within the hair follicle. Lineage tracing studies (Ito M *et al.*, 2005) clearly showed that bulge stem cells regenerate hair follicles during cycling and also contribute to epidermal regeneration in response to wounding. To date, multiple new hair follicle stem cell populations have been described based upon expression of newly defined markers, proliferative potential, skin reconstitution studies and distinct anatomical location along the hair axis (Figure 1). We will review these populations primarily based upon their location along the hair follicle and lineage potential. Later in this review, we will discuss their contributions to wound repair in more detail.

Bulge—The first group of hair follicle stem cells discovered comprises quiescent cells of the bulge (Cotsarelis G *et al.*, 1990). Since these cells divide infrequently, they retain nucleotide analog labels, such as BrdU, and hence, are known as label-retaining cells. Under physiological conditions, bulge cells mainly contribute to the cycling portion of the anagen hair follicle. Following wounding, they can also contribute, at least transiently, to epidermal repair (Ito M *et al.*, 2005). In addition to label-retaining methods, bulge stem cells have also been identified on the basis of expression of keratin 15 (Krt15) (Liu Y *et al.*, 2003), R-spondin receptor *Lgr5* (Jaks V *et al.*, 2008), CD34 (Trempe C *et al.*, 2003), and transcriptional factors *Sox9* (Nowak J *et al.*, 2008), *Lhx2* (Mardaryev *et al.*, 2011), *Tcf3* (Merrill B *et al.*, 2001), *Nfatc* (Horsley V *et al.*, 2008) and *Gli1* (Brownell I *et al.*, 2011).

Krt15 expression defines a large portion of bulge stem cells (Lyle S *et al.*, 1998; Liu Y *et al.*, 2003). Fate-mapping analyses of Krt15+ cells showed their ability to reconstitute all epithelial lineages of the anagen hair follicle (Morris R *et al.*, 2004) and sebaceous glands

(Petersson *et al.*, 2011). In further lineage tracing experiments, Ito M *et al.* (2005) teased out the epidermal contribution of Krt15+ cells to wounding. After skin wounding, Krt15+ bulge cells rapidly contribute to the inter-follicular epidermis, albeit only transiently. Furthermore, Krt15+ cells are dispensable for physiological epidermal maintenance, as genetic ablation of Krt15+ bulge cells does not result in epidermal deficiency. Thus, Krt15+ bulge stem cells can shift their fate towards the epidermal lineage, but only in response to wound-induced signaling (Ito M *et al.*, 2005). Later in this review we will touch upon the potential mechanism of this hair-to-epidermis lineage switch.

Lgr5, an R-spondin receptor implicated in facilitation of canonical WNT signaling (de Lau W *et al.*, 2011), marks a broad population of hair follicle progenitors, in lower bulge and secondary hair germ during telogen, and in the lower outer root sheath during anagen (Jaks V *et al.*, 2008). Lgr5 expression significantly overlaps with that of Krt15. Similar to Krt15+ stem cells, Lgr5-expressing cells physiologically contribute to all lineages of the anagen hair follicle, but not to the epidermis or sebaceous gland. Lgr5+ cells from the outer root sheath repopulate the secondary hair germ during each new telogen phase (Jaks V *et al.*, 2008).

Isthmus and junctional zone—In recent years, the upper isthmus (junctional zone), above the bulge to where the sebaceous gland duct inserts, was shown to house an unexpectedly diverse populations of epithelial cells with stem cell qualities (reviewed in Gordon W and Andersen B, 2011). In the junctional zone, the stem cells co-express transmembrane proteins Lrig1 and Plet1 (Jensen K *et al.*, 2009). Lineage tracing experiments have shown that Lrig1+ stem cells tend to be bipotent, physiologically contributing predominantly to infundibulum and sebaceous gland lineages and only occasionally to the inter-follicular epidermis. Normally, they do not generate hair follicle-fated progeny. Epidermal contribution of Lrig1+ stem cells significantly increases upon retinoic acid-induced epidermal hyperplasia. Interestingly, mice lacking Lrig1 develop spontaneous epidermal hyperplasia, likely stemming from the Lrig1 functioning as a negative feedback loop regulator of pro-proliferative cMyc signaling (Jensen K *et al.*, 2009).

Lgr6+ stem cells (Snippert H *et al.*, 2010) and Gli1+ stem cells (Brownell I *et al.*, 2011) are located immediately below the Lrig1+ compartment. While initially multipotent, Lgr6+ stem cells undergo progressive developmental fate restriction, and in the adult they participate mainly in epidermal and sebaceous gland maintenance (Snippert H *et al.*, 2010). Interestingly, despite very close physical proximity, Gli1+ stem cells only marginally overlap with the Lgr6+ cells, while forming a close association with the peri-follicular sensory nerve endings. Nerve endings surrounding Gli1+ stem cells secrete Shh, which appears to facilitate their epidermal lineage potential (Brownell I *et al.*, 2011). Analogous to Lrig1+ cells, both Lgr6+ and Gli1+ stem cells generate long-lasting epidermal progeny in the wound epidermis, making their contributions a contrasting feature to that observed by Krt15+ bulge stem cells (Ito M *et al.*, 2005).

Recent lineage tracing studies by Petersson *et al.* (2011) revealed that progeny of Krt15+ bulge stem cells en route to the sebaceous gland acquire marker characteristics of the compartments they transit through – Lgr6 in the isthmus, Lrig1 in the junctional zone and Blimp1 at the base of the sebaceous gland. This implies a high degree of plasticity and interchangeability between various hair follicle stem cell populations even under physiological conditions. This feature becomes accentuated in skin reconstitution studies, where the majority of stem cell types mentioned above are able to reconstitute all of the epithelial lineages of the skin and form new hair follicle-bearing skin. Future studies in this direction are required to conclusively determine whether hair follicles contain one multipotent stem cell population at the root of all other follicular stem cell types, or if

several distinct populations coexist and are able to exchange stem cell bidirectionally in response to changes in microenvironmental signaling.

2.3 Stem cells of other ectodermal appendages

Sebaceous glands—Each hair follicle is closely associated with the sebaceous gland and, together, they constitute what is known as the pilosebaceous unit. The predominant cells in the sebaceous gland, sebocytes, secrete lipid-rich products into the infundibular opening of the adjacent hair follicle. Cells expressing the transcriptional repressor *Blimp1* comprise unipotent sebocyte progenitors (Horsley V *et al.*, 2006). Horsley V *et al.* (2006) confirmed that *Blimp1*+ progenitors give rise to terminally differentiated *Ppar γ* + sebocytes via transient amplifying progenitors, yet they do not contribute progeny toward inter-follicular epidermis or hair follicles. Mechanistically, *Blimp1* represses *cMyc* transcription, likely limiting the input of proliferative progenitors toward the gland from the multipotent stem cell populations of the isthmus (Snippert H *et al.*, 2010; Frances and Niemann, 2012) and the bulge (Morris R *et al.*, 2004; Petersson *et al.*, 2011). The key rate-limiting role of *Blimp1* in sebaceous gland homeostasis was revealed upon epithelial *Blimp1* deletion, which led to sebaceous gland hypertrophy and oily hair coat phenotype (Horsley V *et al.*, 2006).

Sweat glands—Ventral paw skin is hairless, but contains sweat glands, a secretory type of ectodermal appendage. Unlike hair follicles, sweat glands are relatively quiescent and until recently very little was known about their regenerative potential. A recent study by Lu C *et al.* (2012) revealed that despite homeostatic quiescence, sweat glands feature several distinct progenitor types, whose regenerative potential can be stimulated by injury. Sweat glands have relatively simple organization and consist of the secretory glandular portion and the duct connected to the skin surface. Basal myoepithelial cells and suprabasal luminal cells comprise the glandular portion of the sweat gland. Curiously, sweat-producing luminal cells are maintained by suprabasal unipotent progenitors, largely independent of the basal myoepithelial cells. Neither myoepithelial nor luminal glandular cells contribute progeny toward the duct which, in turn, is maintained by its own basal unipotent progenitors. Unlike glandular cells, ductal cells become activated upon glabrous skin wounding and help to restore ductal openings onto the skin surface. While ductal progenitors preferentially regenerate the duct itself, they can also regenerate glabrous epidermis immediately surrounding the sweat gland opening (Lu C *et al.*, 2012). In this respect, ductal progenitors of the sweat gland share characteristics with the hair follicle isthmus stem cells, which can also generate permanent epidermal progeny following injury (Jensen K *et al.*, 2009; Snippert H *et al.*, 2010; Brownell I *et al.*, 2011).

3. Re-epithelialization strategies – fast vs. permanent

Ito *et al.* (2005) and Levy V *et al.* (2005) showed that physiological maintenance of the inter-follicular epidermis does not depend on hair follicle stem cells. Ito *et al.* (2005) demonstrated that genetic ablation of *Krt15*+ bulge stem cells leading to effective destruction of all hair follicles in the skin does not affect skin maintenance. The same bulge stem cells, however, become efficiently recruited into the newly forming epidermis during wound healing. Interestingly, bulge-derived epidermal progeny in the wound are short-lived and eventually become replaced by non-bulge epidermal cells. In light of these findings, it is not surprising that hair follicle stem cells are dispensable for wound re-epithelialization in general. In *Edaradd* mice that lack primary hair follicles and have distinctly hairless tails, tail wounds can fully re-epithelialize, although after an initial delay (Langton *et al.*, 2008; reviewed in Ito and Cotsarelis, 2008). This delay is likely caused by the relatively slow recruitment of the peri-wound epidermal progenitors in hairless *Edaradd* mice as opposed to

fast recruitment of bulge stem cells in normal hairy mice. These experiments highlight the existence of two key wound re-epithelialization strategies – fast but transient from hair follicle bulge Krt15+ stem cells and slow but permanent from epidermal and isthmus stem cells (Figure 2a). Below we will discuss experimental evidence for both wound repair strategies in more details.

3.1 “Emergency” repair by hair follicle stem cells

Progeny of bulge stem cells become recruited toward new epidermis during wound re-epithelialization in hair-bearing skin (Ito M *et al.*, 2005). While, to date, numerous types of epithelial stem cells have been identified in the hair follicle, their relative contribution to wound re-epithelialization has only been addressed recently. Pioneering work in this direction was done by Taylor G *et al.* (2000), who employed double pulse-chase labeling strategies to indicate that bulge cells contribute to new infundibular epidermis after wounding. These findings were later verified and expanded by our laboratory. Ito M *et al.* (2005) employed a genetic lineage tracing strategy to firmly establish that Krt15+ bulge stem cells participate in early stages of wound re-epithelialization. Five days following wounding, epidermal progeny of bulge stem cells can be seen migrating toward the wound center from the hair follicles along the wound edge. Interestingly, by day 20 post-wounding, an initially radial distribution pattern of Krt15+ stem cell progeny becomes very sparse, and by day 50, most of the epidermal bulge progeny disappear (Ito M *et al.*, 2005; Cotsarelis, 2006) (Figure 2b).

3.2 Long term epidermal reconstitution by hair follicle stem cells

While hair follicle-fated bulge stem cells divert only transient amplifying progeny toward wound epidermis as part of an “emergency” repair strategy (Ito M *et al.*, 2005), other follicular stem cells can generate long-lasting epidermal populations. The first solid experimental support for this possibility was reported by Levy V *et al.* (2007), who employed a *Shh-Cre* based genetic approach to broadly label the entire follicular epithelium, as opposed to bulge stem cells only. In wounding experiments analogous to those reported by Ito M *et al.* (2005), Levy V *et al.* (2007) showed that follicular *Shh-Cre;R26R* labeled cells generate long-lasting clones in the wound epidermis. In another study, Nowak J *et al.* (2008) used the *Sox9-Cre* model to achieve broad genetic labeling of all hair follicle compartments, similar to that obtained by Levy V *et al.* (2007) with the *Shh-Cre* based approach. Similarly, *Sox9-Cre;R26R* labeled hair follicle progenitors reconstituted long-lasting epidermal clones following wound repair. Taken together, these results suggested that hair follicle progenitors, distinct from Krt15+ bulge stem cells, are able to permanently convert into interfollicular epidermal stem cells in response to wounding. Several recent studies established that such progenitors reside in the isthmus and junctional zone portions of the hair follicle and can be identified based on the expression of Gli1 (Brownell I *et al.*, 2011), Lgr6 (Snippert H *et al.*, 2010) and Lrig1 (Jensen K *et al.*, 2009). In the lineage tracing studies, all three types of isthmus stem cells generate long-lasting epidermal clones in the wound, suggesting that their progeny convert into self-sustainable epidermal stem cells. Brownell I *et al.* (2011) also showed that epidermal conversion of Gli1+ stem cells is heavily dependent on the paracrine Shh signaling from the peri-follicular sensory nerve endings, shedding some light on the mechanism of hair follicle-to-epidermal lineage interconversion in adults.

3.3 Re-epithelialization from bone marrow sources

While, as detailed above, the majority of wound epidermis forms from other epithelial cells at the wound edge, in some instances, new epidermal keratinocytes are thought to derive from remotely recruited non-epithelial progenitors. While many contradictory reports exist on this topic, several groups provide evidence for non-cell fusion-based transdifferentiation

of bone marrow-derived cells into epithelial cell types, including epidermal keratinocytes. For example, Harris R *et al.* (2004) and Borue X *et al.* (2004) showed that in bone marrow transplantation mouse models, bone marrow-derived cells contribute to approximately 4% of keratinocytes in the wound epidermis. Experiments by Wu Y *et al.* (2007), Sasaki M *et al.* (2007) and most recently by Tamai K *et al.* (2011) suggest that bone marrow mesenchymal stem cells, rather than some hematopoietic progenitors, are able to undergo direct epidermal transdifferentiation. Interestingly, bone marrow-derived keratinocytes proliferate and expand in numbers during the re-epithelialization phase of wound healing, yet reduce to 0.1% of total epidermal cells when wound healing is complete (Borue X *et al.*, 2004; Brittan M *et al.*, 2005). In this respect, the role of transdifferentiated bone marrow-derived keratinocytes parallels the role of Krt15+ bulge stem cells that contribute transiently amplifying progeny to assist in “emergency” wound healing, yet do not develop into long-lasting epidermal stem cells. Despite a growing body of evidence for the direct transdifferentiation of remotely recruited bone marrow mesenchymal cell types into epidermal keratinocytes, much more experimental evidence to convincingly rule out other possibilities, such as cell fusion remains to be accomplished (Vogel G, 2004).

4. Wound-induced hair follicle neogenesis

Recently our laboratory showed that morphogenetic potential of epithelial cells upon wound healing extends beyond simple re-epithelialization, and that completely new hair follicles can form in the center of large wounds via a process resembling embryonic development (Ito M *et al.*, 2007; reviewed in Chuong C, 2007). This hair follicle neogenesis is a relatively late regenerative event, contingent upon completion of wound re-epithelialization. Neogenic hair follicles start as the bud-like invaginations of the basal layer (*aka* hair germs) and soon develop into elongated hair pegs that go on to mature into hair shaft producing anagen hair follicles within just a few days. Importantly, like normal hair follicles at the wound edge, neogenic hair follicles in the wound center have a prominent Krt15+ bulge stem cell compartment and are able to undergo multiple repetitive cycles of regeneration. Using several fate mapping approaches, Ito M *et al.* (2007) determined that the neogenic hair follicles and their stem cells do not come from Krt15+ bulge stem cells of the preexisting hair follicles at the wound edge, despite the fact that Krt15+ stem cells significantly contribute transient amplifying progeny toward the wound epidermis (Ito M *et al.*, 2005). Instead, neogenic hair follicles must develop from non-hair follicle stem cells that likely acquire embryonic-like competence and transiently expand their lineage plasticity. While currently it is unclear what specific epidermal or isthmus stem cell type, or combination of stem cells generate neogenic hair follicles, Snippert H *et al.* (2010) showed that Lgr6+ isthmus stem cells may be involved (Figure 3). Ongoing research efforts in our laboratory are focused on understanding how wound epidermal cells acquire expanded lineage plasticity and what signaling events in wound scar tissue are catalysts for the induction of new hair follicles. Interesting clues come from the observations that neogenic hair follicles form in the center of healed wounds and almost never at the wound periphery, where there is always a circular zone of hairless scar tissue. Also, several experiments by Ito M *et al.* (2007) and Myung *et al.* (2012) convincingly demonstrate the key role of canonical WNT signaling in inducing neogenic hair follicles. The search is now to understand what upstream signaling events in the wound environment enable the hair-inducing pattern of WNT signaling.

5. Control of wound re-epithelialization strategies

Robust activation of epithelial stem cells and efficient recruitment of their progeny toward an epidermal lineage are critical for wound healing. In this respect it is essential to understand what molecular mechanisms direct multipotent isthmus stem cells and re-route

hair follicle bulge stem cells toward an epidermal phenotype in response to injury. It is equally important to understand the nature of the wound niche microenvironment, its cellular composition, and key signaling factors. Deeper understanding of the normal wound re-epithelialization process will undoubtedly provide greater insights into various wound healing pathologies, when re-epithelialization and maintenance of wound epidermis are severely compromised. One of the key steps in the early wound healing process is the moment when hair follicle stem cells change their fate toward epidermis, thus assuring a rapid influx of proliferating progenitors into the wound bed. Although multiple signaling pathways have been shown to affect proliferation and migration of epidermal keratinocytes over the wound bed, factors that trigger coordinated stem cell fate switching toward epidermal lineage at the onset of wound healing are just starting to be discovered.

Lhx2, Lim-homeodomain transcription factor, appears to play a key role in directing follicular stem cells toward re-epithelialization. Analysis of wound healing in heterozygous *Lhx2* mutant mice, with reduced Lhx2 signaling, showed that while wound re-epithelialization was delayed, wounding-induced anagen initiation by the telogen hair follicles at the wound edge was accelerated (Mardaryev *et al.* (2011)). Confirming initially reported Lhx2 expression in bulge stem cells and secondary hair germ progenitors (Rhee H *et al.*, 2006), Mardaryev *et al.* (2011) further showed that persistent Lhx2 signaling in these cells is necessary for epidermal fate switching, a process that involves direct upregulation of Sox9 and Tcf4. Supporting key roles by Sox9 and Tcf4 as positive mediators of wound re-epithelialization had previously been established by studies which demonstrated significant retardation in skin healing of epithelium-specific *Sox9* and *Tcf3/4* mutant mice (Nowak J *et al.* (2008), Nguyen H *et al.* (2009)). Additionally, sustained Lhx2 signaling dampens expression levels of Lgr5, an R-spondin receptor and facilitator of canonical WNT signaling (de Lau W *et al.*, 2011). Consistent with the previously reported role of Lgr5 in activation of hair follicle-fated bulge and secondary hair germ progenitors during anagen initiation (Jaks V *et al.*, 2008), Mardaryev *et al.* (2011) suggested that by simultaneously upregulating Sox9 and Tcf4 and downregulating Lgr5, Lhx2 switches lineage identity of activated bulge stem cells from follicular to epidermal. Importantly, this Lhx2-mediated mechanism appears to be restricted to the bulge, as Lhx2 is not expressed in isthmus and junctional zone harboring Lrig1+, Lgr6+ and Gli1+ stem cell populations. Considering that bulge stem cells contribute only transiently amplifying epidermal progeny to wounding (Ito M *et al.*, 2005) whereas isthmus populations contribute long term progeny, this data suggests unique epidermal lineage switch mechanisms for the bulge vs. isthmus stem cell populations.

In light of these findings, it is particularly interesting that wounds in anagen skin re-epithelialize noticeably faster than wounds surrounded by telogen hair follicles (Ansell DM *et al.*, 2011). To understand the nature of this phenomenon, it is important to consider that bulge stem cells become activated and proliferate only during the anagen phase of the hair cycle. Normally, rapid proliferation of secondary hair germ progenitors fuels anagen onset, while bulge stem cells continue to proliferate throughout the rest of the active hair growth phase (Greco V *et al.*, 2009). In doing so, bulge stem cells generate activated stem cell-like progenitors that migrate downward along the outer root sheath and eventually contribute to hair follicle matrix (Hsu Y *et al.*, 2011). It is conceivable that a large portion of these activated upper outer root sheath progenitors in anagen hair follicles become re-routed toward an epidermal fate guided by Lhx2 → Sox9/Tcf4 induction or other similar mechanisms (Mardaryev *et al.* (2011)). Supporting this possibility is the observation that rapid wound re-epithelialization in anagen skin is preceded by marked proliferation in the upper outer root sheath of peri-wound hair follicles (Ansell DM *et al.*, 2011).

6. Conclusions

Recent advances in epithelial stem cell biology have significantly improved our understanding of wound regeneration mechanisms and have provided a basis for developing stem cell-based wound healing therapies. Concurrently, many new questions have arisen which undoubtedly will become the topics of future studies. It will be important to better characterize the inter-follicular epidermal stem cells. Currently, distinct epidermal stem cell markers remain elusive, thus preventing detailed lineage analysis studies under homeostatic and wound healing conditions. Future work on this front should resolve the controversy concerning the existence of distinct quiescent interfollicular epidermal stem cells (Kaur P and Potten C, 2011; Ghadially R, 2012; Doupe D and Jones P, 2012).

Another intriguing aspect of wound healing relates to the mechanism of hair follicle stem cell recruitment for re-epithelialization. We already know that various follicular stem cells contribute to wound re-epithelialization differently. While bulge stem cells produce transiently amplifying epidermal progeny (Ito M *et al.*, 2005), non-hair follicle isthmus stem cells generate long-lasting populations in the wound epidermis (Levy V *et al.*, 2007; Jensen K *et al.*, 2009; Snippert H *et al.*, 2010; Brownell I *et al.*, 2011). Clearly, bulge and non-bulge stem cells contribute to wound re-epithelialization via partially distinct mechanisms. Future studies aimed at dissecting the molecular underpinnings of these processes may enable us to augment wound re-epithelialization through the re-routing of hair follicle stem cells towards an epidermal lineage. Recent discovery of the hair follicle neogenesis phenomenon by our laboratory is also changing the landscape of wound healing research (Ito M *et al.*, 2007). The ability of wound epidermis keratinocytes to engage in embryonic-like hair follicle formation and to regenerate the entire repertoire of epithelial stem cells *de novo* highlights a much broader lineage plasticity than originally imagined. Future studies should concentrate on understanding the cellular and molecular aspects of the mechanism of hair follicle neogenesis. Finally, significant efforts should be directed towards translating epithelial stem cell research to human subjects. Species-specific differences between mice and humans in terms of wound healing and hair follicle biology warrant cautious interpretation of many mouse-specific findings in the context of human wound healing.

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Highlights

- Both epidermal and hair follicle stem cells participate in wound repair
- Bulge stem cells contribute to wound re-epithelialization only transiently
- Isthmus stem cells generate long-lasting progeny in the wound epidermis
- Wound epidermis can regenerate *de novo* hair follicles via an embryonic-like mechanism

Figure 1. Heterogeneity of epidermal and hair follicle stem cells

Skin epithelia feature distinct stem cell populations both in epidermis and, most prominently, in hair follicles. Epithelial stem cells in different micro-anatomical locations have different lineage potentials. Stem cells in the follicular infundibulum and inter-follicular epidermis physiologically are restricted to epidermal fate. Inter-follicular epidermal stem cells can be identified as slow-cycling in label retention studies, but distinct markers remain elusive. The isthmus and junctional zone of hair follicles harbor several distinct epithelial cell populations. Most prominent among them are Lrig1+ (yellow), Gli1+ and Lgr6+ stem cells (green), all of which physiologically maintain the isthmus and contribute to sebaceous gland, infundibulum and in some instance to inter-follicular epidermis. Blimp1 identifies unipotent sebaceous gland progenitors (orange). The bulge stem cells (blue) normally contribute to all hair follicle lineages and can be identified based on the expression of Krt15, CD200, Lgr5, CD34, Sox9, Lhx2, Tcf3 and Nfatc1. The secondary germ of telogen hair follicles (purple) contains committed hair follicle-fated progenitors that express CD200, Gli1 and Lgr5.

Figure 2. Contribution of hair follicle stem cells to wound epidermis

a) Upon wounding, both Lgr1+ (yellow) and Lgr6+ (green) stem cells and Krt15+bulge stem cells (blue) within hair follicles along the wound edge become activated and generate progeny that migrate out of the follicles and participate in rapid wound re-epithelialization.

b) While bulge stem cells contribute only transiently to amplifying epidermal progeny, Lgr1+, Lgr6+ stem cells are able to generate long-lasting epidermal clones. As the consequence of these dynamics, bulge stem cell progeny in wound epidermis significantly decline over time.

Figure 3. The role of epithelial stem cells in wounding-induced hair follicle neogenesis

Following re-epithelialization of large full-thickness wounds, new hair follicles develop from the basal cells of the wound epidermis via a process of embryonic-like neogenesis. Progeny of the Krt15⁺ bulge stem cells from the peri-wound hair follicles do not participate in making *de novo* hair follicles, consistent with their transient involvement in wound re-epithelialization. While currently the original lineage identity of the epithelial stem cells that contribute to hair follicle neogenesis is unknown, preliminary data points at the involvement of isthmus stem cells or possibly interfollicular stem cells.