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Emerging interactions between skin stem cells and their niches

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Abstract

The skin protects mammals from insults, infection and dehydration and enables thermoregulation and sensory perception. Various skin-resident cells carry out these diverse functions. Constant turnover of cells and healing upon injury necessitate multiple reservoirs of stem cells. Thus, the skin provides a model for studying interactions between stem cells and their microenvironments, or niches. Advances in genetic and imaging tools have brought new findings about the lineage relationships between skin stem cells and their progeny and about the mutual influences between skin stem cells and their niches. Such knowledge may offer novel avenues for therapeutics and regenerative medicine.

Adult stem cells reside in niches that provide spatially distinct microenvironments for stem cell maintenance and function. The conceptual framework for stem cell niches, their compositions and their operating logistics is constantly being updated. Initially, niches were thought to be composed solely of heterologous cell populations that originate from a lineage different from the stem cells they regulate[1]. Recent studies have added several important modifications: differentiated progeny and stem cells can coexist within a niche, suggesting that niche signals alone are not sufficient to dictate 'stemness'[2,3]; downstream progeny of stem cells can regulate their stem cell parents and thus become a component of the niche[4,5]; and communications between stem cells and their niches are reciprocal, as stem cells may also regulate the assembly and maintenance of their niches[6].

The skin is a complex organ harboring several distinct populations of stem cells and a rich array of cell types (Fig. 1), making it an ideal model for studying the interplay between stem cells and their niches. The outermost layer is the epidermis, a stratified structure that is maintained by stem cells located at the most basal layer and acts as a protective barrier. Underneath the epidermis is the dermis, enriched for dermal fibroblasts that produce collagens and elastic fibers of extracellular matrix (ECM) and give the skin its elasticity. Below the dermis lies the subcutaneous fat, which acts as protective padding, insulation and an energy reservoir.

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Hair follicles are notable appendages of the epidermis. In addition to generating hairs that facilitate thermal regulation, hair follicles also serve as anchors for sensory neurons, arrector pili muscles (APMs) and blood vessels. Hair follicles undergo cycles of regeneration and rest driven by stem cells located in a region known as the bulge, and in a cluster of cells below the bulge known as the hair germ. Melanocyte stem cells (MSCs) are intermingled with hair follicle stem cells (HFSCs) in the bulge and the hair germ. The MSCs generate mature melanocytes that produce melanin, which absorbs ultraviolet (UV) light to prevent DNA damage and gives skin and hairs their distinctive colors.

In this Review, we focus on various stem cell populations in the skin, summarizing and comparing recent advances in research on skin stem cell niches that have contributed to the emergence of new concepts. We summarize the niche components and signals that regulate the behavior of epidermal stem cells, HFSCs and MSCs. In addition, we discuss how the dynamics of stem cell–niche interactions change during aging, wounding, skin cancer initiation and malignant progression. Lastly, we discuss the clinical implications of recent findings and how studying the stem cell niche might shape the future of regenerative medicine.

Stem cells in the interfollicular epidermis

In mammals, the skin's protective barrier is composed of a stratified epidermis (Fig. 2). The interfollicular epidermis (IFE) between hair follicles is exposed to many external insults, such as UV light, chemicals, allergens and traumatic injuries. To withstand these physical stresses, the epidermal cells, called keratinocytes, form a dense cytoskeletal infrastructure of 10-nm intermediate filaments composed of the keratin subfamily of proteins. Keratin filaments are highly enriched in the vertebrate epidermis and its appendages, but not in the surface epithelium of organisms such as insects, which instead secrete a protective outer shell.

The innermost (basal) epidermal layer consists of undifferentiated proliferative progenitors that express keratins K5 and K14. These progenitors not only replenish the basal layer, but also give rise to nonproliferative, transcriptionally active spinous and granular layers expressing K1, K10 and involucrin, and finally the outer layers of terminally differentiated, dead stratum corneum cells[7] (Fig. 2). As demonstrated by retrovirus- and mutation-based lineage tracing data[8–12], these columnar tissue units are in constant flux, as outer layer cells are continually shed and replaced by differentiating cells from inner layers.

Lineage choices of interfollicular epidermal stem cells: hierarchical versus stochastic

Two distinct models have been proposed to explain the behavior of stem cells within the basal layer of the IFE (Fig. 3). The hierarchical model suggests that the IFE is composed of discrete epidermal proliferative units consisting of a slow-cycling stem cell that gives rise to short-lived transit-amplifying cells (TACs), which then depart the basal layer after several divisions to generate upward columnar units of differentiating cells. The stochastic model

suggests that basal epidermis is composed of a single type of proliferative progenitor whose daughter cells choose randomly to differentiate or remain as progenitors.

When individually labeled basal cells in tail, ear or hindpaw epidermis were marked by lineage tracing and their progeny were then monitored over the long term, the clonal fate data were compatible with the stochastic model[13–15]. However, in these various labeling strategies, a crucial issue left unresolved was whether basal cells were marked randomly or selectively. By contrast, a recent study on tail skin employed two inducible Cre-lineage tracers in which Cre recombinases are fused with mutated estrogen receptors (ERs), rendering their inducibilty by tamoxifen. One Cre driver was driven by the K14 promoter, active in all basal cells, and the other by the involucrin promoter, active only in a discrete subset of $K14^+$ basal cells that precociously express this typically differentiation-specific gene. In this study, purportedly slow-cycling basal cells marked by K14-Cre^{ER} but not involucrin-Cre^{ER} behaved like long-lived stem cells and gave rise to the subset marked by involucrin-Cre^{ER}, which displays features of more committed basal progenitors[16]. These findings support the hierarchical model, yet show that progenitors within the basal layer exist and can behave in a stochastic manner. Although it is tempting to speculate that the differences among studies arise from the tools used to mark the basal cells, it is still possible that variation in the epidermis at different body sites accounts for the differences.

Epidermal stem cell proliferation

Epidermal keratinocytes proliferate before moving upward and differentiating. Dermal fibroblasts facilitate colony formation of human and mouse keratinocytes *in vitro* and are a rich source for mitogens such as insulin-like growth factors (IGFs), fibroblast growth factor-7 (FGF-7), FGF-10 and epidermal growth factor receptor (EGFR) ligands[17–19] (Fig. 2). The physiological importance of these factors in regulating epidermal proliferation has been verified in mouse models: epidermis lacking insulin-like growth factor receptor (IGFR) is impaired in basal epidermal proliferation[20]. Ectopic expression of mesenchymal factor FGF-7 in epidermal cells causes epidermal hyperproliferation[21]. EGF signaling, as its name suggests, is a particularly potent pathway for epidermal growth[22]. In mice, activation of transforming growth factor-α (TGF-α), a positive autocrine regulator of EGFR signaling in the epidermis, or deletion of Mig6, a negative regulator of EGFR signaling in the epidermis, leads to epidermal hyperproliferation[23,24]. In humans, reduction of expression of the EGFR antagonist, *LRIG1*, promotes human keratinocyte proliferation in culture.

ECM proteins deposited by basal epidermal keratinocytes and by underlying dermal fibroblasts form a sheath of basement membrane separating epidermis from dermis. Basal epidermal cells adhere to the basement membrane through receptors known as integrins. $\alpha_3\beta_1$ and $\alpha_6\beta_4$ are the major epidermal integrins that bind the ligand laminin-5, the major ECM component of the basement membrane (Fig. 2). In human basal epidermis, greater expression of β_1 integrin marks a relatively slow-cycling population *in vivo* that has a higher colony-forming efficiency when plated in culture, suggesting that this population has greater stem cell potential[26–28].

The functions of integrins have also been explored in animal models. Ablation of β_1 integrin in mice compromises basement membrane assembly and impairs proliferation[29]. Deletion of

α_6 or β_4 integrin, or their ECM ligand laminin-5, leads to epidermolysis bullosa, a skin condition that results in severe blistering of the skin[30–33]. $\alpha_6\beta_4$ integrin signals through RAC1, a small GTPase in basal cells, to mediate their adhesion to the basement membrane; depletion of Rac1 results in epidermal hyperproliferation[34]. Although the precise mechanisms mediating these phenotypic changes may be complex, these studies demonstrate that ECM is a critical niche component for the stem cells within the basal layer.

Genetic studies in mice suggest that epigenetic factors are also regulators for epidermal proliferation and differentiation[35–40]. Among these, the histone H3 Lys27 (H3K27) methyltransferases EZH1 and EZH2 are essential for epidermal homeostasis and wound repair[38,39], whereas the histone H3K27 demethylase JMJD3 promotes differentiation[41]. Intriguingly, several epigenetic factors including EZH2 have been implicated in regulating the transcription of genes encoding α_6 and β_1 integrins in cultured human keratinocytes[42], raising the possibility that some epigenetic modifiers might balance epidermal proliferation and differentiation in part by altering the attachment of basal cells to their basement membrane. Most, if not all, adult stem cells rely upon integrins and the ECM for adhesion to their niche, and understanding the interactions between skin stem cells and ECMs will provide a paradigm for understanding similar processes in other adult stem cells.

In addition to spatial cues from their niches, epidermal stem cell behavior may also be modulated by temporal cues such as circadian rhythms. In mice, proliferation of basal epidermal cells peaks at night, when the accumulation of reactive oxygen species is the lowest. Keratinocyte-specific deletion of *Arntl* (*Bmal1*), which encodes a transcription factor in the core clock machinery, abrogates this temporal difference by increasing epidermal proliferation during daytime[43]. Expression of core clock genes also oscillates in cultured human keratinocytes, and perturbation of this oscillation reduces colony-forming efficiency and promotes differentiation[44]. Although it remains unclear how these successive peaks of expression are established and how circadian rhythms regulate epidermal stem cells, it is tempting to speculate that temporal regulation has evolved to suppress proliferation during daytime, when there are higher risks of DNA damage owing to UV radiation and elevated concentrations of reactive oxygen species.

Epidermal stem cell differentiation

Epidermal stratification is governed by two mechanisms: delamination, in which basal cells lose their attachment to the basement membrane and move upwards[45], and asymmetrical cell division, in which the plane of division is perpendicular to the basement membrane, generating a committed suprabasal daughter and a proliferative basal cell[46]. Transition from the basal to the spinous layer requires Notch signaling, a highly conserved pathway involved in a wide variety of developmental processes. In mouse epidermis, Notch1, Notch2 and Notch3 receptors and one of their ligands, Jagged1, are expressed suprabasally, whereas Jagged2 is expressed basally[47,48] (Fig. 2). Upon ligand-receptor interaction, Notch proteins are cleaved by γ-secretase, releasing their intracellular domains (NICD1, NICD2 and NICD3), which bind to the transcriptional repressor RBP-J, enabling them to activate the Hes/Hey family of transcription factors and other target genes.

The presence of nuclear NICD and the transcription factor Hes1, as well as the expression of a Notch reporter gene regulated by RBP-J and NICD, have all been used as indicators that Notch signaling is active in spinous cells[49]. Indeed, constitutive activation of Notch signaling in the basal epidermis results in massive expansion of spinous layers, reduced integrin expression and detachment of epidermis from underlying dermis. Conversely, deletion of *Rbpj* in embryonic epidermis suppresses formation of spinous layers and reduces basal cell proliferation[49]. Postnatal loss of Notch signaling paradoxically results in epidermal hyperproliferation, but this turns out to be an indirect consequence of the impaired skin barrier and ensuing inflammation[49,50]. Notch signaling also acts genetically downstream of the asymmetric cell division machinery that basal progenitors use to generate spinous cells[51]. When the core components in asymmetric division are compromised, basal cells fail to activate Notch signaling and spinous cell numbers decline[51]. In humans, the Notch ligand DELTA1 is expressed by basal cells, and DELTA-NOTCH interactions in cultured human keratinocytes promote differentiation[52]. Together, these data suggest that Notch activation determines spinous cell fate and promotes delamination, and asymmetric cell division balances epidermal proliferation and differentiation through Notch signaling.

The exact ligand that activates Notch in the mouse epidermis, and the cellular source of that ligand, remains unclear, although evidence thus far implicates cross-talk between stem cells (basal) and their differentiated progeny (suprabasal cells). One possible cell mediator is the primary cilium, a microtubule-based sensory organelle that typically functions in hedgehog signaling but also enhances Notch signaling[53]. Failure in ciliogenesis leads to compromised Notch signaling and defective epidermal differentiation[53].

Hair follicle regeneration

Unlike the epidermis, which regenerates continually, hair follicles undergo cycles of growth (anagen), degeneration (catagen) and rest (telogen) (Fig. 4a). In mice, the first two cycles are synchronized, making hair follicles an ideal system for understanding how stem cells interact with progeny and heterologous cell types in the niche to transition between quiescence and regeneration.

HFSCs can be subdivided into two populations that share similar molecular signatures: a quiescent one located in the bulge (Bu-SCs) and a primed population within the hair germ just below the bulge, which is more prone to proliferation[54] (Fig. 4). Previous lineage-tracing studies have demonstrated that these two populations are responsible for initiating hair growth[2,55,56], and recently live imaging has provided a more precise means of delineating their relative contributions to these early steps[57].

Neither Bu-SCs nor hair germ give rise to differentiated cells directly. At anagen onset, the hair germ is always the first to proliferate[54,58]. Hair germ develops into matrix, a pool of TACs that proliferate rapidly before terminally differentiating to form the hair shaft and its surrounding channel, the inner root sheath (IRS). By contrast, Bu-SCs primarily give rise to the outer root sheath (ORS), a population of cells that retains many stem cell characteristics and envelops the differentiating core of each hair follicle as well as the bulb of matrix TACs at the base of the mature follicle[2,57] (Fig. 4a).

During catagen, matrix and most lower ORS cells apoptose, middle ORS cells form a new hair germ and upper ORS cells form a new bulge adjacent to the original one[2]. The newly formed bulge and hair germ house the HFSCs for the next hair cycle. By contrast, the previous bulge becomes an HFSC reservoir for injury repair. Interestingly, some lower ORS cells escape apoptosis, follow a short-circuited differentiation program and become the inner layer of the new bulge. Marked by K6, these inner bulge cells anchor the hair shaft but have lost stemness, despite returning to the bulge[2] (Fig. 4a).

Regulation of hair follicle stem cell quiescence and activation

HFSCs are maintained in a quiescent state during most of the hair cycle and only proliferate early in anagen. Several secreted factors from stem cell progeny and dermal cells are important in regulating the proliferative status of Bu-SCs and hair germs. HFSC quiescence is largely maintained by bone morphogenetic proteins (BMPs). Originally named for their functions in bone and cartilage formation, BMPs have now been implicated in many developmental processes and stem cell systems. During telogen, dermal fibroblasts express BMP4, whereas subcutaneous fat expresses BMP2 (ref. 59). The $K6^+$ inner bulge layer secretes high levels of BMP6 and another quiescence factor, FGF-18 (ref. 2; Fig. 4b). Together, these factors maintain quiescence of both Bu-SCs and hair germ.

The dermal papilla located beneath the hair germ is an essential niche component that initiates hair regeneration. Upon dermal papilla ablation, telogen-phase hair follicles never reenter the hair cycle[60,61]. Several dermal papilla–specific factors have been implicated in hair follicle activation. During progression from early to late telogen, levels of hair germ–activating factors, including FGF-7, FGF-10, TGF-β2 and the BMP inhibitor noggin, become elevated in dermal papillae[54,62], whereas levels of BMP4 in dermal fibroblasts and BMP2 in mature adipocytes are downregulated, lowering the overall threshold for HFSC activation[59]. In addition, adipocyte precursor cells secrete platelet-derived growth factor-α (PDGF-α) to activate PDGF signaling in dermal papillae, which then relays a yet-to-be-identified signal to activate hair germ[63].

WNT signaling, a prominent pathway in development and cancer, is also critical for hair germ activation: the downstream WNT effector, nuclear β-catenin, accumulates in the activated hair germ and leads to target gene activation[54]. Without β-catenin, hair follicles arrest in telogen[58,64]. WNT signaling is also required in dermal papillae, as hair follicles regenerate more slowly when β-catenin is conditionally targeted there[65]. Although the exact WNT ligand(s) and source(s) mediating these effects remain to be identified, potential sources include the hair germ itself[54] and dermal fibroblasts[59,66]. Together, these activation cues overpower the inhibitory signals and launch regeneration (Fig. 4b).

Several lines of evidence suggest that Bu-SC activation may rely upon signals distinct from those used in hair germ activation. In contrast to the hair germ, which proliferates to form the matrix TACs upon anagen initiation, Bu-SCs remain quiescent until TACs emerge, a time when dermal papilla–activating cues are further distanced from the bulge. A recent study shows that Bu-SC activation depends on sonic hedgehog (SHH), a potent mitogen secreted by the newly formed TAC matrix. Moreover, by mid-anagen, as hair follicles grow downward and matrix-derived SHH moves away from the bulge, Bu-SCs resume

quiescence. Matrix-derived SHH also signals dermal papillae to intensify expression of noggin and FGF-7, which, together with SHH, maintain the matrix and lower ORS in a highly proliferative state throughout anagen[5]. Thus, although cross-talk between the dermal papilla and the hair germ initiates anagen, Bu-SCs depend upon signals from the emerging TAC pool for their activation (Fig. 4b).

Circadian rhythms also seem to have an impact on hair follicles, as they do on the epidermis. Expression of core circadian clock genes oscillates throughout the day in Bu-SCs, matrix and dermal papillae[67–69]. Furthermore, during the resting phase, the Bu-SC population displays an intriguing heterogeneity in core clock gene expression[67], and depletion of circadian clock proteins in mice delays anagen entry[68]. That said, epithelial lineage–specific knockouts of these genes have only mild effects on hair cycle progression[43,67,69], suggesting that circadian rhythms may not act on HFSCs directly to control their activities. Future studies should help to delineate the actions of the circadian clock on specific niche components and reveal how these mechanisms might regulate hair cycles.

Interactions of melanocyte stem cells and hair follicle stem cells

In mice, HFSCs and MSCs are intermingled in the bulge and hair germ[70], providing a unique opportunity to explore how two different types of stem cells coordinate their actions within a shared niche (Fig. 1). As a new hair cycle initiates, MSCs become activated to generate proliferative committed progenitor melanocytes. In mature hair follicles, these melanocytes reside within the inner core of the matrix, where they produce and transfer melanin pigment to differentiating hair cells. As catagen ensues, melanocytes degenerate along with the rest of the matrix.

Signals from both HFSCs and dermal papillae are essential to synchronize MSC activation and differentiation with those of HFSCs[71–74]. In the hair bulb during anagen, KIT ligand (secreted by the dermal papilla) and endothelins (secreted by the matrix) work in concert to prompt melanocyte differentiation. Interestingly, when telogen-phase HFSCs are conditionally targeted for loss of transcription factor NFIB, they aberrantly upregulate endothelin-2 (*Edn2*), which promotes precocious differentiation of MSCs near the KIT ligand–expressing dermal papilla. Adjacent hair germ cells take up melanin, prompting their apoptosis. Once the hair cycle is launched, these defects are resolved as the dermal papilla moves away from the bulge, thereby sparing the remaining HFSCs and MSCs[74]. Notably, *Edn2* expression in mouse skin can be induced upon UV irradiation[75], suggesting that the synchrony between HFSCs and MSCs might also be uncoupled in stress conditions.

Endothelin-1 is naturally induced by WNT signaling in early-anagen hair follicles[72]. Injection of endothelin receptor B antagonists can rescue the melanocyte expansion that results from either HFSC-specific *Nfib* deletion or elevated WNT signaling[72,74]. HFSCs might also produce WNTs and TGF-βs, offering other potential routes for coordinating MSC and HFSC behaviors[71,72,74]. Whether MSCs release instructive signals to HFSCs is an intriguing question that awaits future studies.

Diverse cell types interacting with skin stem cells

Immune cells in the skin

As our body's first line of defense, the skin is equipped with an impressive arsenal of immune cells. Immune cells and epithelial cells can influence each other's functionality and behavior. Mouse epidermis is populated with dendritic epidermal γδ T cells (DETCs) and Langerhans cells[76,77], whereas dermis is enriched for dendritic cells, mast cells, macrophages, γδ T cells and αβ T cells[77] (Fig. 1).

During injuries, compromising the epidermal barrier triggers inflammatory responses, which cause hyperproliferation of epidermal cells. In mice, DETCs in wounded skin produce FGF-7, FGF-10 and IGF-1, which are important for survival, proliferation and migration of epidermal cells[78,79]. Recently, FGF-9 secreted from dermal γδ T cells has been reported to promote dermal WNT activation and induce hair follicle neogenesis in wounded epidermis[80].

Interestingly, there are some similarities and differences between the injury-induced immune responses in mice and humans. Human epidermal resident T cells also upregulate IGF-1 upon wounding[81]. Nevertheless, a robust population of dermal γδ T cells is lacking in humans, which might account for poor hair follicle neogenesis in humans following injury[80]. Notably, when p120-catenin, a component of adherens junctions, is conditionally ablated in the epidermis, barrier functions remain seemingly intact. However, nuclear factor-κB signaling is induced, triggering inflammation and epidermal hyperplasia[82]. These findings suggest that pathogen-independent, intrinsic mechanisms coexist with pathogen-dependent ones to balance immune responses in skin.

The distribution and function of immune cells change during hair morphogenesis and cycling[77], suggesting that hair follicles might also influence the immune cell composition in skin. Indeed, hair follicles can act as entry points for immune cells to move into the epidermis: upon temporary ablation of epidermal Langerhans cells and inflammation, the junctional zone and infundibulum of hair follicles produce chemokines CCL2 and CCL20, respectively, to recruit new Langerhans cell precursors to the epidermis, whereas bulge cells express high levels of CCL8 that repel them[83].

Intriguingly, both bulge and matrix appear to express immunosuppressants, leading to reduced signaling to immune cells; this has prompted the hypothesis that these sites may be 'immune privileged'[84,85]. If so, such immune privilege must have limits, as both hair follicles and epidermis are targeted by immune cells upon allotransplantation. Moreover, in autoimmune disorders such as alopecia areata, immune cells target the hair bulb and the matrix, sparing only Bu-SCs, which leads to reversible hair loss[86]. In contrast, Bu-SCs are destroyed in discoid lupus erythematosus and lichen planopilaris, resulting in irreversible hair loss[87,88]. As the molecules mediating cross-talk between epithelial stem cells and immune cells continue to be discovered, more specific and effective therapies should emerge to maintain the skin's ability to defeat harmful stimuli caused by injuries and infections, but eliminate excess responses during inflammation and autoimmune responses.

Peripheral nerves in the skin

The skin is the largest sensory organ, innervated by numerous fibers of primary sensory neurons whose cell bodies are located in trigeminal and dorsal root ganglia. These neurons are a heterogeneous population, including nociceptors, mechanoreceptors and proprioceptors (Fig. 1).

Anatomically, sensory nerves are in close contact with cells in the epidermis and hair follicles. Free nerve endings terminate at different layers of the epidermis[89]. Mechanoreceptive nerve endings encase a region of the hair follicle immediately above the bulge[90,91]. In neonatal animals, the temporal sequence of innervation correlates with hair follicle morphogenesis, suggesting that the two processes are interdependent[92]. Skin-derived cues have been shown to have an impact on sensory innervation and dendritic arborization[93,94]. Conversely, signals from peripheral nerves may influence hair follicles. For example, premature hair follicle regression is elicited by the neuropeptides substance P and CGRP, which trigger neurogenic inflammation[95]. In addition, peripheral nerves that innervate the cells above the bulge secrete SHH and may govern the behavior of these cells in wounding[90]. As the complex communication circuits between skin and peripheral nerves become better understood at the molecular level, novel strategies may emerge to restore proper wiring and sensory functions after injuries such as severe burns.

Cutaneous blood vessels

The skin vasculature supplies the skin with nutrients, hormones and immune cells and plays a role in thermal control. Although arteries and veins are located in the lower (reticular) dermis, arborizing capillary networks can be found in the region above the bulge[96] (Fig. 1). These capillary networks might influence hair cycling: when angiogenesis is inhibited, anagen induction is delayed, indicating that angiocrine factors may regulate HFSC activity[97]. Intriguingly, the cells above the bulge express angiogenic factor EGFL6, an ECM protein[96]. Whether EGFL6 recruits blood vessels to the hair follicle remains to be explored. Intriguingly, minoxidil, an active ingredient used to treat androgenic alopecia (male-pattern baldness), is a vasodilator that has been proposed to work by increasing blood flow to the skin and thus potentially stimulating hair follicle growth. Future studies should help to reveal molecular cross-talk between skin vasculature and HFSCs.

Arrector pili muscle

HFSCs also control the formation and attachment of the arrector pili muscle (APM) responsible for piloerection (‘goosebumps’[6]; Fig. 1). Bu-SCs express nephronectin, an ECM protein in the same family as EGFL6. Both nephronectin and EGFL6 are ligands for $\alpha_8\beta_1$ integrin. Nephronectin is enriched in the bulge basement membrane, where it recruits $\alpha_8\beta_1^+$ dermal cells. In turn, nephronectin-integrin activation in these dermal cells induces the expression of smooth muscle actin, an APM marker. In mice lacking nephronectin, fewer APMs are formed and their anchorage is shifted to the EGFL6-expressing cells above the bulge, suggesting that EGFL6 may compensate for nephronectin[6]. Taken together, these studies suggest that different hair follicle compartments recruit and assemble different hair follicle–associated structures, including peripheral nerves, blood vessels and APMs, in part through expression of different ECM proteins.

Aging and pathological conditions

Aging and the niche

The skin shows profound structural and functional changes with age, including dermal and epidermal thinning, reduction in epidermal proliferation and injury repair, loss of dermal elasticity and wrinkling, and graying, thinning and loss of hair. Aged HFSCs maintain their numbers and gene signatures. However, telogen lengthens with age, suggesting that quiescent HFSCs become increasingly resistant to activation[98,99].

In culture, HFSCs from aged mice proliferate more slowly and generate fewer large colonies than their younger counterparts, indicating that intrinsic changes with age affect HFSC proliferation. *In vivo*, prolonged telogen in aged mice might be due to more BMP inhibitory cues and/or fewer WNT-activating signals, the balance of which determines HFSC activity. Indeed, elevated *Bmp2*, *Bmp4* and *Bmp6* expression and protein secretion from aged adipose tissue, and to a lesser extent aged dermal fibroblasts, may further raise the threshold for activating aged HFSCs. Correspondingly, aged HFSCs display upregulation of BMP targets *Nfatc1* and *Id2*, reinforcing HFSC quiescence[98]. The composition of immune cells in skin also changes with age[100], and age-related increases in proinflammatory cytokine signaling have been described[101]. Because immune-related changes are heavily influenced by pathogen exposure and skin barrier integrity, they add a variable component to the age-related decline in HFSC activity.

Lastly, although age-related systemic changes affect various organs, including those of the nervous system, muscle and heart[102–104], only modest increases in HFSC proliferation result from joining the circulatory systems of older and younger mice (parabiosis)[98]. Therefore, much of the age-related decline in HFSC function appears to arise from more local signals or the HFSCs themselves[98]. Moreover, these findings suggest that even though some common mechanisms of the aging process are shared, each stem cell niche and the macroenvironment surrounding it have unique regulators, complicating the search for a ‘fountain of youth’.

Wound repair and the niche

When skin is wounded, its stem cells must respond rapidly to restore the compromised barrier and repair tissue damage. Wound healing involves three overlapping phases: inflammation, tissue formation and tissue remodeling[105]. Inflammation occurs immediately after injury. Following platelet aggregation, various leukocyte lineages, including neutrophils, macrophages, mast cells and T cells, are recruited to the wound site. In addition to clearing dead cells and fighting against infections, these leukocytes secrete cytokines and growth factors such as TGF-βs, IGFs and FGFs that promote angiogenesis, migration and proliferation of keratinocytes and dermal fibroblasts, synthesis of ECMs and sometimes generation of new hair follicles in the process of epidermal regeneration[78–80]. During tissue formation, granulation tissue, consisting of newly formed blood vessels, macrophages and fibroblasts, begins to cover the wound. Epidermal cells then migrate over the granulation tissue to reepithelialize the wound. Adipocyte precursor cells are also activated at this stage to generate mature adipocytes important for fibroblast recruitment[106]. During tissue

remodeling, the epidermis and dermal fibroblasts deposit new ECM proteins to strengthen the repaired tissue[105].

Following full-thickness wounds, cells from both the hair follicles and the IFE migrate into the site of damage[16,56,90,107–109]. Mice lacking either hair follicles or HFSCs show delayed wound healing[110]. That said, very few HFSC-derived cells persist within the reepithelialized epidermis of a full-thickness wound. Lineage tracing of $Gli1^+$ cells above the bulge and $Lrig1^+$ cells in the junctional zone show that their progeny can persist longer[90,109], but even these hair follicle progeny are largely replaced by epidermal progeny following repair[16]. Overall, the data on full-thickness wounds have led to the now widely held view that input from hair follicles plays relatively minor roles, whereas IFE plays a major role in wound reepithelialization.

Cancer and the niche

In humans, exposure to UV damage from the sun increases the risk of oncogenic transformation of long-lived skin stem cells. Basal cell carcinoma (BCC), the most common cancer worldwide, is rooted in deregulated, sustained SHH signaling[111]. By contrast, squamous cell carcinoma (SCC), an aggressive skin cancer with significant risk of metastasis, can arise from oncogenic RAS transformation and accompanying loss of tumor suppressors such as p53, BRCA1 or TGF-β receptors, and/or their downstream effectors[112]. The roots of both BCCs and SCCs have been traced to multiple stem cell populations in the hair follicles and epidermis, whereas matrix seems to lack tumor-initiating capacity[113–116]. In mice, cutaneous injuries have been reported to exacerbate BCC malignant progression[117,118], a finding consistent with the higher risk of cancer in patients with chronic wounds.

Tumor-initiating cells, frequently referred to as cancer stem cells (CSCs), are also influenced by their niches, which are greatly altered by infiltration of blood vessels and immune cells as well as perturbations in the cross-talk of niches with their malignant stem cell residents (Fig. 5a). CSCs have been purified and characterized from mouse skin papillomas and SCCs[119–121]. At a near single-cell level, SCC-CSCs can induce the formation of a new SCC when transplanted into a host recipient mouse[120]. Additionally, the expansion of CSCs and their SCC progeny has been monitored by *in vivo* lineage tracing[122]. CSCs exist at the tumor-stroma interface and express high levels of integrins[120,121]. CSC cycling activities are influenced by cues from their niche, where signals from TGF-β and signals from integrin and focal adhesion kinase counteract each other to inhibit or promote CSC proliferation, respectively[120]. SCC-CSCs express vascular endothelial growth factor (VEGF), which stimulates elaborate tumor vascularization. Interestingly, VEGF may also act on CSCs in an autocrine fashion to increase their proliferation and thereby sustain tumor growth[123] (Fig. 5b). Therefore, CSCs are analogous to their normal counterparts in that they rely upon autocrine and paracrine niche signals for their self-renewal and differentiation.

With the identification of CSCs *in situ*, it is now possible to tackle the question of intrinsic versus extrinsic regulation of CSCs during tumor progression. Gene expression signatures have been reported for purified SCC-CSCs[120]. Large-scale screens, either genome-wide or with preselected gene sets, have allowed researchers to identify which of the myriad changes

in gene expression are causative for SCCs[124,125]. In the future, it will be interesting to discover whether altering some of these drivers in CSCs also affects CSC niches, as seen, for instance, with VEGF[123].

The niche in regenerative medicine

The use of cultured human keratinocytes to treat burn patients has been a success in regenerative medicine[17,126,127]. However, although these autologous grafts fulfill the need for epidermal barrier protection, they lack hair follicles, sweat glands and peripheral nerves. In addition, although the dermis underneath the engrafted epidermis recovers eventually, the entire process takes months, and the dermis is never restored fully.

The considerable progress in understanding stem cell–niche interactions in hair follicles and sweat glands opens new avenues for therapeutic advances, offering possible improvements for skin grafting and alopecia treatments. During embryonic development, WNT signaling within the epidermis and BMP inhibition in underlying mesenchyme triggers hair follicle formation[128,129]. Analogously, similar cross-talk between adult hair germs and dermal papillae stimulates each new round of hair cycling[54]. Intriguingly, fibroblasts from the upper dermis of neonatal skin also possess robust capacity for hair induction, whereas those from the lower dermis are not effective[130]. Collectively, these studies suggest that proper niche cell types, signaling and spatial organization are all important considerations for regenerating hair follicles.

In their native niche, adult HFSCs become active only upon hair cycle initiation, when they function in making new hair follicles. When transplanted onto immunocompromised mice, however, mixtures of dissociated HFSCs and mesenchymal cells regenerate not only hair follicles but also sebaceous glands and epidermis[131]. A three-dimensional culture method has recently been developed that involves seeding a mixture of dissociated hair follicle cells and dermal cells from adult mice or humans in collagen gels. Upon transplantation some of these mini-organoids can grow into functional hair follicles that receive nerve innervation, form APMs and undergo hair cycles and piloerection[132].

Another major advance is the identification of progenitors that give rise to sweat glands in mice. In homeostasis and mild injury, most adult sweat gland progenitors behave unipotently, only giving rise to myoepithelial or luminal epithelial cells. However, upon engraftment, purified myoepithelial stem cells can generate an entire gland, containing both myoepithelial and luminal epithelial layers; this is reminiscent of their multipotent behavior during development[133]. Together, these studies further reinforce the idea that stem cell potential and behavior are not fixed, and can be altered upon exposure to different environments. With ever-increasing knowledge of stem cell populations, regulatory signals within the niche, and the ecosystem of the skin, the ability to regenerate a fully functional skin for tissue replacement in regenerative medicine should continue to improve.

Conclusions

Recent findings have brought forth the complexity of cellular and molecular regulators within the skin stem cell niche during development, homeostasis, injury, aging and cancer.

Several open questions remain. First, although many putative niche factors have been identified, their functional importance can be firmly established only by knocking out specific factors spatially and temporally from specific niche components that express them. To date, this has rarely been achieved for any given mammalian stem cell system. Second, although several cell types, including blood vessels and sensory neurons, make stereotypic and spatially distinct contacts with epithelial cells, specific signals governing these connections remain to be identified. Third, given the heterogeneity and complexity of tumor development, our understanding of the cancer stem cell niche is still in its infancy. Fourth, biological processes such as pregnancy, lactation, hypoxia and circadian rhythms can also impact skin stem cells[44,67,134–136]. Whether these influences are mediated through effects on any niche components remains to be explored.

These hurdles will probably be overcome with continued development of stem cell–specific genetic tools, identification of new markers to characterize specific stem cell populations more precisely, and improvements in imaging strategies. With its rich cellular composition, the skin will continue to serve as an important paradigm in the quest to understand stem cell niches. In the era of tissue engineering—driven by the hope that in the future we will be able to manipulate stem cell behavior *in situ*, suppress tumor formation and progression and grow functional tissues for regenerative medicine—it is even more important and timely to tackle the complexity of niche components within the skin.

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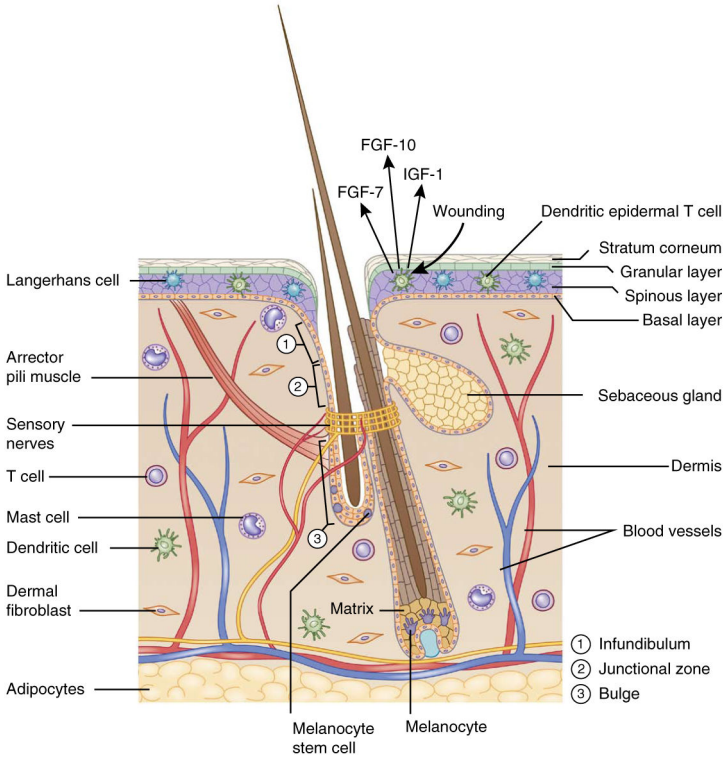

Figure 1.

The skin: an organ with a diverse array of cell types. The hair follicle is a complex appendage of the epidermis. It is composed of an infundibulum that opens to the skin surface, sebaceous glands, and the junctional zone between the glands and the bulge. Hair follicle and melanocyte stem cells reside in the bulge and the hair germ. In full anagen, hair follicle stem cells regenerate the lower two-thirds of the follicle, including the matrix, which produces the hair and its channel. Melanocyte stem cells generate mature melanocytes, which transfer their pigment to differentiating hair cells. The hair follicle also serves as a hub attracting peripheral nerves, blood vessels and arrector pili muscles. The dermis is populated with dermal fibroblasts and various immune cells such as mast cells, dendritic cells and T cells. Deeper in the dermis is a layer of subcutaneous adipocytes.

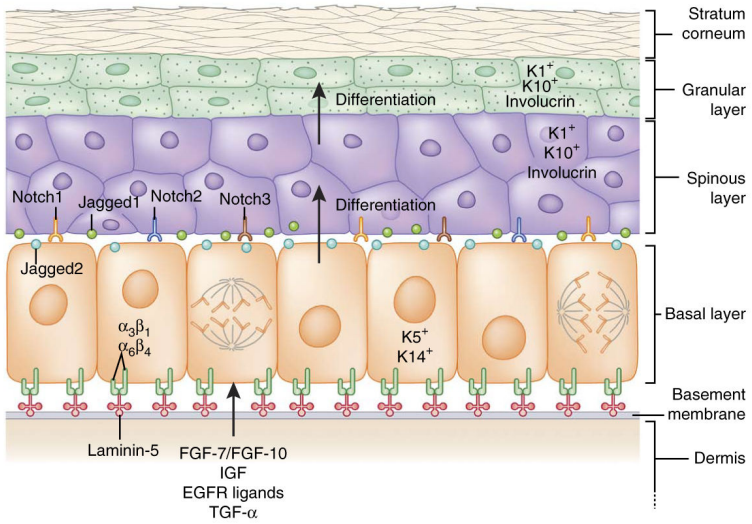

Figure 2.

Interfollicular epidermis: architecture, signaling and lineages. The epidermis is a stratified structure. Self-renewing stem cells reside within the basal layer, which adheres through $\alpha_3\beta_1$ and $\alpha_6\beta_4$ integrins to an underlying basement membrane of laminin-5–rich extracellular matrix that separates the epidermis from the underlying dermis. Secreted factors such as FGF-7, FGF-10, IGF, EGF ligands and TGF-α from dermal fibroblasts promote the proliferation of basal epidermal cells. Proliferative basal progenitors generate columnar units of Notch-activated terminally differentiating cells that go through three stages: spinous layers, granular layers and finally dead stratum corneum layers that eventually are shed from the skin surface. Each cell type expresses a different gamut of keratin (K) proteins.

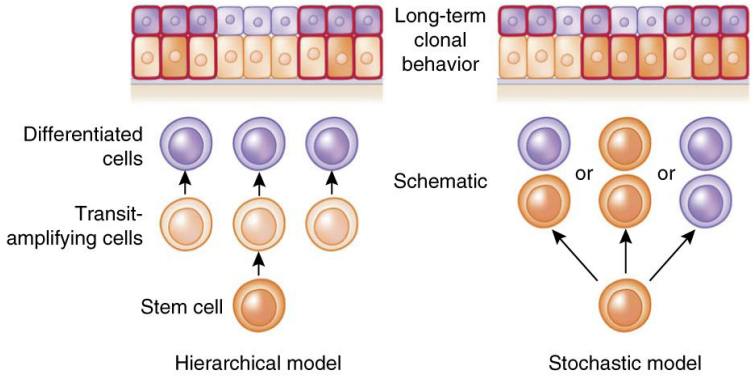

Figure 3.

Hierarchical versus stochastic models of epidermal differentiation. In a hierarchical model, rare divisions by stem cells generate rapidly dividing transit amplifying cells, which then give rise to differentiated cells. During lineage tracing, only clones marking the stem cells are long lived, and thus clone sizes become invariant after a period of time. By contrast, in a stochastic model, all basal cells are the same and each division can yield three different outcomes: (i) one differentiated daughter that withdraws from cell cycle and departs from the basal layer, and one progenitor that remains in the basal layer and continues to divide; (ii) two basal progenitors; and (iii) two differentiated daughters. Although the fate choices are random, the probabilities of different outcomes are similar, so that the generation of differentiated cells and the maintenance of committed progenitor pools are balanced at the population level and long-term homeostasis is ensured. In this model, each individual clone will vary in size. Predictions of lineage-tracing results from each model are shown at the top of the diagram; cells outlined in red are the ones retaining lineage-traced marks.

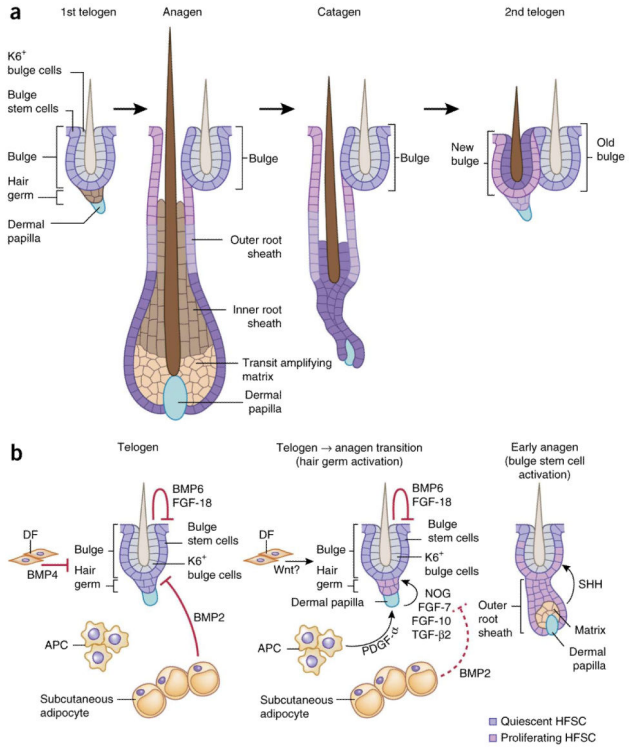

Figure 4.

Hair follicle lineage and niche signals regulate hair follicle stem cells. (**a**) HFSCs can exist in two states. Quiescent bulge stem cells (Bu-SCs) are located in the outer layer of this niche and contribute to the generation of the outer root sheath. Primed stem cells reside in the hair germ, sandwiched between the bulge and a specialized dermal cluster known as the dermal papilla. They are responsible for generating the transit amplifying cell (TAC) matrix, which then gives rise to the hair shaft and its inner root sheath (IRS) channel. Although matrix and IRS are destroyed during catagen, many of the outer root sheath (ORS) cells are spared and generate a new bulge right next to the original one at the end of catagen. The upper ORS contributes to the outer layer of the new bulge, and the middle ORS contributes to the hair germ. Some of the lower ORS cells become the differentiated inner keratin 6^+ ($K6^+$) bulge cells, which provide inhibitory signals to Bu-SCs, raising their activation threshold for the next hair cycle. (**b**) During telogen, $K6^+$ bulge cells produce BMP6 and FGF-18, dermal fibroblasts (DFs) produce BMP4 and subcutaneous adipocytes express BMP2. Together, these factors maintain Bu-SCs and hair germ in quiescence. At the transition to anagen, BMP2 and BMP4 are downregulated, whereas the expression of activation factors including noggin (NOG), FGF-7, FGF-10 and TGF-β2 from dermal papillae and PDGF-α from adipocyte precursor cells (APCs) is elevated. This, in turn, stimulates hair germ proliferation, and a new hair cycle is launched. Bu-SCs maintain their quiescent state until TAC matrix is generated and starts producing SHH.

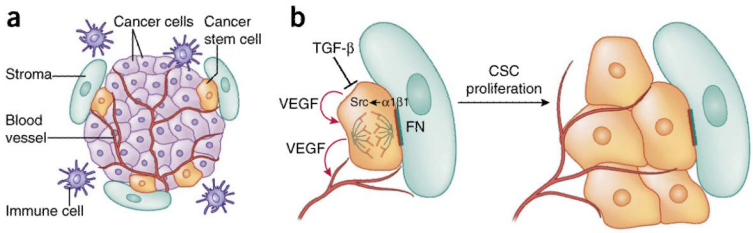

Figure 5.

Signaling pathways in skin cancers. (**a**) The squamous cell carcinoma–cancer stem cell niche. CSCs are often found at the tumor-stroma interface, together with an elaborate vasculature, immune cells and aberrant fibroblasts. (**b**) Upon activation of $\alpha\beta_1$ integrins by extracellular matrix ligands such as fibronectin (FN), focal adhesion kinase (FAK) and its associate tyrosine kinase Src become hyperactivated and promote proliferation of CSCs. By contrast, TGF-β signaling counteracts integrin activity and enhances CSC quiescence. In addition, CSCs secrete VEGF, which acts in an autocrine fashion to enhance CSC proliferation and in a paracrine fashion to promote formation of new blood vessels.