

Stem cell dynamics, migration and plasticity during wound healing

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Tissue repair is critical for animal survival. The skin epidermis is particularly exposed to injuries, which necessitates rapid repair. The coordinated action of distinct epidermal stem cells recruited from various skin regions together with other cell types, including fibroblasts and immune cells, is required to ensure efficient and harmonious wound healing. A complex cross-talk ensures the activation, migration and plasticity of these cells during tissue repair.

The skin is the first barrier protecting animals against UV radiation and pathogens from the external environment. It is composed of an epithelial layer, the epidermis, and the underlying dermis, which are separated by a basement membrane¹. The epidermis contains pilosebaceous units that include a hair follicle and sebaceous glands, and are connected with the interfollicular epidermis (IFE) through the infundibulum¹. The skin epidermis also contains other appendages, such as sweat glands, which regulate body temperature through perspiration¹. The dermis is composed of an upper (papillary) and a lower (reticular) layer of fibroblasts, blood vessels, immune cells and extracellular matrix (ECM)^{2,3}. Specialized fibroblasts form the dermal papilla, which regulates hair follicle growth and the erector pili muscle, responsible for pilo-erection. Partially integrated into the reticular dermis is a layer of dermal adipocytes that form the dermal white adipose tissue⁴. Underneath the dermis, the hypodermis (or subcutaneous adipose tissue) is composed of adipocytes, blood vessels and inflammatory cells⁴. This layer is important for thermoregulation and mechanical protection^{2,4} (Fig. 1a).

Upon tissue damage, the skin has to be repaired as quickly as possible to prevent excessive blood loss and infection. Wound healing occurs through distinct overlapping phases: haemostasis, inflammation, proliferation and remodelling⁵. Haemostasis occurs immediately after tissue damage and results in the formation of a blood clot, which stops the haemorrhage and triggers the recruitment of different immune cells, including neutrophils, macrophages and lymphocytes, to prevent infection and further activate the inflammatory response^{5,6} (Fig. 1b). The proliferation phase coordinates epidermal re-epithelialization and dermal repair⁵ (Fig. 1c). The remodelling phase removes cells that are no longer necessary and induces ECM remodelling⁶. In small excisional wounds (<1 cm in diameter in mice), hair follicles are not reformed and dermal scar tissue compensates for skin loss⁷ (Fig. 1d). However, in large wounds (>1 cm in diameter), regeneration of hair follicles (wound-induced hair follicle neogenesis (WIHN)) can be observed after re-epithelialization during the remodelling phase, resembling hair follicle embryonic development and a regeneration phase, typically 13–14 days after injury in mice^{7,8} (Fig. 1d).

Although the key steps of wound healing are well described at the tissue level, a more in-depth characterization of the behaviour of individual cells at the clonal level and their fate transitions has only yet begun. The emergence of lineage tracing and intravital microscopy, coupled with transcriptional and epigenetic profiling, provide important insights about cellular and molecular mechanisms responsible for wound healing^{9–12}. In this Perspective, we describe recent advances with an emphasis on skin epithelial stem cell populations,

their heterogeneity, clonal dynamics and remarkable plasticity during wound healing. Finally, we discuss the role of fibroblast populations and immune cells during repair and regeneration.

Skin epithelial stem cells during homeostasis

The skin epithelium renews throughout life in a continuous turnover ensured by stem and progenitor cells that balance proliferation and differentiation to replace dead and terminally differentiated cells^{1,13,14}. Epithelial stem cells reside in a specific microenvironment called the niche that is composed of various cell types. Niche cells influence stem cell behaviour directly by cell contact or indirectly via ECM components and growth factors¹⁵. Although skin stem cells are able to regenerate the entire repertoire of skin epithelial lineages upon transplantation, lineage tracing has demonstrated that, during physiological conditions, epidermal compartments are sustained by their own pool of resident stem cells^{14–16} (Fig. 2a).

During adult homeostasis, hair follicles undergo cycles of growth (anagen) and degeneration (catagen), followed by a resting stage (telogen)¹. The hair follicle stem cells (HFSCs) responsible for cyclic regeneration are located in the permanent non-cyclic follicle portion called the bulge^{17–20}. HFSCs were first identified based on their slow-cycling properties^{19,21,22}. They have higher clonogenicity in vitro and give rise to IFE, hair follicle and sebaceous gland lineages upon transplantation^{17,18,20,23,24}. Slow-cycling HFSCs were first isolated and characterized using *K5-tTA/TRE-H2BGFP* and *Krt15-EGFP* transgenic mice^{25,26}, and several studies revealed the expression of bulge specific markers, such as *Cd34* (refs 18,23), *Krt15* (refs 27,28), *Krt19* (ref. 29), *Lgr5* (ref. 30), *Sox9* (refs 31,32) and *Tcf3* (ref. 33). In sharp contrast to transplantation experiments, lineage tracing using *Krt15-CrePR*³⁴, *Shh-Cre*³⁵, *Lgr5-CreER*³⁰, *K19-CreER*²⁹, *Sox9-Cre*³² and *Tcf3-CreER*³³ mouse strains established that HFSCs only contribute to hair follicle regeneration during physiological conditions and do not maintain the sebaceous gland, infundibulum or IFE (Fig. 2a).

The IFE is composed of a single layer of proliferative basal cells and several layers of differentiated non-proliferative cells¹. Basal cells replenish the suprabasal cells that are lost as terminally differentiated squames. In mice, it takes about 1 week for a basal cell to transit to the surface of the skin and about 1 month to replenish the whole IFE³⁶. Early proliferation kinetic experiments reported maintenance of the IFE by small proliferative units that contain stem cells and progenitors³⁷. However, lineage tracing at clonal density later demonstrated that these units do not have a fixed size or predictable proliferation kinetics^{38,39}. Instead, several studies, showed that IFE homeostasis was sustained by a single population of committed progenitors that balance renewal and differentiation in a stochastic manner^{38,40–43}. However, further studies provided evidence that

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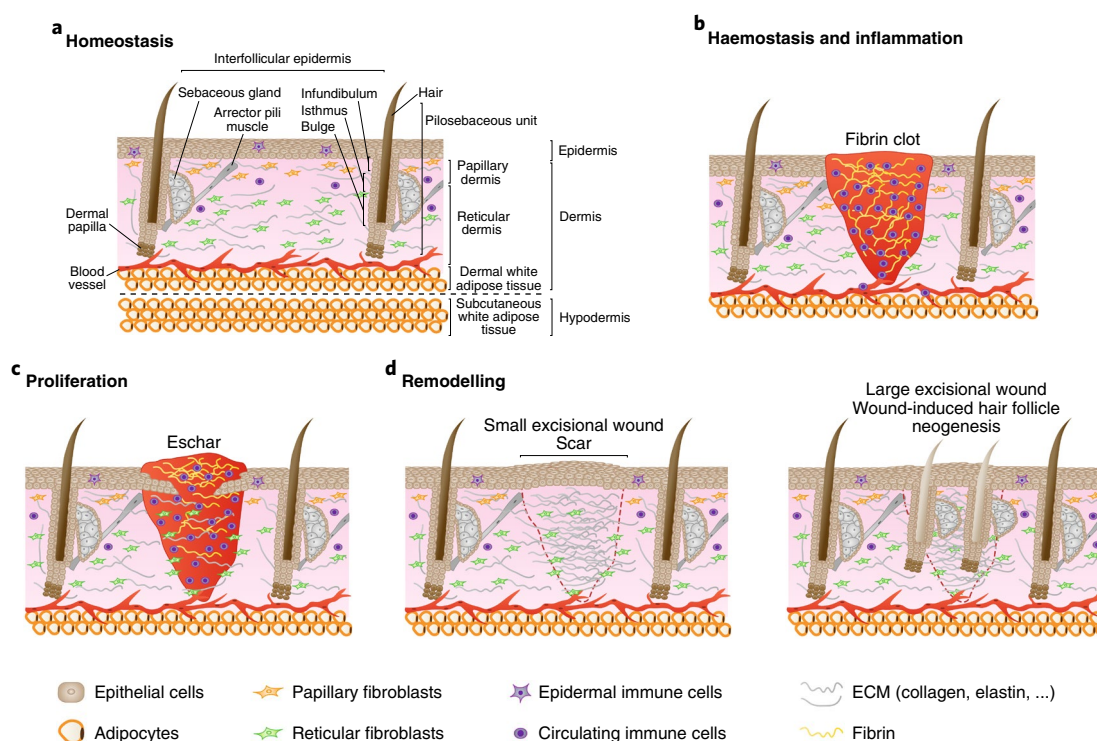


Fig. 1 | Overview of skin homeostasis and wound-healing phases. **a**, The skin is composed of the dermis and the epidermis. In the epidermis, epithelial cells are organized into a pilosebaceous unit (the hair follicle and its associated sebaceous glands) and the surrounding tissue (the IFE). The dermis consists of a papillary and a reticular layer located in the upper and lower part, respectively. Dermal papilla controls the hair follicle cycle and the arrector pili muscle ensures its movement. The dermis includes fibroblasts, blood vessels, immune cells, sensory nerves and, in its lower portion, the dermal white adipose tissue, which contains adipocytes. Below the skin lies the hypodermis or subcutaneous white adipose tissue. **b**, Haemostasis and inflammation start immediately after wounding. The fibrin clot prevents further blood loss and provides a scaffold for the migration of immune, dermal and epidermal cells. **c**, During the proliferation phase, keratinocytes, fibroblasts and endothelial cells proliferate and migrate to the wound site and reform the ECM. **d**, During the remodelling phase, the collagen in the dermis is remodelled and cells from earlier stages are removed. In small excisional wounds in mice, hair follicles are not regenerated and dermal scar tissue compensates for skin loss (left panel). In large excisional injuries, WIHN can be observed after complete re-epithelialization (right panel).

basal epidermal cells are heterogeneous and some cells, depending on the skin regions, exhibit stem cell characteristics^{44,45}. These IFE stem cells (IFSCs) were more quiescent, persisted longer and could give rise to more rapidly cycling committed progenitors with a shorter lifespan^{44,45} (Fig. 2a). Profiling of murine stem and progenitor cells showed that the two populations are molecularly different and that stem cells express a higher level of basal integrins, as do human epidermal stem cells, whereas committed progenitors are primed towards differentiation^{44,46}.

The isthmus, a region located between the bulge and sebaceous gland, contains its own pool of resident stem cells that express *Blimp1* (ref. 47), *Lgr6* (ref. 48), *Lrig1* (ref. 49), *Gata6* (ref. 50) or *Plet1* (ref. 51). These multipotent cells give rise to all epidermal lineages upon transplantation^{48–50,52}. Lineage tracing using *Blimp1-Cre*⁴⁷, *Lgr6-CreER*^{48,53} and *Lrig1-CreER*⁵⁴ has confirmed that these cells maintain the isthmus and sebaceous gland during homeostasis (Fig. 2a). In addition, *Lrig1*-expressing cells also give rise to cells of the infundibulum⁵⁴ (Fig. 2a), whereas *Gata6*-expressing stem cells only contribute to the maintenance of the sebaceous gland ducts but not the gland itself during homeostasis⁵⁰ (Fig. 2a). Altogether, these data show that, during physiological conditions, skin stem cells are confined to restricted compartments. Presently, the molecular mechanisms that restrain the movement of these cells across different territories remain unclear. As in the intestine⁵⁵, cell-specific expression of different guidance molecules might confine cell types in specific territories.

Skin epithelial stem cells during wound healing

During wound healing, stem cells are activated and recruited from different skin regions. Interestingly, lineage restriction and spatial confinement of resident skin stem cells are transiently lost during repair, allowing contribution of multiple epidermal stem cells^{15,34,54,56,57} (Fig. 2b).

The involvement of HFSCs in wound healing was already proposed 40 years ago after dermabrasion experiments in mice⁵⁸ and further confirmed by more-recent analyses of proliferation kinetics²². Lineage tracing targeting labelled retaining cells²⁶ or using *Krt15-CrePR*³⁴, *Shh-Cre*⁵⁷, *K19-CreER*⁵⁴ and *Lgr5-CreER*⁵⁴ reporter strains showed that HFSCs rapidly migrate from the bulge to the wound and contribute to epidermal repair (Fig. 2b). These data demonstrate that HFSCs are highly plastic during wound healing, similarly to their expanded fate potential upon transplantation^{17,20,30,49,52}.

Clonal analysis of IFSCs and committed progenitor cells following injury of mouse tail skin showed that IFSCs are recruited to the wound, contribute to epidermal repair and persist up to 35 days⁴⁴ (Fig. 2b). By contrast, committed progenitors are initially recruited, but their progeny do not remain in the wound long term⁴⁴. Additional lineage tracing with *Dlx1-CreER* and *Slc1a3-CreER* reporters, which mark slow-cycling and rapidly cycling stem cells from different microdomains of tail and back skin IFE, demonstrated that, upon wounding, both stem cell populations repopulate the two IFE regions⁵⁹. However, in the long term, both cell populations only persist in their region of origin and not in the region that

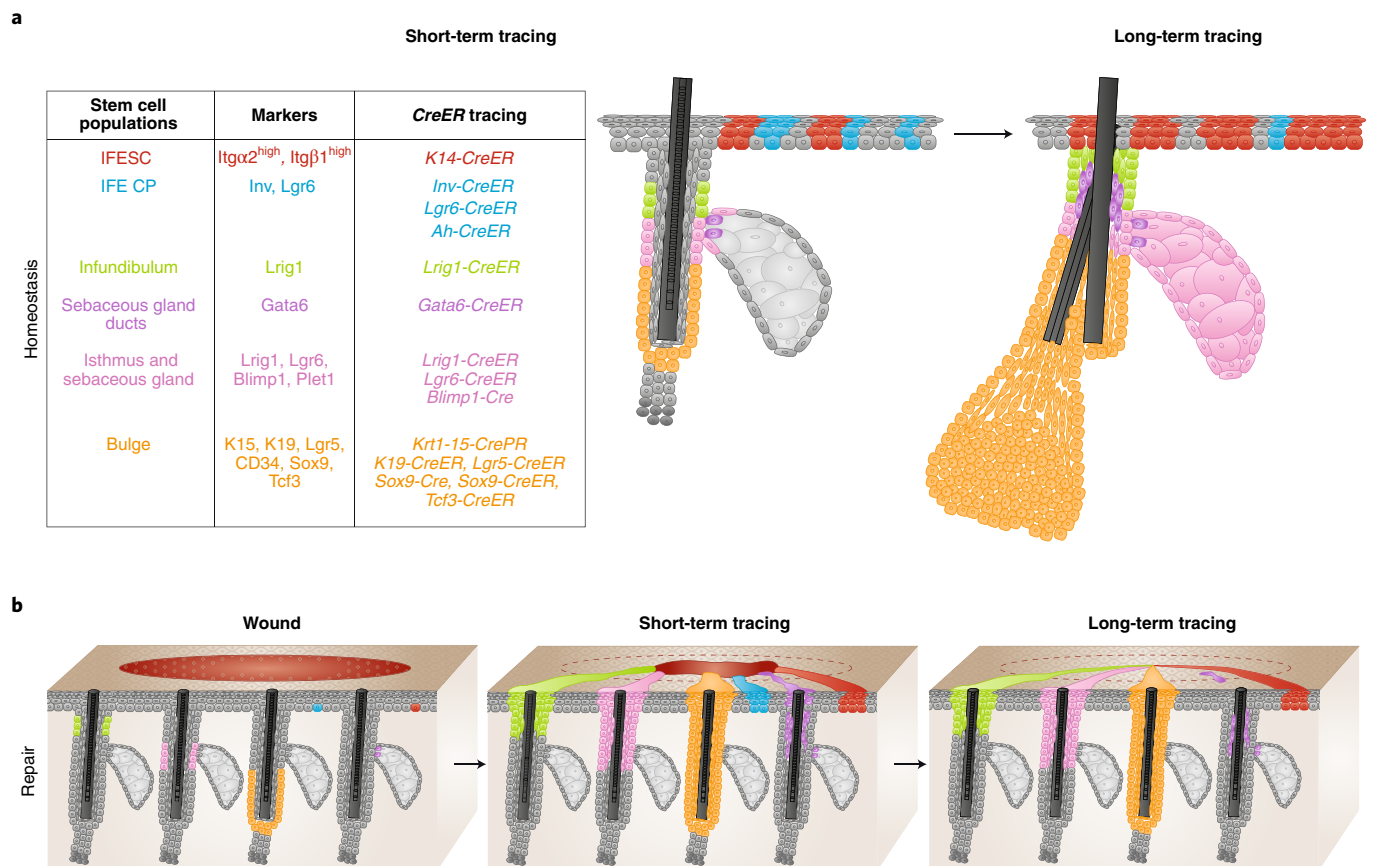


Fig. 2 | Skin epithelial stem cell populations during homeostasis and repair. a, Skin epithelial stem cells express specific markers and can be lineage traced with *CreER* mouse strains (left table). IFESCs and committed progenitors are located in the basal layer of the IFE and give rise to suprabasal, differentiated cells. Stem cells and committed progenitors can be traced using *K14-CreER* or *Inv-CreER* mouse strains induced at low dose, respectively. IFE committed progenitors (CPs) also express *Lgr6*. Infundibulum stem cells are located in the upper part of the isthmus and express *Lrig1*. A population of sebaceous gland duct stem cells expressing *Gata6* are located at the entrance of the gland but only maintains the junctional zone. Isthmus and sebaceous gland stem cells are basal cells located at the junction between the hair follicle and the gland, express *Lrig1*, *Lgr6* and *Blimp1* and give rise to the entire sebaceous gland and the isthmus. Bulge stem cells are located in the permanent lowest portion of the hair follicle, express *K15*, *K19*, *Lgr5*, *Cd34*, *Sox9* and *Tcf3* and give rise to the entire hair follicle. **b,** Upon wounding, both IFESCs and committed progenitors are recruited and contribute to tissue repair. Only IFESCs will reside in the newly formed IFE long term. Isthmus, sebaceous gland and infundibulum stem cells are recruited, contribute to IFE repair and remain long term. Bulge stem cells are recruited to the IFE and a small proportion can remain long term as IFESCs. *Gata6*-expressing sebaceous gland duct stem cells are recruited to the IFE, migrate suprabasally, dedifferentiate and are re-established as IFESCs in the long term.

they migrate to during wound healing⁵⁹. These observations suggest that, during repair, all basal cells present some degree of plasticity, a change in behaviour and functional contribution, but the wound does not reset the clock completely and cells keep a memory of their original location and hierarchy.

Similarly to HFSCs, *Lrig1*-expressing and *Lgr6*-expressing stem cells from the upper isthmus are mobilized following wounding and are possibly activated even more rapidly than HFSCs⁵⁴. HFSCs have been assumed to be quickly lost during regeneration and to only serve as a transient bandage that allows other stem cells from the IFE and upper isthmus/infundibulum to sustain long-term repair³⁴. However, a more recent study showed that the proportion of hair follicle and *Lrig1*-derived cells located in the epidermis drops dramatically 3 weeks after an injury, whereas remaining cells can persist up to 1 year thereafter⁵⁴ (Fig. 2b). The persistence of these cells in the re-epithelialized IFE is proportional to the amount of stem cells labelled in the beginning and suggests a stochastic competition between equipotent stem cells rather than a hard-wired process⁵⁴. Importantly, glabrous skin, such as the ventral (or palmar) part of the paw, heals correctly with slower kinetics than human skin, showing that HFSCs are dispensable for wound healing⁶⁰.

Moreover, similar to the contributions of hair follicles and the infundibulum in skin with hair, sweat gland duct progenitors help to regenerate the injured epidermis in mouse paws⁶¹. Altogether, these studies suggest that the vacant niche created by an injury activates a broad range of stem cells to assume characteristics that differ from their homeostatic roles. Additional studies will be required to better understand the signals that activate distant stem cells, respective timelines and the mechanisms that disrupt and re-establish the boundaries between skin compartments. It further remains unclear how differentiation programmes get rewired during wound healing and how the balance between proliferation, migration and differentiation is achieved.

Migration, proliferation and compartmentalization. Epidermal injury is typically followed by increased keratinocyte proliferation⁵. Interestingly, proliferation is not observed at the wound edge but rather at a distance of 0.5–1.5 mm away from the edge^{9,10,62}, in a proliferative zone that surrounds the wound. At the leading edge, keratinocytes do not proliferate but migrate as a cellular sheet^{9,10} (Fig. 3).

Intravital microscopy during wound healing demonstrated that both basal and suprabasal layers migrate during wound healing¹⁰.

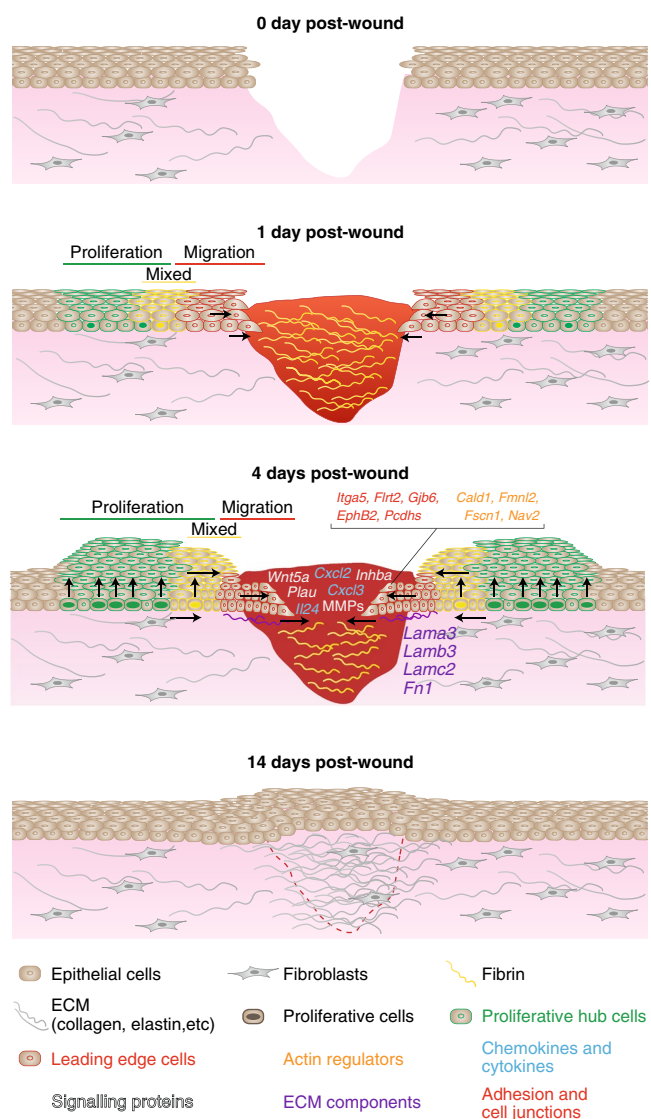


Fig. 3 | Epidermal migration, proliferation and compartmentalization during wound healing. Epithelial cells start to migrate into the wound bed within 12 hours after injury. The day after wounding, IFE cells located close to the wound show an elongated shape towards the direction of the wound (horizontal arrows) and are quiescent, whereas cells located at a distance start to proliferate, which leads to the establishment of a proliferative and a migrating leading edge compartment. Between these two zones, a mixed region is observed containing both migrating and proliferative cells. Four days after wounding, leading edge cells are compressed and upregulate the expression of specific genes that promote inflammation and regeneration. This gene signature is transient and disappears when the IFE is healed. Vertical arrows represent the movement of basal cells toward the differentiated layers. MMPs, matrix metalloproteinases.

The speed of migration is greatest closer to the leading edge and decreases thereafter¹⁰. At a distance of 0.5 mm from the edge, both migration and proliferation co-occur¹⁰. In this mixed region, basal cells are elongated towards the wound and orient their division in this direction¹⁰. In the tail epidermis, cells present at the leading edge are initially elongated parallel to the direction of the wound, suggesting active migration, but assume a perpendicular orientation 2–4 days after wounding, possibly because they are pushed and compressed by cells behind⁹. Whether proliferation is necessary for cell migration remains unclear. Pharmacological inhibition of cell

proliferation prevents wound closure and cell compression at the leading edge in tail skin⁹. By contrast, proliferation is dispensable for wound closure in the mouse ear epidermis¹⁰. However, cells also display a more elongated shape in wounds with inhibited proliferation, suggesting a compensatory effect¹⁰. Differences in wound size and region-specific dermal populations could explain the discrepancies observed between the ear and tail IFE. However, in *Rac1*-knockout mice with perturbed cell migration and elongation, a defect in the orientation of cell division is evident, suggesting its control by migration of the leading edge¹⁰. Altogether, these observations imply that cell migration at the leading edge comes first after wounding and that the displacement of cells in this region triggers orientated cell division of the cells following behind. Increased proliferation can itself generate a surplus of migrating cells that later push the leading edge towards the wound centre.

Transcriptional profiling of cells from migration and proliferation zones indicated two molecularly distinct and transient regions⁹. Cells at the leading edge expressed transiently higher levels of matrix metalloproteinases, pro-inflammatory molecules, genes controlling the cytoskeleton, microtubule and actin remodelling, ECM ligands and cell-adhesion molecules, such as integrin α_5 (ref. ⁹) (Fig. 3). The constant size of the leading edge and its independence of wound size or skin area suggest that the signals controlling marker expression are local and potentially propagated from cell to cell within the epidermis. The leading edge might act as a transient scaffold enabling harmonious wound healing. By secreting a higher level of proteins that control ECM remodelling and blood clot dissolution, the leading edge might promote the progression of tissue regeneration towards the wound centre and protect stem cells and their progeny from tissue remodelling.

In addition, the acquired migratory phenotype of keratinocytes displays some features of epithelial-to-mesenchymal transition, including downregulation of cell-adhesion molecules, increased motility and upregulation of epithelial-to-mesenchymal transition markers, such as *Slug*^{63,64}. Whether epithelial-to-mesenchymal transition is required for efficient wound healing or leading edge migration will require further analysis.

Stem cell population dynamics. During homeostasis, IFSCs and committed progenitors divide asymmetrically at the population level to maintain a constant number of epidermal cells⁶⁵. However, during wound healing, cell numbers need to increase to compensate for lost cells until re-epithelialization is completed. Excess of renewal over differentiation can be achieved by increasing symmetric renewal or decreasing the proportion of cells that undergo differentiation, as during oesophageal wound repair⁶⁶ or in vitro culture of keratinocytes⁶⁷. Upon tail injury, clonal analysis of *K14-CreER* (IFSCs and progenitors) and *Lrig1-CreER* (infundibulum stem cells) mouse strains demonstrated streaks of labelled cells arising from single IFE or infundibulum cells, both basal and suprabasal, that project towards the wound centre⁹. IFE-derived clones decreased by more than 90% during the first week, due to rapid terminal differentiation of committed progenitors, but overall cell ratios demonstrated that committed progenitors continued to divide mostly asymmetrically at the population level⁹. The proportion of basal and suprabasal cells from *Lrig1-CreER*-derived clones was similar to IFE-derived clones, although the size of the infundibulum-derived clones was slightly bigger⁹. Clonal persistence, clone size and the basal/suprabasal cell ratio were consistent with a hierarchical model in which rare stem cells reside at the top of the hierarchy, dividing asymmetrically at a much more rapid pace than homeostasis and give rise to progenitors, so that the equilibrium between proliferation and differentiation remains balanced. Irrespective of their initial locations, wounding seems to induce the activation of a minor stem cell population, whereas lineage hierarchy and balance between self-renewal and differentiation of committed progenitors remain unchanged from homeostasis⁹.

Recently, clonal analysis of the human skin epidermis was performed after grafting in vitro reconstructed, genetically engineered skin into a patient with a severe form of the genetic skin disorder epidermolysis bullosa⁶⁸. Sequencing of the integration site of the retrovirus used to replace the mutated gene revealed that viral integration in keratinocyte colonies originating from progenitors rapidly decreased over time⁶⁸. By contrast, the integration sites of the most clonogenic colonies derived from stem cells increased, supporting the notion that only human skin stem cell-derived colonies are able to renew and expand long term in vivo, whereas progenitor cells possess limited potential to self-renew and revert back into a stem cell-like state⁶⁸.

Stem cell plasticity. When stem cells from the hair follicle and infundibulum are recruited to the IFE upon injury, they progressively lose their initial identity and are reprogrammed to an IFE fate³⁴. The molecular mechanisms responsible for this plasticity are still incompletely understood. In a comparison of chromatin landscapes of injured IFE and homeostatic HFSCs and IFSCs, the wounded IFE exhibited a hybrid signature between HFSCs and IFSCs, in which the open chromatin regions were enriched for both IFSC (Klf5) and HFSC (Sox9) transcription factors¹². This hybrid stage called 'lineage infidelity' seems to ensure proper re-establishment of the epidermal barrier¹². Although this hybrid state is transient during repair, it persists in skin cancer^{12,69}.

Differentiated suprabasal epidermal cells are able to revert back to a stem cell state upon wounding^{70,71}, a phenomenon also observed in airway epithelium after lineage ablation of basal stem cells⁷². However, lineage tracing and photolabelling of suprabasal IFE cells demonstrated that these cells cannot adopt a basal state again under wound-healing conditions^{9,10}. Contrastingly, a population of *Gata6*-expressing cells residing in the isthmus, which during homeostatic conditions give rise to the sebaceous duct, can be mobilized during wound healing to migrate towards the injured IFE and revert from a differentiated to a basal stem cell fate⁵⁰. This reversion does not occur immediately after injury, as the suprabasal cells require a few days to access the basal layer and undergo stem cell reprogramming⁵⁰. This intriguing observation raises the question of whether other differentiated epidermal cells are also able to revert back to a stem cell state or whether this is a unique property of the *Gata6*-expressing population. It is possible that the timing of reversion is important and that experiments performed on the tail and ear epidermis induced the labelling of the suprabasal cells too early to observe the reversion^{9,10}. Further experiments will be necessary to identify the mechanisms underlying this cellular plasticity and reprogramming of differentiated cells during wound healing. Other cases of dedifferentiation have been previously described in the hair follicle^{73,74}. After depilation or laser ablation to induce the loss of bulge HFSCs, hair germ cells⁷³ and infundibulum or sebaceous gland cells⁷⁴ are able to repopulate the stem cell niche and establish functional HFSCs. Similarly to skin, cells in the gut epithelium that are committed to terminal differentiation can revert back to a progenitor-like state and contribute to tissue repair following injury^{75–77}. However, intestinal stem cells are required to ensure tissue repair following ionizing radiation⁷⁸, demonstrating that, despite the ability of committed cells to re-assume stemness, regular tissue-resident stem cells are essential for repair.

The degree of damage can also influence cellular plasticity. In relatively small wounds, re-epithelialization occurs without reforming hair follicles, whereas de novo hair follicle formation is apparent in large wounds⁸. Lineage tracing confirmed that these de novo hair follicles do not originate from HFSCs but from IFE cells⁸. Analogous to hair follicle morphogenesis during embryonic development^{79–81}, WIHN depends on Wnt signalling, as overexpression of Dickkopf Wnt signalling pathway inhibitor 1 or β -catenin deletion in IFE basal cells prevents de novo hair follicle regeneration⁸. Epidermal deletion

of *Wntless*, a gene required for the secretion of Wnt ligands, suppresses WIHN, suggesting that keratinocyte-derived Wnt is essential⁸². Different mouse strains have different susceptibilities to WIHN^{83,84}. Toll-like receptor 3 (Tlr3) levels are increased in mouse strains with enhanced WIHN, and double-stranded RNA is a key signal that triggers skin regeneration through Tlr3 (ref. ⁸⁴). Msh homeobox 2 is also crucial for WIHN⁸⁵. Fibroblast growth factor 9, secreted by $\gamma\delta$ T cells, triggers Wnt expression by wound-induced fibroblasts, further amplifying the Wnt signal required for WIHN. These data illustrate the importance of a crosstalk between immune cells, fibroblasts and keratinocytes to enable successful WIHN⁸⁶.

Plasticity upon wound healing is also observed in other skin lineages. In the dermis, myofibroblasts, located close to de novo formed hair follicles, are converted into adipocytes in large wounds⁸⁷. This cell-fate conversion depends on bone morphogenetic protein signalling originating from newly formed hair follicles that activate the expression of Zfp423, a transcription factor that regulates adipocyte development⁸⁷. Cell plasticity has also been described in other epithelia, such as the mammary gland, lung and intestinal epithelium⁸⁸. It will be important to define whether generic mechanisms conserved across different tissues and species control cellular plasticity after lineage ablation and during tissue repair, inflammation or tumorigenesis.

Crosstalk between stem cells and the niche. During wound repair, fibroblasts are responsible for ECM synthesis in the dermis and for the fibrotic response leading to scar formation⁵. However, during embryonic development and in certain body locations, such as the oral cavity, wounds heal without forming scars⁸⁹. The dermis of the mouse back skin is composed of fibroblasts of different developmental origin^{90,91}. At least two fibroblastic lineages give rise to the upper (papillary fibroblasts, dermal papillae and erector pili muscle) and lower (reticular fibroblasts, preadipocytes and hypodermal adipocytes) dermis, respectively⁹⁰. Upon wounding, fibroblasts of the lower dermis are recruited first, followed by fibroblasts of the upper dermis⁹⁰ (Fig. 1). Reticular fibroblasts secrete the collagens responsible for scar tissue formation and are unable to regenerate the hair follicle after transplantation⁹⁰. This observation may potentially explain why de novo hair follicle formation rarely occurs during wound healing. The upper papillary dermis is the only fibroblast lineage competent to regenerate hair follicles after transplantation, but it is recruited late during repair⁹⁰. Interestingly, activation of Wnt- β -catenin signalling in epidermal cells increases the recruitment of papillary fibroblasts and also the number of hair follicles regenerated in the wound bed⁹⁰. By sharp contrast, inhibition of β -catenin in fibroblasts promotes hair follicle regeneration during wound healing and correlates with a decrease in the number of reticular fibroblasts and an increase in the number of papillary fibroblasts⁹². In addition to different locations, two distinct fibroblastic lineages can also be identified on the basis of the embryonic expression of Engrailed-1 (*En-1*)⁹¹. The En-1-positive lineage of fibroblasts produces ECM composed of collagen type I and type III, becomes more abundant postnatally and is responsible for fibrotic scarring after injury⁹¹. By contrast, the En-1-negative lineage becomes less abundant after birth but has a higher regenerative capacity and their dominance during embryonic development might explain why embryos are able to regenerate scarless skin^{91,93}. Of note, the preferential location of En-1-positive lineage fibroblasts in the reticular dermis after birth suggests that they encompass the reticular populations previously described^{90,91}. Altogether, these data support the importance of surrounding dermal fibroblasts in skin regeneration.

A crosstalk between epidermal and immune cells also plays a major role during wound healing. Interestingly, after epidermal stem cells have been challenged with an inflammatory stimulus, the skin retains a memory of past inflammation and heals faster upon wounding. This memory is associated with chromatin remodelling

that primes stem cells to respond more rapidly upon injury, in particular, by promoting cell migration, which is partly mediated by the AIM2 inflammasome⁴¹. This remarkable observation is reminiscent of an 'alert state', as described in muscle stem cells, in which stem cells contralateral to the injured muscle undergo accelerated cell cycle entry upon tissue damage⁹⁴. By contrast, ageing is associated with a defect in wound healing, linked to a decrease in epidermal migration and impaired signalling between epidermal and dendritic epidermal T cells⁹⁵. Aged epidermal cells demonstrate a defect in signal transducer and activator of transcription 3 (Stat3) activation following wounding, which in turn prevents the activation of *SKINTS* genes that encode immunoglobulins and activate dendritic epidermal T cells in the wound bed⁹⁵.

Upon wounding, skin stem cell populations are also activated by activin A, which is overexpressed in the dermis and the epidermis⁹⁶. Overexpression of activin A enhances wound healing^{96,97}, promotes keratinocyte migration and the formation of granulation tissue by dermal cells^{98,99}. Moreover, activin A recruits Foxp3-expressing regulatory T cells, which further supports accelerated healing⁹⁷. These data illustrate the importance of the crosstalk between immune cells, dermal cells and keratinocytes during wound healing⁹⁷.

Conclusions and outlook

Taken together, this body of work provides previously unappreciated insights into the cellular and molecular mechanisms regulating wound healing and the importance of a crosstalk between different skin cell populations. However, many open questions remain regarding the signals that stimulate stem cells to migrate to the wound centre. We also know little about the control of skin compartmentalization during homeostasis and repair. How rapidly after wounding are these boundaries re-established? Mechanistically, it will be interesting to further elucidate how cells move between and within basal and suprabasal layers and learn more about the role of the leading edge. Factors controlling chromatin remodelling and lineage infidelity in chronic wounds and cancer also remain to be discovered.

Lineage ablation experiments at the leading edge should aid in defining the role and function of involved compartments during wound repair. Moreover, single-cell RNA sequencing and ATAC (assay for transposase accessible chromatin with high-throughput) sequencing of stem cell populations isolated during different regeneration stages should provide insights into the chromatin and transcriptional landscape associated with cellular heterogeneity. These studies might also elucidate further mechanisms that mediate the reprogramming of cells from the hair follicle and infundibulum as they are recruited to the IFE. Finally, understanding the role of the niche in the reprogramming of differentiated cells towards a stem cell fate will be an important challenge for the future.

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Competing interests

The authors declare no competing interests.

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