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Pathways to improving skin regeneration

Christelle Adolphe and Brandon Wainwright

A significant proportion of the human population suffers from some form of skin disorder, whether it be from burn injury or inherited skin anomalies. The ideal treatment for skin disorders would be to regrow skin tissue from stem cells residing in the individual patient's skin. Locating these adult stem cells and elucidating the molecules involved in orchestrating the production of new skin cells are important steps in devising more-efficient methods of skin production and wound healing via the ex vivo expansion of patient keratinocytes in culture. This review focuses on the structure of the skin, the identification of skin stem cells, and the role of Notch, Wnt and Hedgehog signalling cascades in regulating the fate of epidermal stem cells.

The skin is the largest organ in the body and functions as a barrier against infection and dehydration; skin appendages such as hair follicles, sebaceous glands and sweat glands also protect the body against harsh environmental effects. Mammalian skin has the remarkable ability to regenerate itself every two weeks, replacing dead sloughed skin and healing wounds, as a result of the activity of the adult epidermal stem cell population located deep within the skin. Adult stem cell populations remain relatively quiescent in vivo, yet they are capable of giving rise to large amounts of new epidermal tissue via daughter stem cell populations. When daughter stem cells commit to differentiation they enter a transient state of rapid proliferation. Following exhaustion of their limited proliferative potential, epidermal transit amplifying (TA) or committed progenitor cells withdraw from the cell cycle and begin the complex process of terminal differentiation.

Although keratinocytes of the skin can be propagated in vitro, and have been engrafted to replace the damaged epidermis of burn patients, graft skin is usually void of associated structures such as hair follicles and sweat glands, leading to anomalies in epidermal barrier homeostasis. Thus, before scientists can make the most of patient stem

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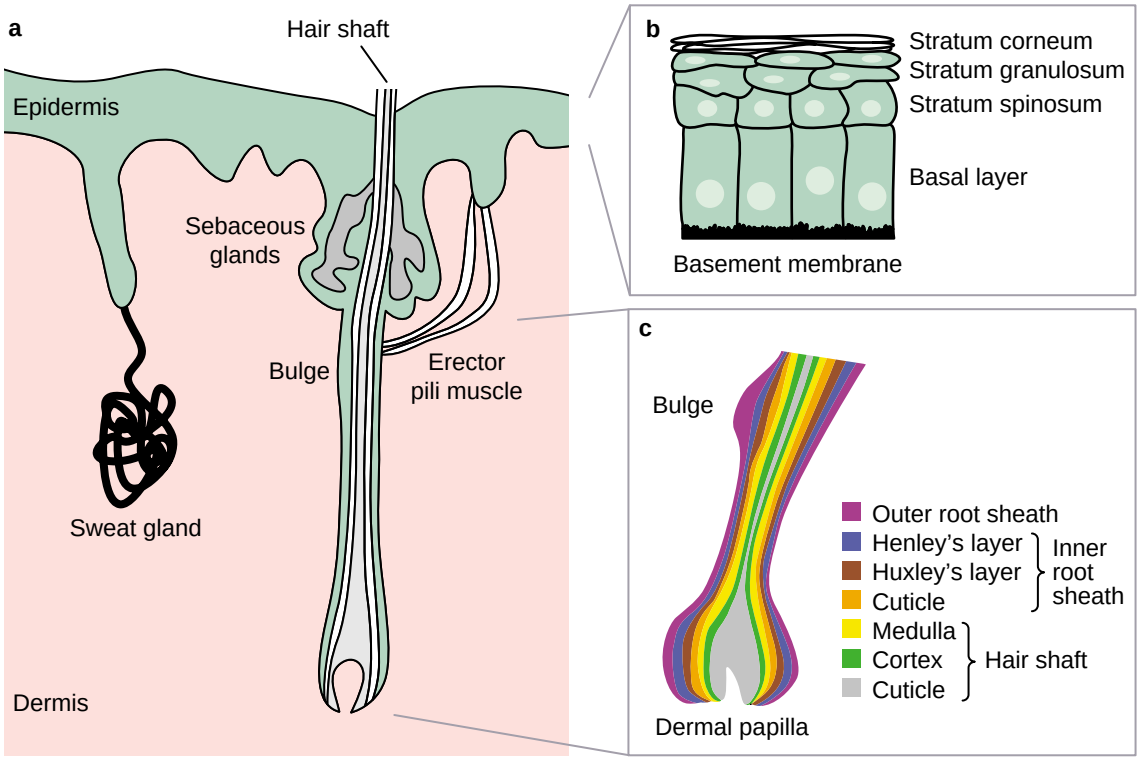
cells to repair lost or injured skin, we need to gain a better understanding of the molecular cues governing epidermal stem cell self-renewal and differentiation and those involved in the formation of hair follicles, sebaceous glands and sweat glands.

Structure of mammalian epidermis

Mammalian skin is composed of two distinct layers – the epidermis and dermis – separated by a basement membrane (Fig. 1a). The nonvascularised epidermis receives nutrients from the underlying dermis via the basement membrane. The epidermis consists of a layer of undifferentiated basal cells that contact the basement membrane, in addition to several layers of stratified epidermal tissue (Fig. 1b). Epidermal stem cell and TA cell populations reside in the basal layer of the epidermis and within a specialised region of the hair follicle known as the bulge (Fig. 1a and c).

The epidermal differentiation program involves changes in cell morphology and gene expression patterns (Fig. 2). Mature epidermal



Structure of the mammalian skin and hair follicle

Figure 1. Structure of the mammalian skin and hair follicle. (a) The skin is a large, complex organ system composed of a thick layer of connective tissue (dermis) and several layers of epidermal cells (epidermis). The basement membrane, which is in direct contact with the proliferating basal cells of the epidermis, separates the epidermis from the dermis. The skin's appendages (sweat glands, sebaceous glands and hair follicles) play an important role in ensuring normal skin function, including barrier protection, thermoregulation and sensory detection. (b) The epidermis consists of a layer of undifferentiated basal cells that contact the basement membrane, as well as layers of stratified epidermal tissue: the squamous cells (stratum spinosum), granular cells (stratum granulosum) and cornified cells (stratum corneum). (c) The hair follicle is made up of concentric sheaths. The outer root sheath is contiguous with the basal layer of the epidermis; the inner root sheath and hair shaft are each made up of three distinct layers. An important structure of the hair follicle is the bulge, which is where the hair folllicle stem cells have been shown to reside.

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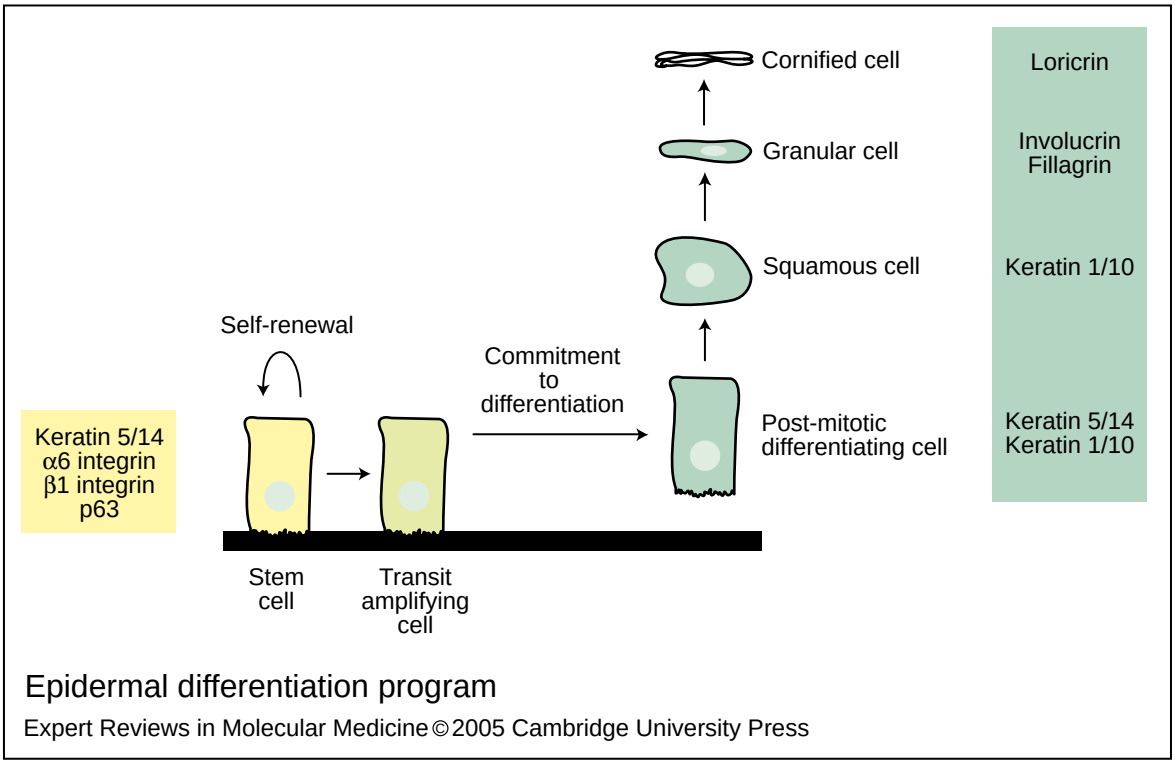

Figure 2. Epidermal differentiation program. Keratinocyte stem cells reside in the basal layer of the epidermis. Stem cells can undergo self-renewal, thereby giving rise to another stem cell, or can divide to give rise to transit amplifying cells, which are rapidly dividing cells that provide the skin with new epidermal basal cells. New epidermal basal cells subsequently detach from the basement membrane and begin the process of terminal differentiation. As cells differentiate, they move up to form the different layers of the epidermis (squamous, granular and cornified layers – see also Fig. 1b). Stem and transit amplifying cells can be distinguished from terminally differentiated cells on the basis of protein expression (yellow box). Each stage of the differentiation program (epidermal layers) can also be distinguished according to the proteins they express (green box).

basal cells express the basal keratins 5 and 14 (Ref. 1). Basal cells also express α3β1, α2β1 and α6β4 integrin adhesion molecules, which function primarily in cell–extracellular-matrix and cell–cell adhesion (Ref. 2). The α6β4 integrin is exclusively expressed in the basal surface of basal cells, in specialised adhesion structures called hemidesmosomes (Ref. 3) that bind to the laminin 5 component of the basement membrane (Ref. 4). As basal cells commit to differentiation, they downregulate their expression of α6β4 integrin (Ref. 5), begin to express keratins 1 and 10 (Refs 1, 6), and detach from the basement membrane to form the stratum spinosum (squamous cells). As squamous cells move towards the surface of the skin they begin to express involucrin and fillagrin (Ref. 7), which provides the stratum granulosum (granular cells) with additional strength. As cells approach the outer surface they produce and secrete lipids important in establishing the waterproof and barrier functions of the stratum corneum (cornified cells). When these cells reach the body surface of the skin, they are dead, flat, enucleated cells that are sloughed off, continually being replaced by new inner tissue produced from stem cells.

Structure of hair follicles

An important appendage of mammalian skin is the hair follicle. The mature hair follicle is a complex structure consisting of several layers of concentric epidermal cells, known as root sheaths (Fig. 1c) (Ref. 8). The outermost layer, known as the outer root sheath, is contiguous with the basal layer of the interfollicular epidermis. The matrix, located at the bottom of the hair follicle in the region of the hair bulb, is composed of proliferating cells that terminally differentiate to generate the inner root sheath (comprising three layers: the cuticle, Huxley's layer and

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Henley's layer) and hair shaft (also comprising three layers: the medulla, cortex and cuticle) (Ref. 8).

In mammals, hairs do not persist throughout life, but are periodically shed and regrown in a 'hair cycle' (Ref. 8). The lower portion (two-thirds) of the mature adult hair follicle undergoes cycles of complete destruction (catagen), quiescence (telogen) and restoration (anagen). The upper 'permanent' portion of the hair follicle consists of the sebaceous gland and arrector pili muscle (Fig. 1a). The hair follicle bulge marks the lowest point of the 'permanent' region of the hair follicle, and is where the multipotent stem cells of the skin reside (see below) (Ref. 9). During anagen, stem cells exit the bulge and migrate down to regenerate the 'cycling' portion of the hair follicle (Refs 9, 10), while matrix cells proliferate and differentiate to generate the inner root sheath and hair shaft. During catagen, the lower portion of the hair follicle undergoes apoptotic cell death, and the dermal papilla, a ball of specialised dermal cells that are essential for follicle formation (Ref. 11), is drawn up towards the bulge where it remains during telogen. Activation of a new anagen cycle occurs when the dermal papilla recruits stem cells of the bulge to commence a new process of proliferation and differentiation, thereby regenerating a new hair follicle (known as the bulge-activation hypothesis) (Ref. 12).

Stem cells of the epidermis

Epidermal stem cell populations of mammalian skin reside in at least two locations: a specialised region of the hair follicle known as the bulge, as mentioned above; and in a nonrandom distribution within the basal cells of the interfollicular epithelium (the keratinocyte stem cell population). The basal layer of mammalian skin is a heterogeneous population of proliferating and differentiating cells. Keratinocyte cultures derived from the interfollicular epithelium of human epidermis have different capacities for sustained growth, as determined by the types of clones derived from plating single cells (Ref. 13). Holoclones give rise to large colonies where fewer than 5% of colonies abort and terminally differentiate; paraclones consist of cells with a short replicative lifespan, after which they uniformly abort and terminally differentiate; and meroclones contain a mixture of cells with different growth potential, marking a transitional stage between holoclones and paraclones. Hence, holoclones are presumed to be derived from putative stem cells, meroclones from the TA cell population, and paraclones from terminally differentiated cells (Ref. 13). In support of these observations of colony formation, stem cells located in the bulge of the hair follicle also give rise to the largest colonies (holoclones) (Ref. 14).

Multipotent stem cells of the hair follicle

The 'slow-cycling' nature of stem cells allows them to retain label or dyes over extended periods of time. Cotsarelis and co-workers (Ref. 15) were the first to show that a population of label-retaining cells that were undifferentiated and slow-cycling existed exclusively in the bulge region of the hair follicle. Retention of labels such as tritiated thymidine ([^{3}H]thymidine) or bromodeoxyuridine (BrdU) for 8 weeks or more has since become a well-accepted definition of quiescent stem cells in vivo (Refs 16, 17, 18). The ability of stem cells to retain dye or labels has proved a useful tool for analysing the epidermal stem cell compartment. Taylor and colleagues (Ref. 9) devised a double-label technique – first labelling epidermal cells with BrdU and, when only the bulge cells retained this label, re-labelling these cells with [^{3}H]thymidine – thus allowing them to trace the movements of double-labelled stem cells. Oshima and colleagues (Ref. 10) performed similar stem cell analyses by using transgenic mice that expressed the β-galactosidase gene, and transplanting follicle segments into the bulge region of wild-type vibrissae follicles. Together, these studies demonstrated the ability of stem cells from the bulge to migrate upwards to re-populate the epidermis and generate sebaceous glands, and downwards, to populate the cells of the growing hair follicle (Refs 9, 10). Thus, bulge stem cells are multipotent and can give rise to epidermis, hair follicle and sebaceous gland lineages. Recent work from Fuchs and colleagues (Ref. 19) has uncovered an asymmetry of bulge stem cells, whereby some remain attached to the basal lamina of the hair follicle, while others appear suprabasal. Both populations of bulge stem cells expressed keratin 14 and CD34, can be stimulated to self-renew and terminally differentiate in vitro, and are multipotent (able to give rise to epidermis and hair follicles upon grafting) (Ref. 19). These data clearly demonstrate that cells within the hair follicle bulge possess the classical defining features of bona fide stem cells.

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Keratinocyte stem cells

The interfollicular epidermis is also thought to contain its own population of stem cells, given the ability of human interfollicular keratinocytes to generate normal epidermis when grafted in vivo. Cell kinetic studies of murine epidermis estimate that the keratinocyte stem cell compartment comprises around 1–10% of epidermal basal cells (Refs 20, 21). Together the keratinocyte stem cell and TA cell populations comprise 70–80% of epidermal basal cells, express high levels of keratins 5 and 14, and β1 and α6β4 integrins (Refs 22, 23), and represent the interfollicular epidermal proliferative unit. The remaining 20–30% of basal cells are postmitotic differentiating cells, which have ceased cell cycle activity and begun the process of terminal differentiation, and nonepithelial cells such as leukocytes and melanocytes.

Markers of the epidermal stem cell population

Although stem cells are likely to exist in keratinocyte primary cell cultures, their isolation and characterisation remains a controversial issue. A true stem cell marker should be able to distinguish between stem cells and TA cells, with expression restricted to the stem cell population. As mentioned above, epidermal stem cells and their TA cell progeny express high levels of the basal keratins 5 and 14. Fractionation of cultured keratinocytes via fluorescent-activated cell sorting has shown that cells expressing high β1 integrin (Ref. 22) and α6 integrin (Refs 23, 24) have the greatest proliferative capacity in vitro. However, although epidermal stem cells express two-to threefold higher surface levels of β1 integrin than TA cells (Ref. 22), β1 integrin expression is not restricted to a stem cell phenotype. β1 integrin thus remains a marker of epidermal progenitor (stem and TA) cell populations. Keratinocytes placed in suspension initiate terminal differentiation but this process can be inhibited by ligating β1 integrin, suggesting that β1 integrin acts to maintain keratinocytes in a stem or TA (undifferentiated) state (Ref. 25).

Melanoma chondroitin sulphate proteoglycan (MCSP) has also been shown to be expressed in the human hair follicle stem cell compartment of the bulge; however, it does not enrich keratinocytes expressing high levels of β1 integrin for stem cells (Ref. 26). Even though label-retaining cells (previously mentioned as a well-accepted definition of quiescent stem cells in vivo) can be distinguished from β1 integrin^{bright} TA cells by their characteristic low levels of CD71 transferrin receptor expression in adult skin (Refs 18, 23), this is not the case during embryonic development.

Perhaps the most controversial stem cell marker is the p53 homologue p63. The ΔN-p63 isoform of p63 has been shown to be highly expressed in cultures generated from holoclones, and expression is barely detectable in cultures generated from meroclones and paraclones (Ref. 27). In support of p63 regulating the epidermal stem cell compartment, mice lacking p63 activity presented with skin resembling primitive ectoderm and lacking keratin 5 expression, representative of a defect in epidermal stem cells (Ref. 28). However, as with many proposed stem cell markers, the widespread expression of p63 in up to 70% of basal cells is not consistent with the low incidence of label-retaining cells. Recent evidence suggests that p63 plays an important role in maintaining the proliferative potential of epidermal basal cells and in regulating the onset of terminal differentiation, in response to the balance in expression between two p63 isoforms: ΔN-p63 and TA-p63 (Ref. 29). Hence p63 expression acts as a marker of the epidermal proliferative unit, which consists of proliferative stem and TA cells.

Regulation of epidermal stem cell fate and lineage specification

Stem cells and their daughter cells undergo constant cell-fate decisions. Theoretically, stem cell numbers could be kept constant by ensuring that every stem cell division is asymmetric, giving rise to one daughter stem cell and one TA cell. However, this mechanism of stem cell division does not account for the expansion of stem cells following tissue damage. Stem cells therefore have two recognised fates: either to remain quiescent or to proliferate. Daughter stem cells can also remain as stem cells or differentiate into TA cells. The interactions of a stem cell with its microenvironment (or 'stem cell niche'), comprising neighbouring cells and the surrounding growth factors and ligands, are responsible for regulating the cell fate of stem cells and their daughter cells. Although many different signalling pathways have been implicated in stem cell renewal and differentiation throughout a variety of organ systems, three signalling

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pathways appear to play a crucial role in regulating the fate of epidermal stem cells: Notch, Wnt and Hedgehog signalling activity (Fig. 3). The possible roles of molecular signals in the regulation of cell fate lineages during differentiation of epidermal stem cells are summarised in Figure 4.

Notch signalling in the skin

Cell–cell interactions mediated by the Notch pathway play an important role in cell fate decisions during embryonic development and adult tissue homeostasis. Notch signalling is achieved by interaction between a Notch cell-surface receptor and a ligand expressed on the surface of an adjacent cell. This interaction triggers a series of proteolytic processing events and results in the release of the Notch intracellular domain (Ref. 30). The activated form of Notch translocates into the nucleus and triggers a cascade of downstream signalling events by interacting with the DNA-binding protein RBP-Jk, which is subsequently converted from a repressor to an activator of gene transcription (Ref. 31) (Fig. 3a).

In mammalian skin, Notch cell-surface receptors (Notch1, Notch2, Notch 3 and Notch4) are expressed in suprabasal differentiated keratinocytes. In human epidermis, the Notch ligand Delta1 localises to the basal layer, with highest expression in putative 'stem cell'

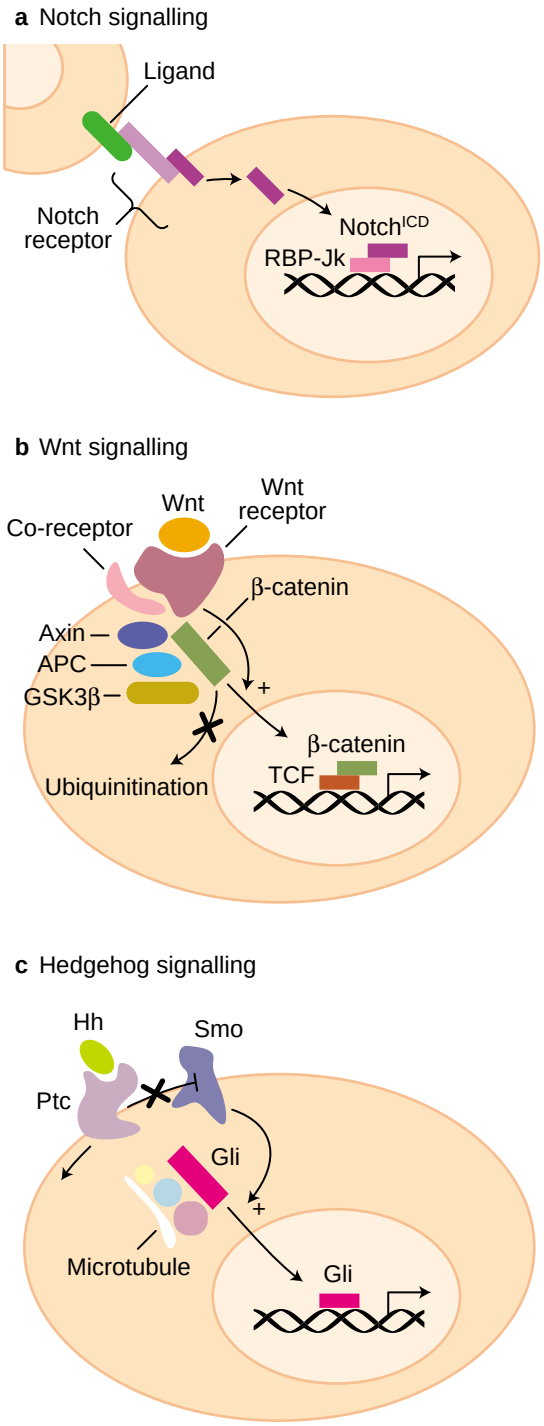

Figure 3. Pathways involved in regulating the epidermal stem cell compartment.

Figure 3. Pathways involved in regulating the epidermal stem cell compartment. (a) Notch signalling. Interaction of the Notch receptor with ligand results in Notch cleavage and translocation of the intracellular domain (Notch^{ICD}) into the nucleus, which interacts with the DNA-binding protein RBP-Jk to alter gene expression. (b) Wnt signalling. In the absence of a Wnt signal, β-catenin is targeted for degradation via the ubiquitination pathway, via the action of a multiprotein destruction complex, including axin, the adenomatous polyposis coli (APC) protein, and glycogen synthase kinase 3β (GSK3β). In the presence of a Wnt signal, the degradation machinery is inhibited and β-catenin translocates into the nucleus where it forms a transcriptional complex with TCF (T-cell factor) and activates target gene expression. (c) Hedgehog signalling. In the presence of Hedgehog (Hh), Patched (Ptc) inhibition of Smoothened (Smo) activity is released. Smoothened is therefore free to induce the dissociation of Gli transcription factors from a microtubule-associated multiprotein complex, and Gli enters the nucleus to induce transcriptional activation of target genes.

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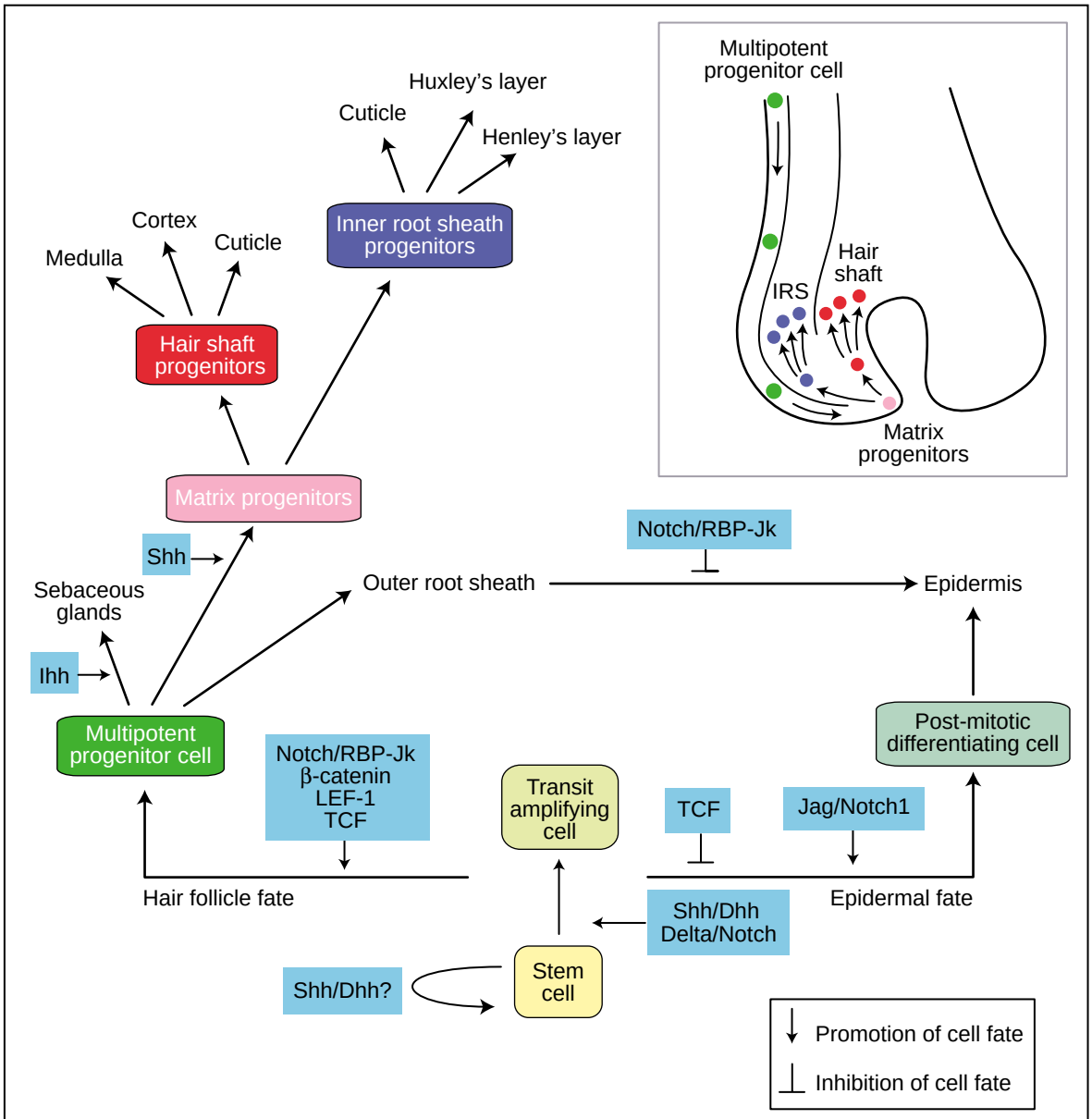

Molecules involved in the differentiation of an epidermal stem cell along the different cell fate lineages

Figure 4. Molecules involved in the differentiation of an epidermal stem cell along the different cell fate lineages. There are a host of molecules thought to play an important role in determining the fate of epidermal stem cells. Hedgehog family members are implicated in stem cell self-renewal and, together with Delta–Notch, a transit amplifying cell fate. Once transit amplifying cells have ceased cell cycle activity they are instructed by a variety of molecules (including Notch/RBP-Jk, β-catenin, LEF-1 and TCF) to differentiate along the hair follicle lineage (left-hand side), giving rise to sebaceous glands and all the different layers of the hair follicle. Alternatively, activation of Jag/Notch signalling instructs transit amplifying cells to differentiate along the epidermal lineage (right-hand side), giving rise to the stratified layers of the epidermis. The inset diagram shows the migration of a multipotent progenitor cell along the hair follicle fate lineage. Abbreviations: Dhh, Desert Hedgehog; Ihh, Indian Hedgehog; Jag; Jagged; LEF, lymphoid enhancer binding factor; Shh, Sonic Hedgehog; TCF, T-cell factor.

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populations (Ref. 32). By contrast, the Notch ligands Jagged1 and Jagged2 are co-expressed with the Notch receptors in the differentiating keratinocytes of the suprabasal layers (Refs 33, 34). Activation of Notch signalling in keratinocytes is sufficient to cause cell cycle arrest by inducing the expression of the cyclin-dependent kinase $p21^{WAF1}$, and promotes keratinocyte terminal differentiation, as shown by the expression of keratin 1, keratin 10 and involucrin (Ref. 33). An earlier role for Notch signalling in epidermal stem cell regulation has been indicated by analysis of Delta1–Notch signalling interactions. Delta1–Notch signalling appears to promote exit from the stem cell compartment towards a TA cell fate, and subsequent terminal differentiation after a limited number of cell divisions (Ref. 32). Delta1 localises to putative stem cell patches, and those cells surrounding the epidermal stem cells (committed to differentiation) are Delta1-negative yet express the Notch1 receptor (Ref. 32). Keratinocytes expressing high levels of Delta1 suppress the growth potential of neighbouring Notch1-expressing cells (Ref. 32), thus acting to promote exit from the cell cycle and commitment to terminal differentiation. Given Jagged1 and 2 and Notch1 and 2 are co-expressed in differentiating keratinocytes of the suprabasal layers (Ref. 33), it has been suggested that Jagged–Notch signalling promotes terminal differentiation.

Downregulation of Notch signalling molecules is evident in keratinocytes undergoing rapid epidermal proliferation, as observed in pathological conditions such as basal cell carcinoma (BCC) (Ref. 35) and psoriasis, alongside the initial re-epithelialisation process associated with wound healing (Ref. 36). However, the transcription of Notch ligands and receptors is progressively reactivated once wounded skin begins to stratify or when skin pathologies undergo treatment (Ref. 36), indicating that activation of Notch signalling promotes the differentiation of receiving cells.

Mice with a mosaic disruption in the transcription factor RBP-Jk, resulting in loss of Notch signalling activity, exhibit epidermal hyperkeratinisation, hair loss and epidermal cysts (Ref. 37). These results suggest that Notch–RBP-Jk signalling acts to negatively regulate hair follicle stem cells in the bulge towards an epidermal cell fate, thereby promoting hair follicle formation.

Although $p21^{WAF1}$ was originally identified as a mediator of p53-induced cell cycle arrest, p21-null mouse keratinocyte cultures contain a higher fraction of keratinocytes with clonogenic properties than their wild-type counterpart (Ref. 38). p21 expression has since been shown to play an important role in determining the balance between mouse keratinocyte stem cells, TA cells and irreversibly differentiated cells (Ref. 39).

In conclusion, Notch signalling appears to act as a positive regulator of keratinocyte differentiation, and down modulation results in uncontrolled proliferation.

Wnt signalling in the skin

Wnt proteins play an important role in embryonic patterning by controlling cell proliferation and regulating stem cell fate. In mature tissues, Wnt signalling has been implicated in the self-renewal of adult intestinal and haematopoietic stem cell populations (Refs 40, 41). In the absence of a Wnt signal, cytoplasmic β-catenin is phosphorylated and targeted for degradation by a complex of proteins including axin, adenomatous polyposis coli tumour suppressor protein (APC) and glycogen synthase kinase 3 β (GSK3β). In the presence of a Wnt signal, the degradation machinery is inhibited, and β-catenin accumulates in the cytoplasm and translocates to the nucleus. There it forms a transcriptional complex with the LEF/TCF (lymphoid enhancer binding factor and T-cell factor) family of DNA-binding proteins, and regulates transcription of target genes such as the cellular oncogenes c-Myc and cyclin D1 (Ref. 42) (Fig. 3b).

Tcf-3, a member of the TCF family of DNA-binding proteins, is expressed in the bulge (stem cell compartment) of the hair follicle. Tcf-3 appears to act as a repressor protein, perhaps to inhibit c-Myc or cyclin D1. When cells migrate out of the bulge and towards cells of the sebaceous gland or epidermis, they lose Tcf-3 expression (Ref. 43). If Tcf-3 is overexpressed in basal cells (using a keratin 14 promoter), epidermal differentiation is repressed and cells adopt a hair follicle or stem cell phenotype (Ref. 43). These data suggest that Tcf-3 acts as a repressor of epidermal differentiation, and repression of its activity is required for epidermal fate. If β-catenin is ablated in mice (Ref. 44), or a mutant Lef-1 that lacks the β-catenin-binding site is expressed in mice (Ref. 45), hair follicle differentiation is impaired, and cells differentiate into epidermal or sebaceous cell lineages. These results suggest that β-catenin and Lef-1 signalling promote

Accession information: DOI: 10.1017/S1462399405009890; Vol. 7; Issue 20; 23 September 2005

hair follicle morphogenesis, and repression of signalling is required for epidermal development. Transgenic mice overexpressing the c-*myc* target gene in the basal layer of the skin also exhibit epidermal hyperproliferation alongside impaired wound healing and hair loss, possibly reflecting stem cell depletion (Refs 46, 47).

Thus, the Wnt signalling pathway plays a role in maintaining the phenotype of hair follicle bulge stem cells and in the commitment of stem cells to a hair follicle lineage.

Hedgehog signalling in the skin

The current model for Hedgehog–Patched signalling is that in the absence of Hedgehog ligand, the Patched receptor acts to repress the signalling activity of the pathway by interacting with the Smoothened protein (Ref. 48). Binding of Hedgehog to the Patched receptor is thought to induce a conformational change in Patched, causing it to dissociate from the Patched–Smoothened complex, and Smoothened is then free to interact with associated proteins and induce transcription of downstream target genes, via a complex interaction of Gli transcription factors and associated molecules (Fig. 3c).

The mammalian Sonic Hedgehog (Shh) ligand is required to induce the proliferation and down-growth of hair follicles into the dermis (Refs 49, 50, 51). Mice lacking Shh develop hair placodes and dermal condensates, but hair follicle formation is arrested at the hair bud stage. The abnormalities in development were not only restricted to the hair follicles of these mice. Hyperplasia and abnormal keratin expression was also seen in the interfollicular epidermis. Proliferation was localised to basal cells of the epidermis, but keratin 14, normally a marker of basal cells, was observed in suprabasal layers of the thickened epidermis (Ref. 50). Overexpression of Shh in the basal cells of the mouse epidermis (using the keratin 14 promoter) results in extensive epidermal proliferation of the skin, and lesions reminiscent of BCCs (Refs 52, 53). Mice that were severely affected exhibited epidermal proliferations associated with invaginating hair follicles, whereas mice that were only mildly affected displayed one or two epidermal growths interspersed with six to eight normal hair follicles (Ref. 53). Interfollicular epidermis appeared to differentiate normally, suggesting increased Shh activity does not affect epithelial stratification but induces growths of the invaginating hair follicle (Ref. 53). Recent evidence also suggests that Indian hedgehog (Ihh), another mammalian Hedgehog homologue, has epidermal tumourigenic potential. Mice overexpressing ΔN-Lef-1 in the basal skin cells, which blocks β-catenin signalling, develop sebaceous tumours that express Ihh (Ref. 54), leading to the hypothesis that Ihh might play a role in stimulating the proliferation of undifferentiated sebocytes. Thus, it appears that both Shh and Ihh signalling pathways might play a role in the proliferation of different epidermal stem cell fate lineages. Recent data have also shown that high levels of ectopic Shh expression in the basal cells of mouse skin directly increased the number of epidermal progenitor cells and elevated their proliferation in culture (Ref. 52). Regions of transgenic epidermis expressing the highest levels of Shh were completely devoid of regenerative and proliferative potential and consisted of solely differentiated epidermal cells, indicating a loss of the epidermal stem cell compartment (Ref. 52).

These results show that Hedgehog is a key factor whose expression must be temporally regulated to ensure epidermal stem cell homeostasis.

Stem cells and skin cancer

Stem cells have been postulated to be the source of tumour formation, largely because both tumours and stem cells are capable of self-renewal and have the capacity for unlimited replication. Similarly, the increased incidence of cancer with age could be explained by hypothesising an accumulation of mutations in skin cells that persist throughout life. In addition, many tumours seem to present with cell types that are clonally related, such as hair follicle and sebaceous structures often observed in epithelial tumours of the skin (Ref. 55). Although little is known about the specific targets of cancer-inducing genes, current theories suggest that they exert their proliferative effect by disrupting the balance between growth and differentiation pathways. For example, the Shh signalling pathway is involved in regulating epidermal proliferation and when pathway members are mutated, resulting in deregulated Shh activity, the common skin tumour BCC develops. Forced expression of Shh in human keratinocytes also results in BCC-like structures when skin is reconstituted from these cells and grafted onto nude mice (Ref. 56). BCCs have several characteristics in common with

Accession information: DOI: 10.1017/S1462399405009890; Vol. 7; Issue 20; 23 September 2005

immature hair follicles, including histology, ultrastructure and pattern of keratin expression, thus suggesting they result from a deregulation of normal Shh activity on multipotent epidermal cells. Disruption to Wnt signalling, such as overexpression of stable β-catenin in the basal cells of the epidermis, also gives rise to a common skin tumour, pilomatricoma, which consists of pure matrix and hair cells (Ref. 57). In addition, suppression of β-catenin signalling, by expressing ΔN-Lef-1, results in sebaceous and squamous tumours (Ref. 45). These results suggest that tumours also derive from disruption to normal β-catenin signalling in epidermal cells. Thus, perturbation of the signalling pathways involved in regulating the balance between stem cell proliferation and differentiation appear to play a significant role in the onset of tumour formation.

Clinical implications and applications

The biology of epidermal stem cells and their role in epidermal regeneration is the subject of extensive research with the aim of improving current skin transplantation procedures to restore normal skin function. In vitro skin cultivation was first reported by Rheinwald and Green in 1975; they described the successful expansion of skin epithelium from single-cell suspensions of epidermal cells using a mesenchymal feeder cell layer of terminally irradiated mouse 3T3 cells (Ref. 58). This significant finding has allowed scientists to increase the amount of skin tissue obtained from a patient biopsy in three to four weeks and has formed the basis of cultured skin autografts used in the treatment of burn injuries for almost 20 years.

Significant progress has already been made in the genetic modification of epidermal stem cells to treat hereditary skin diseases. Different strategies have been developed to deliver a gene of interest to epidermal stem cells, including naked DNA, adenovirus and lentivirus (Refs 59, 60). For example, dystrophic epidermolysis bullosa results from a deficiency in anchoring fibres and causes chronic skin wounds and skin cancer. Epidermal stem cells obtained from patients with this disease have been successfully transduced with a lentivirus vector containing the *COL7A1* gene, which results in the correction of anchoring fibres following skin transplantation (Ref. 61). Similarly, epidermal stem cells from patients suffering from junctional epidermolysis have been corrected with a laminin 5 gene using a nonviral approach (Ref. 62). However, it is important to note that not all epidermal skin regeneration techniques are aimed at genetically modifying a patient's own stem cells. This may be necessary in the case of an underlying genetic defect such as the skin diseases mentioned above, but for the treatment of burn or wound victims, genetic manipulation may be unnecessary.

A recent and significant advance in epidermal biology comes from recent work by Fuchs and colleagues (Ref. 19) showing that a single skin stem cell isolated from mouse hair follicles can regenerate epidermis, hair follicles and sebaceous glands, following in vitro cultivation. These results clearly show for the first time the multipotent nature of skin stem cells and support the use of adult stem cells for skin augmentation. Although the idea of identifying the stem cell population to further in vitro epidermal culture is appealing, it has been shown that stem cells are not required to expand the epidermis in culture. Kaur and colleagues (Ref. 63) have demonstrated that all classes of keratinocyte progenitors (stem cells, TA cells and early differentiated cells) can regenerate epidermal tissue in an in vivo setting. These results clearly show that extensive proliferation and tissue-regenerative capacity is not restricted to epidermal stem cells. However, the isolation of epidermal stem cells to treat skin injury remains a firm favourite, given stem cells have the intrinsic ability to respond to appropriate environmental cues and that they have the ability to differentiate along all epidermal lineages.

Recent evidence has also shown that the neural stem cell marker nestin is expressed in the bulge region of the hair follicle, and that these bulge-region stem cells can differentiate into neurons, glial cells, keratinocytes, smooth muscle cells and melanocytes following manipulation in vitro. These results clearly identify the multipotent nature of skin stem cells and suggest that skin stem cells are an accessible autologous source of undifferentiated multipotent stem cells for therapeutic application (Ref. 64).

Current methods of skin regeneration are costly and time-consuming, making their use inefficient for the treatment of acute wounds and skin disorders. However, recent evidence has shown that overexpression of the Shh ligand can significantly increase the number and proliferative potential of epidermal progenitor cells (Ref. 52). This finding might lead to a method for increasing the rate of production of skin

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tissue from in vitro epidermal cultures, thereby favouring the use of skin substitutes for a variety of applications.

Research in progress and outstanding research questions

There is a fundamental requirement to further our understanding of epidermal stem cell biology – in particular stem cell self-renewal and stem cell proliferation. A better understanding of these processes will provide the opportunities to improve current skin explant systems, especially from a cell- and gene-therapy perspective. As previously discussed, transplantation of cultured epidermal stem cells can permanently reconstitute an epidermal barrier and has saved the lives of many burn patients worldwide. However, current epidermal transplants fail to generate hair follicles and sweat glands, which is not surprising given the complexity of these epidermal structures. Freshly isolated keratinocytes have been shown to produce hair follicles when combined with isolated dermal papilla cells and implanted into the skin of host animals (Ref. 65); however, it remains to be determined whether long-term keratinocyte cultures maintain the ability to differentiate along the hair follicle lineage. The hair follicle, sweat glands and sebaceous glands play an important role in maintaining correct integument function by offering additional external protection from abrasion and ultraviolet light, and playing a crucial role in temperature regulation, sweat evaporation and sensory detection; thus, the development of new therapeutic techniques that promote the production of fully functional skin is important. The ultimate aim of skin regeneration is to isolate patient stem cells from an initial biopsy and manipulate keratinocyte growth in culture so that correct skin function is restored, thereby providing transplanted skin grafts with the aesthetics of normal skin. A major challenge over the next decade will be to identify the molecular signals governing stem cell self-renewal, proliferation and differentiation and apply this knowledge to current ex vivo epidermal cultures.

Although significant advances are occurring in identifying markers of the adult epidermal stem cell population, much remains to be elucidated about early (embryonic) epidermal stem cell morphogenesis and self-renewing activity. The analysis of early epidermal stem cell activity will allow us to understand the mechanisms involved in programming a functional epidermis.

Much work is required before it is possible to reprogram adult stem cells at will. Issues include the isolation and identification of signalling molecules, the identification of target (responsive) cells and the detailed analyses of spatial and temporal gene expression patterns. The overall objective is to be able to manipulate the dose of gene expression in order to promote rapid epidermal growth without deleterious effects. Another important factor is to correctly identify the timing of when certain genes must be turned on and off, thereby allowing for the correct developmental cues to occur in each cell, thus ensuring correct differentiation into epidermal, hair follicle and sebaceous gland development. Gaining knowledge about the mechanisms that control stem cell regulation is the first step in improving current skin regeneration methods. Perhaps the biggest challenge in epidermal stem cell therapy is to decrease public preoccupation with genetically modified cells and raise the awareness of their use in clinical applications.

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Further reading, resources and contacts

Links to worldwide stem cell information, addressing both general and technical stem cell issues:

http://www.nscc.edu.au/stem_what.html

General information about adult stem cells versus embryonic stem cells, and discussion of obstacles to be overcome before the potential of stem cell technologies can be realised:

http://stemcells.nih.gov/info/basics

Features associated with this article

Figures

Figure 1. Structure of the mammalian skin and hair follicle.
Figure 2. Epidermal differentiation program.
Figure 3. Pathways involved in regulating the epidermal stem cell compartment.
Figure 4. Molecules involved in the differentiation of an epidermal stem cell along the different cell fate lineages.

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