



Published in final edited form as:

Biol Cell. 2005 March ; 97(3): 173–183.

Epidermal stem cells: the cradle of epidermal determination, differentiation and wound healing

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Abstract

The field of epidermal stem cells has dramatically advanced in the last decade, leading to a better understanding of the molecular factors, signalling pathways and cellular events that identify and characterize stem cells, thus revealing their immense potential for therapeutic use. Furthermore, multipotent epidermal stem cells present the major advantage of easy accessibility with the discovery of their specific location within the bulge of the hair follicle. This review focuses on the most recent findings on epidermal stem cells, and their potential role in initial epidermal commitment, differentiation and wound healing processes in the skin.

Keywords

differentiation; epidermis; hair follicle; stem cell; wound healing

Abbreviations used

EDC, epidermal differentiation complex; EGF, epidermal growth factor; GFP, green fluorescent protein; HSE, human skin equivalent; IFE, interfollicular epidermis; IFN γ , interferon γ ; IL-1 (etc.); interleukin-1 (etc.); LRC, label-retaining cell; SC, stem cell; TA, transit amplifying (cell); TNF α , tumour necrosis factor α ; TGF β , transforming growth factor β

Introduction

Skin is composed of an underlying dermis, of mesodermal embryonic origin, separated by a basement membrane from the multilayered overlying epidermis, of ectodermal origin. Epidermis is a stratified squamous epithelium primarily composed of keratinocytes: cells that express the characteristic intermediate filament known as keratin. Stratification of the epidermis commences during embryonic development and is a process that continues to occur throughout the life of the organism. The process entails the outward movement of the proliferative basal cells that are adjacent to the basement membrane towards the surface of the skin. The stratification is concurrent with a Ca²⁺-dependent differentiation process and the layer-specific expression of structural and enzymatic markers, with the basal cell

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EF-hand domain: A helix-loop-helix (HLH) structural motif in the calcium-binding site of calcium-binding proteins.

S100 family: EF-hand-containing proteins of 10–14 kDa, in which calcium-binding motifs function to transmit calcium-dependent regulatory signals.

differentiating first to a spinous cell, then to a granular cell, to ultimately terminally differentiate as a cornified, anucleated cell (Figure 1).

Studies over the last two decades have demonstrated that epidermal-specific genes expressed in keratinocytes are regulated in a temporal and spatial manner during differentiation (reviewed in Presland and Dale, 2000). In humans, many of these epidermal genes, which encode putative cornified envelope precursors, are closely linked in the epidermal differentiation complex (EDC), a 2.5 Mbp region located on chromosome 1q21 (Volz et al., 1993); in mice a syntenic region is on chromosome 3. Three types of proteins are clustered in the EDC: (1) proteins characterized by relatively small sizes with short tandem peptide repeats in the central region, (2) proteins termed fused-type which have Ca^{2+} -binding EF-hand domains at the N-terminal region followed by multiple tandem repeats, and (3) members of the S100 family, characterized by presenting only Ca^{2+} -binding EF-hand domains (Kalinin et al., 2002; Eckert et al., 2004). Recently, a second cluster of epidermal-specific genes has been described on the human chromosome 19q13.12 and in the syntenic region of chromosome 7 in the mouse (Park et al., 2002; Michibata et al., 2004; Moffat et al., 2004).

The outermost layer of the epidermis is the product of covalent cross-links of envelope precursor proteins by Ca^{2+} -dependent transglutaminases and attachment of lipid molecules (Madison, 2003). This cornified layer provides the protective and water barrier functions between the body and the environment (Kalinin et al., 2002) (Figure 1). The differentiation of keratinocytes and subsequent generation of a protective cornified layer are processes that must be accomplished continuously, thus requiring the contribution of the primary stem cells (SCs) for the renewing epidermis. Additionally, in mammals, the epidermis gives rise to a specialized, derived structure, the hair follicle.

During embryonic development and epidermis formation, a series of signals between the surface epithelial cells and the underlying dermal cells generate hair placodes in the epidermis (reviewed by Hardy, 1992; Paus and Cotsarelis, 1999; Millar, 2002; Rogers, 2004). These placodes subsequently give rise to the lineages for all the hair follicle layers that ultimately differentiate into the hair shaft and inner root sheath (Hardy, 1992; Figure 1). The hair follicles vary in size and shape depending on their location. The cycling and regeneration of each hair follicle depend on specialized mesenchymal dermal papilla cells and proliferating matrix cells located at the base of the follicle. During normal development, each hair follicle goes through three stages: growth (anagen), involution (catagen) and rest (telogen) (Paus and Cotsarelis, 1999; Figure 1). After the resting telogen period, signals from the dermal papilla cells initiate the regeneration of a new hair.

SCs in the epidermis

An important question that has been the focus of great interest is where do the multipotent SCs reside within the epidermis and what molecular markers can be used for their detection? Reports have shown there are a number of SC repositories within the undamaged adult epidermis: cells from the bulge region of the hair follicle, keratinocytes of the interfollicular epidermis (IFE) and sebaceous gland (Ghazizadeh and Taichman, 2001).

Several lines of evidence indicate that the primary source of multipotent SCs in the epidermis that ultimately replenishes the hair, IFE and sebaceous lineages rests within the bulge region of the hair follicle (Taylor et al., 2000; Oshima et al., 2001). Several reports have demonstrated the enrichment of a population with label-retaining cells (LRCs) or SC-like cells based on their slow cycling, hence their ability to retain bromodeoxyuridine or [^3H]thymidine labelling (Cotsarelis et al., 1990; Morris and Potten, 1999). The identification and characterization of multipotent murine hair follicle SCs, as well as what regulates their commitment and maintenance, was shown utilizing transgenic methodologies that allow for labelling, tracking

and isolation of the slow-cycling subpopulation of cells from the bulge region. Tumber et al. (2004) accomplished this by generating transgenic lines: a line expressing histone H2B–green fluorescent protein (GFP) controlled by tetracycline-responsive element to be crossed with a second line expressing a tetracycline repressor–V16 under the control of a keratinocyte-specific keratin 5 (K5) promoter. Morris et al. (2004), used the well-characterized keratin 15 (K15) promoter (Liu et al., 2003) to express the Cre recombinase or the GFP marker in the bulge cells to allow for lineage analysis and isolation. *In vitro* studies, performed with the isolated putative SCs, demonstrated their ability to reconstitute all epithelial cell types within the skin (Morris et al., 2004). Clonal analysis of individual bulge cells will be necessary to determine if there is a mix of multiple types of unipotent SCs or if all the SCs that localize to the bulge are multipotent.

The secondary SCs dispersed throughout the basal layer in the IFE are somewhat less effective and multi-potential than the bulge SCs (Costarelis et al., 1999; Watt, 2001; Fuchs and Raghavan, 2002). Within the basal proliferative layer of the IFE there are subpopulation cell types that differ in their capacity for self-renewal and ability to undergo terminal differentiation (Barrandon and Green, 1987; Jones and Watt, 1993). The slow-cycling IFE SCs that have high self-renewal capacity give rise to non-SCs. These non-SCs are known as transit amplifying cells (TAs, Figure 1), and are characterized by their limited proliferative capacity, dividing only a few times before undergoing terminal differentiation.

Epidermal SC markers

Although several characteristics such as capacity of self-renewal and multipotency have been defined for the epidermal SCs, the determination of a specific marker, or combination of markers, that would assist in their direct identification within the epidermis is still focus of much interest. Much as surface markers for SCs in the haematopoietic system (e.g. CD34 and CD38) and for neural SCs (e.g. CD133) have aided in their isolation and purification, several studies have characterized putative epidermal SC markers. Based on recent reports (Tumber et al., 2004 and Morris et al., 2004), the comparison of the transcriptional profiles of SCs identified several genes (i.e. *ESP8*, *COL18A1*, *PKD2*) common to hair follicle, neural, haematopoietic and embryonic SCs (Turksen, 2004). It remains to be determined if these genes can be used as a ‘footprint’ to indicate the ‘stemness’ potential of the epidermal or other particular cell population.

Below we summarize results on specific factors and signalling pathways that have been used in the enrichment of a cell population with epidermal SCs.

Integrins

Notwithstanding the important structural and regulatory roles that integrins ($\beta 1$ and $\alpha 6\beta 4$) have in the epidermis, there are questions with regard to their specific role as SC markers. In the epidermis, basal cells are maintained in a spatio-temporal orientation by attachment to the basal lamina through hemidesmosomes, anchorages formed by laminin 5 from the basal lamina and integrin $\alpha 6\beta 4$ that are intracellularly linked to the keratin cytoskeleton (Borradori and Sonnenberg, 1999). In addition, they also transduce signals from the extracellular matrix to the interior of the cell, thus participating in the overall organization of the cytoskeleton, proliferation, apoptosis, and differentiation (Mainiero et al., 1996; Goldfinger et al., 1999).

Li et al. (1998) reported that high levels of $\alpha 6$ integrin and low expression of the transferrin receptor is a combination that identifies epidermal SCs characterized by a long-term proliferative capacity. However, others did not find a direct correlation between the levels of integrin $\alpha 6\beta 4$ heterodimer and the epidermal proliferation capacity (Jones and Watt, 1993; Jones et al., 1995) and $\alpha 6\beta 4$ integrin-null mice show no defects in proliferation. The phenotype

of these mice, characterized by epidermal blistering, is consistent with a role for $\alpha 6\beta 4$ integrin in anchorage (DiPersio et al., 2000).

Another proposed epidermal SC marker is $\beta 1$ integrin, which is expressed throughout the basal layer of the epidermis, and its expression is downregulated in keratinocytes that have initiated the differentiation program. Utilizing human keratinocytes and IFE, it is possible to enrich for cells with SC qualities by selecting those with high levels of $\beta 1$ integrin expression (Jones and Watt, 1993; Jones et al., 1995). $\beta 1$ integrin has a role in maintaining keratinocytes in an undifferentiated state (Levy et al., 2000) and a conditional knockout using K14cre mice lead to defects in epidermal proliferation and basement membrane formation (Raghavan et al., 2000). These findings indicate a prominent role of $\beta 1$ integrin in the proliferative capacity of keratinocytes, but whether this protein identifies the SC compartment within the epidermis remains to be determined.

Despite the conflicting findings when using $\beta 1$ or $\alpha 6\beta 4$ integrin as SC markers, several studies indicate that both markers can be used to enrich a population for SCs. Some of the reported differences noted above might be attributed to differences between the mouse and human keratinocytes. Ultimately, it is plausible that a more complex combination of markers will be needed to distinctively profile the epidermal SC.

p63

p63 belongs to the p53 family of transcription factors, with p53 having a well-established role as a tumour suppressor gene (Yang et al., 2002). The pivotal role for p63 in epidermis was demonstrated by ablating it during embryonic development. This leads to prenatal lethality with a lack of all stratified epithelia, including severe defects in epithelial–mesenchymal-derived structures, such as mammary and prostate glands (Yang et al., 1999; Mills et al., 1999). Mutations in p63 are associated with human ectodermal dysplasias (Celli et al., 1999; van Bokhoven et al., 2001). The implications are that functional p63 is essential for commitment of the ectoderm to epidermal lineages (Mills et al., 1999) and/or for maintenance of the epidermal SC population necessary for epithelial morphogenesis and renewal (Yang et al., 1999; Pellegrini et al., 2001).

In contrast with the related p53 protein, p63 is transcribed from two different promoters (TA and ΔN) as alternatively spliced isoforms (α , β and γ). The TA isoforms (TAp63 α , β and γ) contain an N-terminal transactivating domain that is absent from the ΔN isoforms ($\Delta Np63\alpha$, β and γ). p63 is expressed mainly in the basal layer of the epidermis, predominantly as the $\Delta Np63\alpha$ isoform (Yang et al., 1998); however, other isoforms are expressed at lower levels (Nylander et al., 2002). In the mouse ablation reports mentioned above, both groups disrupted exons common to all isoforms (TA and ΔN), resulting in phenotypes that are a consequence of the loss of all possible gene products (Yang et al., 1999; Mills et al., 1999). Thus it is not possible to determine from these data whether each isoform contributes a distinct function during epidermal determination and differentiation.

In vitro data suggest that ΔN isoforms might act in a dominant-negative fashion versus the TA isoforms (Yang et al., 1998), with $\Delta Np63\alpha$ acting as a positive and negative regulator blocking differentiation (King et al., 2003). Findings reported by Koster et al. (2004) showed that TAp63 isoform overexpression blocks keratinocyte differentiation while they are required for the process of commitment and initiation of epithelial stratification during embryogenesis. The authors also showed that the ability of TAp63 to drive commitment extended to non-keratinocyte cell types. There are several potential reasons for the different findings, including the influences of genetic backgrounds and/or variation in methods of isolation of cells to be studied *in vitro*. Nonetheless, the functional analysis of the specific isoforms should provide

critical data to unravel their role in the processes of epithelial SC determination and maintenance.

Wnt signalling, β -catenin and c-Myc

β -Catenin is a multifunctional protein that plays important roles during embryonic development and neoplasia as a mediator of the Wnt signalling pathway. When the Wnt pathway is quiescent, β -catenin participates only in adherens junctions. When β -catenin moves into the cytoplasmic compartment it gets phosphorylated and targeted for ubiquitination and degradation. Under certain circumstances, such as the activation of the Wnt pathway, phosphorylation of cytoplasmic β -catenin is inhibited, leading to an accumulation of stabilized cytoplasmic protein. As a consequence, β -catenin translocates to the nucleus where it binds Tcf/Lef transcription factors and regulates transcription (Merrill et al., 2001; Jamora and Fuchs, 2002; Alonso and Fuchs, 2003; Jamora et al., 2003).

Using diverse mouse models has demonstrated that the Wnt pathway is involved in regulating SC fate decisions, with the level of β -catenin correlating with different epidermal SC fates (Huelsenken et al., 2001). One of the downstream targets of the β -catenin transcriptional pathway is the oncogene c-Myc (Honeycutt and Roop, 2004). c-Myc is required for transition from the G1 to the S phase of the cell cycle and it promotes the proliferation of epidermal TA cells. However, deregulation of c-Myc transcription depletes epidermal SCs, with the consequent inability of SCs to react to injury (Waikel et al., 1999, Arnold and Watt, 2001, Frye et al., 2003). Furthermore, targeted overexpression of c-Myc in basal keratinocytes leads to impairment of keratinocyte migration and inhibition of wound healing (Waikel et al., 2001). The majority of genes suppressed by c-Myc in epidermis encode cell adhesion and cytoskeleton proteins, resulting in profound changes in keratinocyte adhesion and motility. Lastly, c-Myc can redirect epidermal cells to exit the SC compartment and differentiate into sebocytes and IFE at the expense of the hair lineages (Frye et al., 2003), uncovering an additional role for c-Myc in epidermal SC fate determination.

As highlighted in this section, the determination of specific epidermal SC marker(s) remains a challenge; however, crucial progress has been made in recent years: (1) the detection of the major repository for SCs in the bulge, (2) the determination of the plasticity of the adult epidermal SC, and (3) the identification of factors and pathways involved in the commitment and maintenance of the epidermal SC population. These discoveries should prove essential in the usage of SCs in therapeutic procedures.

Response to injury: the keratinocyte activation cycle

The normal process of keratinocyte differentiation is an invaluable biological programme that constantly restores an important barrier between the organism and its environment. However, this perpetual programme of self-renewal is interrupted when an injury occurs. Keratinocytes represent the body's 'first line of defence' from the outside environment, and as such they are the first responders to injury. After injury, keratinocytes become activated (Figure 2A). This means that they secrete various cytokines and growth factors and, at the same time, respond to them. Therefore, these responses alter the keratinocyte phenotype from basal/differentiated to what is generally called the activated keratinocyte (Tomic-Canic et al., 1998).

Given that there are different types of possible injuries, there are multiple ways in which keratinocytes are equipped to respond to them. Upon UV radiation, differentiation of keratinocytes is stimulated, possibly because enhanced cornification would provide additional protection (Li et al., 2001; Sesto et al., 2002). After mechanical injury, keratinocytes will start migrating and proliferating instead (Tomic-Canic et al., 2004). Upon interferon γ (IFN γ) stimuli, keratinocytes will activate the expression of tight-junction genes thus augmenting their

junctions in order to prevent paracellular penetration of viral particles (Banno et al., 2003). Therefore, keratinocytes possess biological tools to first assess the damage and then to appropriately respond to it. How is this accomplished?

Upon mechanical injury, keratinocytes release pre-stored interleukin-1 (IL-1), which is the first signal upon wounding and has a dual function: to activate keratinocytes and to signal-alert the surrounding tissues. The keratinocyte activation cycle is governed by extracellular signals including tumour necrosis factor α (TNF α), transforming growth factor β (TGF β), epidermal growth factor (EGF), HG-EGF, IL-8 and IFN γ , and is characterized by changes in expression of keratin proteins. One of the markers of activated epidermal keratinocytes is the expression of keratins K6 and K16. K6 is activated by cytokines and growth factors such as IL-1, EGF and TNF α (Jiang et al., 1993; Komine et al., 2000; 2001). It has been proposed that cytoskeletal changes that include the expression of K6 and K16 keratin filaments provide plasticity and flexibility while maintaining resilience of the intracellular scaffold that is important for migration (Coulombe, 1997; Wong and Coulombe, 2003). Once the activation cycle is completed, keratinocytes become deactivated and revert to normal differentiation.

Very little is known about which signals participate in ending the activation cycle. One possibility is that once the activation signals cease, as a consequence, so does the activation cycle. Alternatively, factors such as corticosteroids may participate in inhibition of the activation cycle (Lee and Tomic-Canic, 2002). Corticosteroids are known to participate in the inhibition of wound healing, but the molecular mechanism of this inhibition is not understood (Muller-Decker et al., 2002). Preliminary studies indicate that corticosteroids act as dominant inhibitors of EGF leading to suppression of K6/K16 expression as well as keratinocyte migration (B. Lee, C. Vouthounis, O. Stojadinovic, H. Brem, M.J. Im and M. Tomic-Canic, unpublished work).

The process of wound healing

During the process of tissue repair, keratinocytes undergo significant changes of their junctions and adhesion molecules: hemidesmosomes have to be dissolved in order to allow for keratinocyte migration. The migrating keratinocytes produce a different set of membrane molecules, such as vitronectin, fibronectin receptors and integrin $\alpha 5 \beta 1$, and they replace the collagen receptor allowing keratinocytes to migrate (Cavani et al., 1993; Haapasalmi et al., 1996; Singer and Clark, 1999). Many factors active during wound healing such as EGF, keratinocyte growth factor (KGF) and TGF β , are potential regulators of these processes (Werner et al., 1994; Zambruno et al., 1995; Abraham and Klagsburn, 1996; Nanne and King, 1996).

The activated keratinocyte phenotype is broadly defined, but which keratinocyte phenotypes can become activated? Based on early studies it was believed that because of their mitotic ability, basal keratinocytes rather than keratinocytes already committed to differentiation take part during wound healing (Galvin et al., 1989). However, the use of conditional knockouts, which allow the targeting of genes specifically in epidermis, and viral transductions with biomarkers such as GFP or β -galactosidase, permit a much 'closer look and better resolution', therefore revisiting this question. Epidermal SCs, TA cells and early differentiated cells have the ability to form fully differentiated epidermis both *in vivo* and *in vitro* (Li et al., 2004). Although each of these keratinocyte types has different capacity and matrix requirements, all of them can reproduce stratified epidermis (Figure 2A) (Li et al., 2004). If early differentiating keratinocytes possess regenerative capacity, the question is at which point do they become irreversibly committed, i.e. what is the 'point of no return'? Although there is no conclusive answer, one can speculate that once a differentiating keratinocyte is unable to respond to activating signals it is irreversibly committed to terminal differentiation. Once keratinocytes

process profilaggrin into filaggrin they undergo specific changes that mark late (terminal) differentiation (Presland et al., 2001), and perhaps this process demarcates the 'line of regenerative potency' among the differentiating keratinocyte population (Figure 2A).

Failure to heal: chronic wounds from an epidermal prospective

Under certain circumstances a wound fails to heal, thus leading to the development of a chronic wound (ulcer). Incidences of chronic wounds are higher among the elderly and diabetic, as well as among people with vasculature problems; therefore failure to heal may have various underlying pathologies. The epidermis of a chronic wound has a typical appearance (Figures 2B and 3). It is thick and hyperproliferative, with mitotically active cells located in the suprabasal (differentiated) layers. Furthermore, the cornified layer is hyperkeratotic (thick cornified layer) and parakeratotic (presence of nuclei in the cornified layer). Keratinocytes on a chronic wound edge are capable of proliferating but are unable to migrate properly. In the need to restore the 'injured' barrier, keratinocytes keep differentiating but the presence of nuclei in the cornified layer (parakeratosis) indicates incomplete differentiation. Taken together, the histology of epidermis at the non-healing edge of chronic wounds suggests that chronic wound keratinocytes do not successfully complete either of the two possible pathways: activation or differentiation (Figures 2B and 3). Instead, keratinocytes are caught in a 'loop' of trying, but not succeeding, to accomplish either of the two processes. Results from our laboratory have shown that non-healing keratinocytes of the chronic wound edge are marked by induction of c-Myc and nuclearization of β -catenin, which may contribute to the inhibition of migration (O. Stojadinovic, C. Vouthounis, B. Lee, H. Brem, A. Merchant, J. Fallon, M.R. Stallcup and M. Tomic-Canic, unpublished work). These results are consistent with reports in transgenic animals, in which the persistent activation of c-Myc in basal keratinocytes led to inhibition of keratinocyte migration and development of a chronic wound (Waikel et al., 2001; Arnold and Watt, 2001). Furthermore, overexpression of c-Myc has been implicated in promoting the cycling of SCs that leads to depletion of the local epidermal SCs (Ganderillas and Watt, 1997; Waikel et al., 2001; reviewed by Honeycutt and Roop, 2004). Given that keratinocytes of the wound edge are hyperproliferative and express c-Myc, our current hypothesis is that local depletion of epidermal SCs at the non-healing edge of a chronic wound may be responsible for the persistence of a wound.

One of the major issues in chronic wound treatments is how to revert the chronic wound keratinocyte phenotype to a proper differentiating phenotype. One possible approach is to surgically remove (debride) non-healing keratinocytes (purple in Figure 2B) and allow the freshly wounded keratinocytes to become exposed and permissive to the activation signals. The intent is that these cells will resume their activation cycle followed by proper differentiation. Another important issue is to provide and maintain the source of the activation signals in a chronic wound environment. In the past decade many clinical trials have utilized a variety of growth factors and cytokines in potential treatment of chronic wounds (Martin, 1997). Currently, platelet-derived growth factor (PDGF-BB) and human skin equivalents (HSEs), containing either both dermis and epidermis or only dermis, are the only Food and Drug Administration (FDA)-approved therapy for chronic wounds (Brem et al., 2003; Nagai and Embril, 2002; Sibbald, 1998). HSEs may consist of either living fibroblasts suspended in a native collagen matrix (resembling dermis), or, in addition, may have their surface covered with viable, stratified keratinocytes (dermis and epidermis). These are viable cells fully capable of secreting and responding to a variety of growth factors/cytokines, similarly to normal skin (Ehrlich, 2004). Although there are many questions that remain to be answered on the molecular mechanism of how HSE works as therapy of chronic wounds, it is believed that it acts as temporal cell therapy that provides local sustained release of stimulating factors (Brem et al., 2003; Ehrlich, 2004). The chronic wound environment is very complex and involves pathogenic changes in underlying tissues such as dermis, endothelium and peripheral nerves,

all of which also contribute to keratinocyte impairment. Therefore, the appropriate treatments should have a broad and complex approach, and will require the targeting of more than one cell population.

Epidermal SCs and chronic wounds

Using HSE as a therapy for chronic wounds also raises a question of the use of SCs as a therapeutic approach in wound healing. The intriguing question is: what is the fate of epidermal SCs provided by HSE therapy? As mentioned above, both epidermal SCs and TA cells have the ability to fully restore epidermis (Li et al., 2004). Epidermal SCs have the potential to regenerate epidermis, and if stimulated adequately, develop different cell types and tissues (Liang and Bickenback, 2002). Embryonic SCs can form an epidermal equivalent when seeded on extracellular matrix in the presence of bone morphogenic protein-4 (BMP-4) or ascorbate (Coraux et al., 2003). Furthermore, the potency of skin SCs in tissue repair is not limited to epidermis. Recent reports indicate that human skin-derived SCs are capable of neural differentiation (Belicchi et al., 2004; Joannides et al., 2004). Conversely, bone-marrow-derived cells also are able to incorporate and differentiate into skin structures, suggesting that bone marrow might be a valuable source of SCs for skin repair (Badavias et al., 2003).

Conclusions

Although many questions remain to be answered, it is clear that because of their plasticity and accessibility, epidermal SCs provide a major resource for therapeutic applications in tissue repair and regeneration, as well as mediators for gene therapy approaches. In addition to their multi-potency, SCs can be genetically engineered using viral vector constructs (Ghazizadeh and Taichman, 2001), which allows for usage in gene therapy. The recent advances in biotechnology, such as genomics, proteomics, *in vivo* epidermal targeting, GFP labelling and adeno- and retro-viral gene transfers, will allow for much better understanding of SC biology. Questions such as how specific SCs respond and create appropriate niche(s) are now being successfully answered (Brouard and Barrandon, 2003; Gambardella and Barrandon, 2003; Blanpain et al., 2004; Fuchs et al., 2004). Lastly, these novel technological approaches will allow for identification of specific molecular markers which will aid further in the isolation of SCs, thus opening unlimited possibilities for therapeutic approaches.

Acknowledgements

We thank members of the Morasso and Tomic laboratories, especially Dr Olivera Stojadinovic. Special thanks to Dr Harold Brem and Dr John Fallon for chronic wound histology, and Mr John Ward for photography assistance. We apologize to our colleagues whose contributions were not cited due to the space limitations. Our research is supported by the Intramural Research Program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases (M.M.) and by the National Institutes of Health grants AR45974, NR08029 (M.T.-C.).

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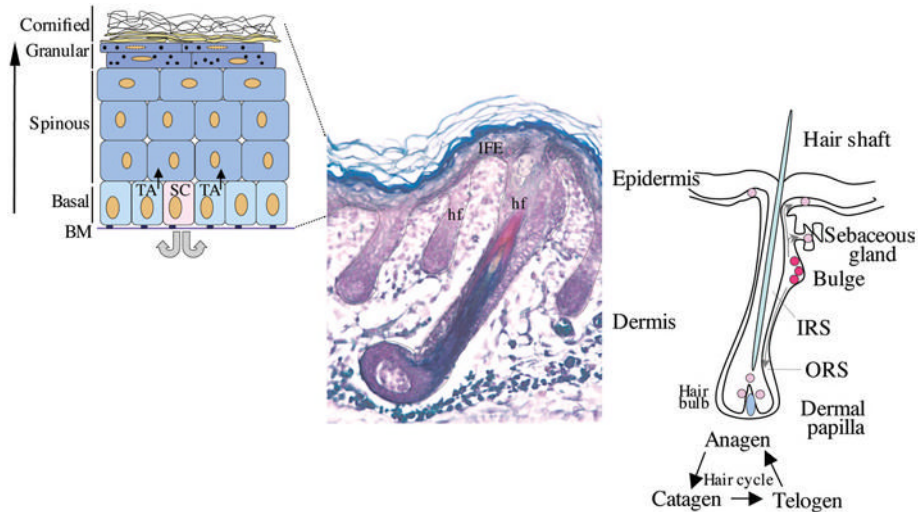


Figure 1. Stem cells in the interfollicular stratified epidermis and the bulge region of the hair follicle
 Left-hand panel, schematic representation of the stratified layers of the epidermis (basal, spinous, granular and cornified layers). The proliferative basal layer is adjacent to the basement membrane (BM). Stem cells (SC) generate transit amplifying (TA) cells which will differentiate to form the stratified layers. Middle panel, section of mouse skin stained to distinguish the different compartments of the hair follicle (hf) (Magnification 10 \times). Right-hand panel, schematic representation of a hair follicle and hair cycle, with the multipotent SCs (red) localizing to the bulge region. These cells migrate to populate the bulb region of the follicle, the sebaceous gland and the interfollicular epidermis (IFE; pink). IRS, inner root sheath; ORS, outer root sheath.

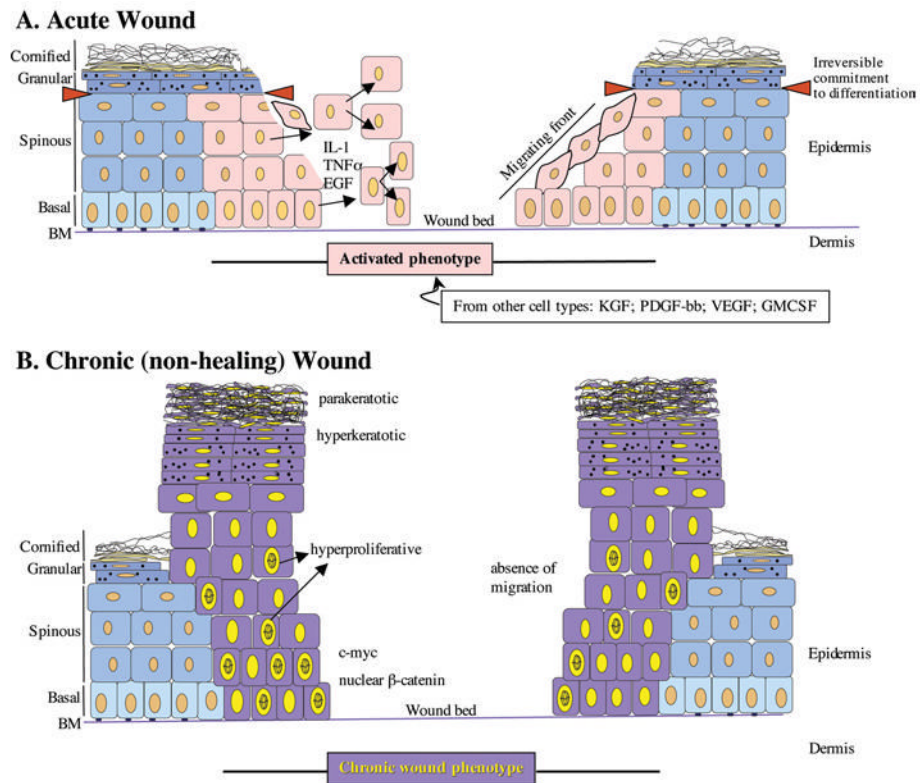


Figure 2. Schematic representation of keratinocyte phenotypes in acute (A) and chronic (B) wounds (A) In the acute wound, keratinocytes become activated (pink), and release/respond to various pro-inflammatory cytokines and growth factors. In response to these stimuli, keratinocytes from the basal and suprabasal layers start proliferating and migrating. Red triangles demarcate the point of irreversible commitment of keratinocytes to differentiation after which they are unable to participate in the wound healing response. (B) Chronic wound keratinocytes (purple) are hyperproliferative (indicated by mitotically active cells present throughout the suprabasal layers), hyperkeratotic (indicated by thick cornified layer) and parakeratotic (indicated by presence of nuclei in the cornified layer). Chronic wound keratinocytes are unable to migrate.

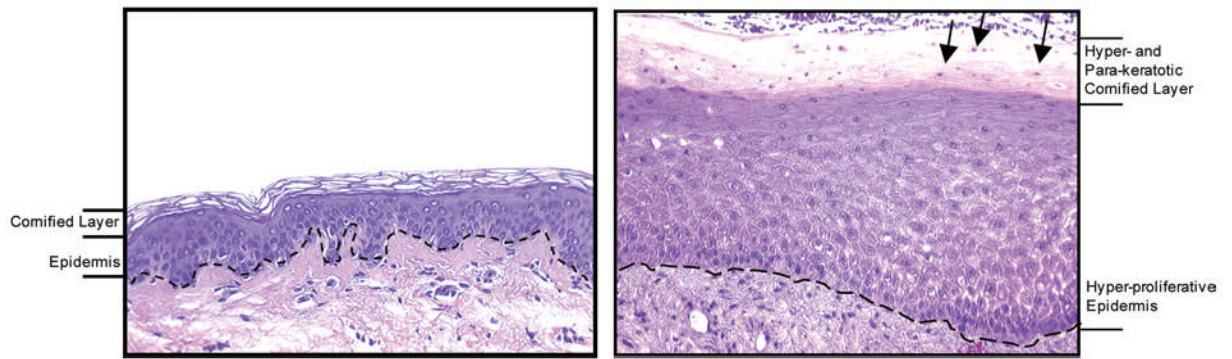


Figure 3. Histology of human epidermis in normal skin (left-hand panel) and at a non-healing edge of a chronic ulcer (right-hand panel)
In the chronic ulcer, the epidermis is hyperproliferative, as indicated by increased thickness. The cornified layer is both thicker and contains cell nuclei (arrows). Dashed line: basement membrane. Magnification 20 \times .