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REVIEW ARTICLE

Wound Healing by Keratinocytes: A Cytoskeletal Perspective

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Abstract | Skin, being the protective barrier against the environment, can be subject to frequent trauma and stress, hence has the ability to heal itself rapidly. This capacity is attributed to a large number of resident stem cells and progenitors in the skin, which are activated to proliferate, migrate and differentiate to recreate the cellular diversity and regain tissue integrity. The barrier function of the skin is maintained by the epidermis, a multilayered epithelial compartment formed of keratinocytes. Wound repair evokes the capacity of cells to sense and respond to environmental cues. Tissue damage demands rapid cellular action, wherein spatio-temporally regulated cellular responses are critical determinants of the outcome of healing. Hence cells surrounding the wound have to immediately sense the damage and must activate the key signaling pathways to launch the wound-healing response. Emerging data is indicating that mechanical tension release is one of the first cues sensed by the neighboring cells of the damage. This cue is relayed by the cytoskeleton and converted into biochemical and cellular signals, which help the cells to respond accordingly to the trauma. In this review, we will focus on the role of keratinocytes and keratinocyte stem cells in wound healing, and the cytoskeletal dynamics involved therein.

Keywords: *Wound healing, Cytoskeleton, Keratinocyte, Migration*

1 Introduction

Tissue development, homeostasis, and repair evoke the capacity of cells to sense and respond to environmental cues. Tissue damage demands rapid cellular action, wherein spatio-temporally regulated cellular responses are critical determinants of the outcome of healing. Wound microenvironment-derived cues are sensed by neighboring cells to activate key signaling pathways that impact their cytoskeletal dynamics, which not only influence cell migration, but also proliferation and differentiation, parameters which are deregulated in abnormal tissue transformation. Hence, research into understanding mechanisms of regulating the cellular dynamics in wounded tissue opens up avenues to probe into pathological conditions such as abnormal healing and tumorigenesis The skin has been the tissue of choice to understand wound healing not only because healing in the skin represents all the stages of wound-healing, but also due to ease of experimental access. Cytoskeletal dynamics and signaling contribute towards range of cellular behaviors such as proliferation, differentiation, and migration which are initiated upon wounding.

Skin is the largest organ of the human body, which is a composite of both epithelial and mesenchymal tissue types. The outermost layer, the epidermis, is primarily composed of keratinocytes which are of epithelial origin. Following this is the dermis of mesenchymal origin, primarily composed of fibroblasts which secrete the collagen and elastin-rich connective tissue. The dermis also has blood vessels, adipose tissue and nerves. The epidermis and dermis are separated by a

Homeostasis: A relatively stable state of cell and tissue physiology.

Tumorigenesis: Formation of an abnormal mass of cells as a result of uncontrolled proliferation.

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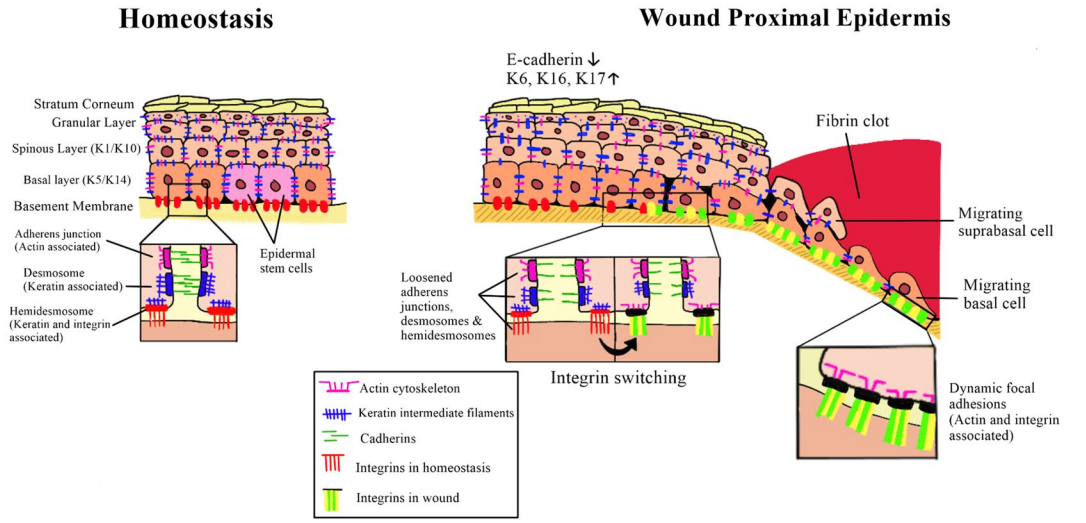

Figure 1: **Keratinocytes in homeostasis and wound healing.** The homeostatic epidermis is shown as a stratified layer of keratinocytes; the basal layer harboring stem cells, followed by differentiating cells of the spinous and granular layers ending in the cornified layer. Keratinocytes adhere to each other and the basement membrane through multiple junctions (represented in different colors). Attachment of the actin and keratin filaments to the junctions provides mechanical strength to the intact epidermis. Wounding leads to junctional remodeling from stable keratin-based hemidesmosomes to actin-based dynamic focal adhesions, a process marked by change in integrin expression (represented in different colors) that is concomitant to change in wound extracellular matrix. Wound proximal keratinocytes express stress keratins that aid the switch to the migratory phenotype marked by active cell protrusions.

basement membrane, which is a layer of extracellular matrix rich in laminin and collagen. The epidermis itself is multilayered (Fig. 1), the bottom-most layer being anchored to the basement membrane and known as the basal layer (stratum basale). The cells of this layer proliferate and differentiate creating the upper layers of the epidermis, viz., the spinous layer (stratum spinosum), the granular layer (stratum granulosum), and the cornified layer (stratum corneum)[1,2] through the process of cornification. As keratinocytes progress through these layers, they change shape from columnar or cuboidal basal cells to large flattened dead cells at the cornified layer. The cytoskeletal elements within keratinocytes play an important role in both homeostasis and wound repair. The cellular cytoskeleton not only provides mechanical support to cells but integrate both outside-in and inside-out signaling by associating with cell membrane. The cellular cytoskeleton is typically categorized into three kinds: the microtubules, the actin filaments, and intermediate filaments, which in keratinocytes comprise of keratin filaments. To exemplify, both microtubules and actin filaments are made up tubulin and actin monomers, respectively, wherein the filamentous form is in a state of dynamic equilibrium maintained by polymerization and depolymerization at the ends. The filament dynamics is influenced by the monomers and their interactions with associated proteins. Actin filaments form a plasma membrane-proximal cortical meshwork, as well as interact with microtubules to regulate cell polarity, cell division, migration and tension sensing.

Cornification: The process of keratinocyte differentiation leading to the formation of keratin-filament rich dead cells packed against each other creating a physical barrier.

The signature cytoskeletal element in keratinocytes is the intermediate filament protein keratin, from which these cells derive their name. Keratin filaments are critical for intercellular junction stabilization and differentiation process in keratinocytes[3]. Keratinocytes express two classes of keratins that form heteropolymers: type I comprising of K9–K20, K23–K28 and K31–K40 and type II comprising of K1–K8 and K71–K86[3]. The unique combination of keratin pairs expressed in keratinocytes determines their identity in the epidermal layers. In comparison to the mechanical properties of actin and microtubules, keratin filaments are highly flexible and elastic, thereby majorly contributing to the mechanical resistance of the cells[4]. The basal layer expresses K5–K14, while the suprabasal layers express K1–K10, which are considered markers of differentiation (Fig. 1). Moreover, the expressions of these keratin subtypes change when cells sense changes in matrix stiffness, undergo differentiation, sense inflammatory stress, and activate the wound

Inflammation: The ability of cells to sense external stressors and launch an immune response leading to the activation of the innate and adaptive immunity.

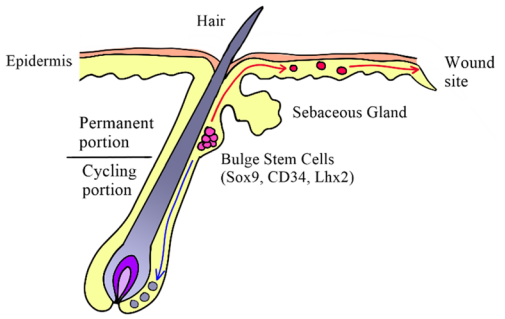

Figure 2: **Hair follicle stem cells in wound healing.** The pilosebaceous unit is represented here, with the multipotent bulge localized hair follicle stem cells (pink) below the sebaceous gland. The portion of the hair follicle below the sebaceous gland undergoes cyclical growth and regression. Hair growth signals activate bulge stem cell proliferation and migration towards the hair bulb (downward arrow) creating progenitors (gray) that differentiate to form hair shaft. Upon sensing a wound, the bulge stem cells show migration towards the epidermis (upward arrow) wherein they differentiate to epidermal progenitors (red) and contribute to faster healing of the epidermis.

repair program[5]. Other than keratin, cytoskeletal elements, such as loricrin and profilaggrin, mark the cells of the granular layer, and filaggrin is present in the stratum corneum[6].

Keratinocytes are attached to each other through intercellular junctions such as desmosomes, adherens junctions, and tight junctions. While desmosomes interact with keratin filaments, adherens junctions interact with actin cytoskeleton[7] (Fig. 1). Keratinocytes of the basal layer attach to the basement membrane via hemidesmosomes, anchoring keratin filaments to the cytoplasmic face of $\alpha_6\beta_4$ integrins which interact with laminin-5 of the basement membrane[8]. The intercellular junctions help to create a network of cells in the epidermis, maintaining mechanical integrity via the keratin filaments[9]. In several inherited skin blistering diseases, it is the junctional components that are mutated, causing epidermal destabilization, splitting from the dermis and barrier dysfunction[8]. Epidermal homeostasis is maintained by epidermal stem cells, located in the basal layer of the epidermis, which maintain a balance of self-renewal and committed progenitor formation[10,11]. The extent of asymmetric division maintains the balance between self-renewal and daughter cell formation. In the adult epidermis, perpendicular mitotic spindle orientation with respect to the basement membrane creates a daughter cell targeted for differentiation, while the progenitor cell is retained in association with the basal layer[11,12].

A very important component of the skin is the pilosebaceous unit comprising of the hair follicle and the sebaceous gland (Fig. 2). The lower part of the hair follicle below the sebaceous glands undergoes cyclical phases of growth (anagen), retraction (catagen) and rest (telogen) which marks the hair fall and growth cycle. This periodic regenerative process is fueled by hair follicle stem cells (HFSCs) located in the bulge niche (Fig. 2) below the sebaceous gland[13]. They express surface markers like α_6 integrin CD34 and transcription factors such as Sox9[14,15] and Lhx2[16], which are important determinants of the HFSC specification and maintenance processes. Other prominent stem cell populations present in the hair follicle are at the isthmus (marked by expression of Lgr6, Plet1, and Gli1), junctional zone (marked by Lrig1), hair germ stem cells (marked by Lgr5), and sebaceous gland stem cells (Blimp1)[17]. The HFSCs of the bulge reveal their multipotent nature upon tissue injury, wherein they migrate upwards to the wounded epidermis (Fig. 2) and aid in regenerating the epidermis, sebaceous glands and hair follicles[18]. During the process of re-epithelialization, all the hair follicle stem cell pools contribute to the newly formed epidermis in varying degrees, wherein the earliest response occurs from the bulge HFSCs[17]. Similar to the epidermal stem cells, polarity and spindle orientation are important in determining hair follicle stem cell divisions to ensure the balance between self-renewal and differentiation. Lhx2 knock-out from bulge HFSCs affects the expression of cytoskeletal modulators and adhesion molecules reduces cellular F-actin, affects apicobasal polarityand cell division symmetry, and further lineage specification[19]. Lhx2 is reported to play a key role in determining the epidermal regeneration capacity of HFSCs by suppressing Lgr5 expression (hair germ specification) and promoting Sox9 expression and lineage specification to epidermal keratinocytes[20].

2 Wound Healing in the Skin

The process of wound healing in any tissue is typically divided into three stages that are temporally overlapping and well represented during skin healing[21]. Immediately after a cut on the skin occurs, blood clotting (hemostasis) and localized inflammation are accompanied by infiltration of immune cells, hence termed the

Sebaceous gland: It is an exocrine gland associated with the hair follicle, which secretes sebum that lubricates the hair and skin.

Multipotent: A stem cell which is capable of giving rise to more than one cell type.

Apicobasal polarity: This marks a difference in cellular cytoskeletal architecture and junctional distribution in epithelial cells along an axis perpendicular to the basement membrane where the cell is attached (basal side) to top.

Lineage specification: The phenomenon of stem cells differentiating into a specific cell type.

inflammatory phase. The release of mitogens and chemo-attractants by degranulating platelets and inflammatory cells initiates new tissue formation, which marks the proliferative phase. During this phase, epidermal keratinocytes and dermal fibroblasts should not only proliferate, but must also migrate into the wound to recreate the lost tissue. Reformation of the epidermis is described as re-epithelialization which ends in completion of barrier formation. To aid rapid reformation of the epidermal barrier, epidermal stem cells, as well as stem cells from the pilosebaceous unit, are activated to proliferate and migrate into the wounded epidermis[17]. Fibroblasts in the dermis secrete collagen and transform to myofibroblasts which cause contraction of the wound edges to aid wound closure. The last and the longest phase is the remodeling phase, wherein the newly deposited extracellular matrix is remodeled by secreted enzymes in an attempt to gain original tissue architecture, but typically wound healing ends with scar formation in adult humans. Poor outcome of chronic wounds result from a major defect in re-epithelialization, and hence, there has been a lot of research towards understanding keratinocyte responses upon wounding[22].

Mitogens: A peptide or protein which induces mitotic cell division.

Chemoattractants: A molecule that stimulates directed cellular migration towards it.

Extracellular matrix: A complex network of extracellular molecules which provides anchoring mechanism to cells.

3 Keratinocytes in Re-epithelialization: Cytoskeletal Mediators of the Wound Response

3.1 Wound Sensing by Keratinocytes

The epidermal barrier breach can send a wound signal to keratinocytes in more ways than one, the quickest being changes in mechanical tension due to loss of neighboring cells and junctions being disrupted at the wound edge[23,24]. Within the keratinocytes, the cytoskeleton is the primary structure mediating mechanical force transmission[25]. The "tensegrity" model states that the cytoskeletal structures, along with adhesion structures that link the cytoskeleton to the extra cellular-matrix, provide cells with a "pre-tension" which is necessary for the cells to responds to extrinsic tension[26]. Hemidesmosomes, composed of integrins (well-known molecular sensors of mechanical forces and regulators of actin dynamics[27]) have been largely studied in the context of keratinocyte attachment to the ECM, while very little is known from the perspective of mechanotransduction and possible role of hemidesmosomal integrin signaling onto YAP–TAZ pathway (see below)[28]. Seminal work using keratinocytes expressing normal keratin or its mutant version incapable of forming proper filaments reveals the role of this cytoskeletal entity in maintaining cadherin-based junctions and cellular connectivity, an important determinant of epidermal integrity[29]. Keratin filaments has been shown to sense tension and recruit Tensin molecules on them upon application of force, in a manner similar to focal adhesion strengthening due to tension sensing by actin filaments[30]. Tension changes can affect the proliferation rate of keratinocytes, as observed in keratinocyte hyperproliferation on stiff keloid-scar tissue[31]. This proliferative edge resulted from activation of Epidermal Growth Factor Receptor signaling as a by-product of integrin activation at focal adhesions. Frequent stretching of human keratinocytes grown on flexible silicone gels can stimulate proliferation, along with suppression of differentiation marker K10 and increased expression of wound-associated K6 which is expressed by wound-proximal keratinocytes[32,33].

A major development in understanding the molecular basis of mechanotransduction was the identification of YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif) as mechanical signal relays[34]. YAP and TAZ are key downstream coactivators in the Hippo pathway, and their knockdown in full-thickness skin wounds was shown to delay wound closure as well as reduce TGF-β expression at wound site[35]. In the absence of mechanical stimulus, YAP/TAZ is inactive and is localized in the cytoplasm. It has been shown that in MCF-10A cells (human mammary epithelial cells), sense mechanical tension through integrin and actin cytoskeleton and translocate YAP/TAZ to the nucleus, thereby upregulating the cell proliferation[36]. The nuclear translocation of YAP/TAZ controls proliferation and differentiation, also rate of wound healing in cultured mouse keratinocytes as well as in the adult mice epidermis and control the TGF-β signaling which is necessary for the wound healing [37,38]. Another transcription factor, activator protein-1 (AP-1), which is involved in regulating gene expression (such as that of keratins) necessary for proliferation and differentiation in epidermal keratinocytes[39], can be activated by mechanical forces[40,41].

Mechanotransduction: The conversion of mechanical stimuli to biochemical signals to elicit a cellular response.

3.2 Wound Response by Keratinocytes

Keratinocytes of the wound-proximal epidermis show a range of cellular responses such as proliferation, migration and differentiation, which is also spatially segregated. The ultimate goal is for the missing epidermis to reform in its entirety, including the top differentiated layers. The re-epithelializing wound-edge, as studied in mouse models of

excisional wounds (both epidermis and dermis removed using a biopsy tool), is composed of migrating keratinocytes only, followed by an intermediate mixed region where both proliferation and migration coexist (mixed zone) and finally the peripheral proliferative zone supplying cells to the growing epidermal sheet[42,43]. These studies have revealed that not only the basal cells but suprabasal cells also actively migrate, evident from lamellipodia formation observed in both the layers (Fig. 1). Moreover, the polarized migration is a cue to drive differentiation and proliferation in the wound-proximal keratinocytes[42]. In vitro 3D organoid-based models of skin equivalents (having multilayer epidermis and dermis) have been used effectively to understand the above-mentioned parameters of re-epithelialization such as proliferation, migration and differentiation, with the added benefit of ease of manipulation of wound-edge cells[44].

The epidermal keratinocytes, which maintain strong intercellular adhesions in homeostatic conditions, must undergo a host of changes upon wounding to become mobile, yet migrate collectively. The molecular changes they undergo has been compared to attaining partial epithelial-to-mesenchymal transition (EMT), which is a transient and reversible process[45]. The expression of Slug, a Snail family member is required for proper wound re-epithelialization by promoting the downregulation of E-cadherin, a component of adherens junctions, thus promoting motility in the migrating edge keratinocytes[46]. Cytokines present in the wound environment including IL-1α[47] and growth factors like EGF and TGFα[48] initiate the synthesis of 'keratins of the wound healing response' such as K6, K16 and K17 in the proximal keratinocytes of the wound[48,49] (Fig. 1). Expression of these wound-healing keratins weakens intercellular adhesion[50]. The decrease in desmosomal adhesion is regulated by Protein kinase C α (PKCα), and its loss is reported to delay re-epithelialization due to formation of hyper-adhesive desmosomes[51]. Keratinocytes remodel hemidesmosomes by switching integrin expression to form focal adhesions, which are more dynamic than desmosomes and hemidesmosomes, and help to promote the migratory phenotype[52]. Directional migration is achieved by coordinating focal adhesions disassembly at the trailing edge and formation at the leading edge, processes sensitive to force generation by non-muscle myosin-II and Arp2/3 complex driven actin polymerization[53]. Actin filaments interact with focal adhesions through regulatable, force sensitive links such as paxillin, vinculin, talin, actinin and plectin[54]. The keratinocytes switch expression of integrins from laminin and collagen binding ($\alpha_6\beta_4$ and $\alpha_3\beta_1$) to those binding fibronectin, vitronectin, and tenascin rich wound matrix binding ($\alpha_5\beta_1$, $\alpha_v\beta_5$, and $\alpha_v\beta_6$) (Fig. 1). Though the keratinocytes become migratory and downregulate intercellular adhesions, they do not break out of the "epidermal sheet", and upon wound closure, revert back to their homeostatic state, upregulate E-cadherin and initiate the epidermal differentiation program[54,55].

Lamellipodia: Flat membranous projections on leading edge of migrating cells consisting of branched actin filaments.

To attain efficient polarized migration, keratinocytes must dynamically remodel all three cytoskeletal components; the actin filaments, microtubules and intermediate filaments in coordination with the junctions they associate with[56]. Manipulating microtubule dynamics by affecting microtubule associated proteins (MAP) and regulatory proteins that influence (de)polymerization have shown impact on cell proliferation and migration in wound healing[57]. Promoting microtubule depolymerization by activating depolymerizing agents such as MAP4[58] and stathmin[59] by hypoxia and growth factor signaling, respectively, promotes keratinocyte proliferation and migration at the wound edge. In contrast, reducing the levels of another microtubule depolymerizing agent, Fidgetin-Like 2 (FL2), which specifically shears cortically directed microtubules influencing focal adhesion dynamics, enhances the rate of wound healing both in tissue culture and mouse models[60]. Spectraplakins are an interesting class of crosslinkers, which can interact with all three cytoskeletal types, and regulate their dynamics[61]. Actin crosslinking factor (ACF7), a spectraplakin that can bind F-actin and microtubules plays a critical role in wound re-epithelialization by regulating directed migration of keratinocytes. Targeted deletion of ACF7 in epidermal layer or specifically in the hair follicle stem cells was found to affect migration of the keratinocytes and stem cells and delay wound healing. ACF7 functions as a linker between+tip protein EB1 and actin to polarize microtubule growth along actin filaments associated with focal adhesions; hence, the loss of ACF7 slowed down focal adhesion turnover at the leading edge of migrating cells. Interestingly, GSK3β was determined to be the kinase maintaining a phosphorylation–dephosphorylation cycle of ACF7 at the leading edge. The nature or identity of the chemotactic signal leading to the GSK3β–ACF7 axis regulating HFSC migration to wound epidermis is elusive[62,63].

4 Conclusion

The phenomenon of wound healing involves a well-orchestrated cross-talk between multiple cell types via signaling mechanisms that impact

a range of cellular behaviors leading to recovery of the lost and damaged tissue. In the skin, damage to the epidermis activates wound-healing program in keratinocytes, which is marked by signals impacting their cytoskeletal dynamics, which further affects keratinocyte proliferation, differentiation, and migration. It must be noted that other important cellular components of the wound-healing process have not been mentioned here due to limited scope. The interested reader may refer to several excellent reviews on understanding the role of fibroblasts[64], immune cells[65], adipocytes[66], and endothelial cells in wound healing[67].

A healthy healing wound is typically self-limiting wherein cells in the healed region return to their homeostatic state. However, when that fails, the outcome could be a chronic wound, over-scarring and even cancerous transformation. In fact, historically, cancer has been described as an over-healing wound[68] as a consequence of obvious similarities to the stages of wound healing. In the context of re-epithelialization, the wound responses of keratinocytes have several parallels to the processes of tumorigenesis and invasion[69,70]. At the other end of the spectrum is ineffective wound healing in diabetics marked by reduced efficiency of keratinocyte migration and proliferation[71]. Cytoskeletal dynamics and cross-talk are the critical determinant of efficient cell migration, thus appearing as a central theme drawing our interest in finding therapeutic strategies aimed at manipulation at the molecular level. Such therapeutic targets can be identified upon deciphering the molecular basis of both physiological and aberrant keratinocyte responses in wound healing. Categorically two avenues may be explored, one where specific cytoskeletal modulators can be precisely targeted and the other where upstream signaling modules impinging on those cytoskeletal responses can be manipulate. Regulation of keratin filament dynamic and their mechanical signaling is largely unexplored in comparison to actin and microtubules. Stem cells in the pilosebaceous unit and epidermis are important populations that can be targeted for improving epidermal repair. What signaling mechanisms drive these stem cells to the site of healing is a very important avenue to explore for both fundamental and applied research.

Publisher's Note

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Compliance with ethical standards

Conflict of interest

On behalf of all the authors, the corresponding author states that there is no conflict of interest.

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