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Wound re-epithelialization: modulating keratinocyte migration in wound healing

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1. ABSTRACT

An essential feature of a healed wound is the restoration of an intact epidermal barrier through wound epithelialization, also known as re-epithelialization. The directed migration of keratinocytes is critical to wound epithelialization and defects in this function are associated with the clinical phenotype of chronic non-healing wounds. A complex balance of signaling factors and surface proteins are expressed and regulated in a temporospatial manner that promote keratinocyte motility and survival to activate wound re-epithelialization. The majority of this review focuses on the mechanisms that regulate keratinocyte migration in the re-epithelialization process. This includes a review of cell attachments via desmosomes, hemidesmosomes, and integrins, the expression of keratins, the role of growth factors, cytokines and chemokines, eicosanoids, oxygen tension, antimicrobial peptides, and matrix metalloproteinases. Also reviewed are recently emerging novel mediators of keratinocyte motility including the role of electric fields, and signaling via the acetylcholine and beta-adrenergic receptors. These multiple regulators impact the ability of keratinocytes to migrate from the wound edge or other epidermal reservoirs to efficiently re-epithelialize a breach in the integrity of the epidermis. New discoveries will continue to uncover the elegant network of events that result in restoration of epidermal integrity and complete the wound repair process.

2. INTRODUCTION

Although cutaneous wound healing is a complex, multi-step process, involving many cell types, an essential feature of a healed wound is the restoration of an intact epidermal barrier. Indeed, a wound that is not epithelialized is not considered “healed” no matter how perfectly restored the underlying dermal structures may be. Thus, wound epithelialization, also called re-epithelialization, is a critical and defining feature of wound repair.

Re-epithelialization of the wound can be conceptually viewed as the result of three overlapping keratinocyte functions: migration, proliferation, and differentiation. The sequence of events by which keratinocytes accomplish the task of re-epithelialization is generally believed to begin with dissolution of cell-cell and cell-substratum contacts. This is followed by the polarization and initiation of migration in basal and a subset of suprabasilar keratinocytes over the provisional wound matrix. A subset of keratinocytes immediately adjacent to, but not within the wound bed, then undergo mitosis. Finally, there is multilayering of the newly formed epidermis and induction of differentiation specific gene products to restore the functionality of the epidermis (recently reviewed (1)). The most limiting is migration, since defects in this function, but not in proliferation or differentiation, are associated with the clinical phenotype of chronic non-healing wounds (2-4). Thus, the majority of

this review will focus on some of the mechanisms that regulate keratinocyte migration in the re-epithelialization process.

3. CELL ATTACHMENTS: DESMOSOMES, HEMIDESMOSOMES, INTEGRINS

In normal, uninjured epidermis, keratinocytes are attached to one another by desmosomes and to the extracellular matrix (ECM) of the underlying basement membrane by hemidesmosomes. Once the epidermis is disrupted by wounding, these interactions must be released to allow for migration of keratinocytes from the wound edge to participate in the re-epithelialization process (5). The mechanism of this process is still the subject of investigation.

There is evidence that activation and relocalization of protein kinase C alpha (PKCalpha) to the cytoplasmic desmosomal plaque is responsible for decreasing its adhesive properties (6, 7). Additionally, the transcription factor Slug, appears to be required for the desmosomal dissolution. It is upregulated in keratinocytes at the wound edge, and wound re-epithelialization is impaired in Slug knock out animals (8). A complete understanding of the temporal mechanisms regulating both desmosomal dissolution and reconstitution in the reforming epidermis will require further investigation.

Hemidesmosomes, the protein complexes that link the intracellular keratin filaments of the basal layer keratinocytes to the underlying basement membrane, must also be disassembled to allow for keratinocyte migration (9). The alpha6-beta4 integrin of the keratinocyte contributes to the adhesive property of the hemidesmosome and binds with laminin-332 (previously known as laminin-5) in the lamina densa of the basement membrane (reviewed in (9)). Coordinated modulation of hemidesmosome expression and dissolution must occur for keratinocyte migration and wound re-epithelialization. The mechanisms that control these processes are also not fully understood. One proposed mechanism involves differential affinity of integrins to laminin-332. A non-proteolytically processed form of laminin-332 is deposited beneath the leading edge of keratinocytes at the wound margin (10, 11). The alpha6-beta4 integrin has reduced affinity for this non-processed version of laminin-332, and this may contribute to the reduction in adhesion of basal cells to the basement membrane (12). On the other hand, another integrin that is upregulated in migrating keratinocytes, alpha3-beta1 (13), has an increased affinity for the unprocessed laminin-332 (12, 14), and thus may contribute to the motile phenotype. Another mechanism proposed to facilitate hemidesmosome disassembly may lie with "inside-out" modulation of alpha6-beta4 affinity for ligand by intracellular signaling pathways that alter the cytoplasmic portion of the integrin. Targeted deletion of the cytoplasmic signaling domain of the beta4 partner results in keratinocytes that are unable to migrate and proliferate (15). Serine phosphorylation of the cytoplasmic domain of the beta4 integrin likely occurs through the action of PKCalpha, which interferes with the binding of a stabilizing protein, plectin, (16, 17) and suggests that this results in the relocalization of alpha6beta4 from the hemidesmosome to the lamellipodia of the keratinocyte's leading edge(18). In this scenario, macrophage stimulating protein (MSP) and EGF, both found in the wound environment, can modulate the PKCalpha-dependent phosphorylation of the beta4 subunit to increase disassembly of hemidesmosomes and increase keratinocyte motility(18). Once relocalized to the lamellipodia, both the alpha3-beta1and alpha6-beta4 integrins are thought to be important in maintaining the keratinocyte's directional migratory trajectory, mediated in part by their intracellular association with Rac1 (19-21).

In addition to the change in distribution of integrins in the migration cells, keratinocytes actively migrating in the wound also demonstrate phenotypic shift in the repertoire of expressed integrins (22). Migrating cells express alpha5-beta1, alphaV-beta6 (receptors for fibronectin) (23) and alphaV-beta5 (receptor for vitronectin) (24, 25) as well as the aforementioned alpha3-beta1 (which binds multiple ECM proteins, including the unprocessed laminin-332) .The integrin that binds the ECM protein tenacsin, alpha9-beta1, is also upregulated in migrating epithelium (26, 27). Beta1-containing integrins are required for wound re-epithelialization, as its absence in genetically engineered animals results in severe impairment of this function (28). Surprisingly, migrating keratinocytes do not express the alphaV-beta3 integrin specific for binding fibrinogen, a major component of the provisional wound matrix (29). Complicating our understanding of the roles of individual integrins in wound repair is the recently noted phenomenon of "transdominant" inhibition, wherein activation of one integrin can negatively control the function of another (reviewed in (1)). Multiple associated proteins and regulatory cascades also modulate integrin binding in migrating keratinocytes, as do the changes in the composition of the underlying ECM.

4. KERATINS

Keratinocytes of the wounded epidermis express a different array of keratins than do those residing in intact epidermis. The basal layer of the intact epidermis is characterized by the expression of the keratin pair of K5 and K14, and suprabasilar layers by the expression of K1 and K10 (30, 31). When wounded, human skin keratinocytes express K6, K16 and K17 (31), and recent investigations have not agreed on whether the newly expressed keratins are localized only in the suprabasilar keratinocyte of the re-epithelializing wound (32), or if they are also expressed in the basal cells that migrate across the wound bed (33). The signals that initiate the synthesis of the unique "wound" or "hyperproliferative" keratins are likely cytokines and growth factors that are abundant in the wound environment, including IL-1 (34), EGF and TGFalpha (35). Although it is undisputed that K6, K16 and K17 are upregulated in response to injury, the functional significance of this switch in keratin expression is less clear. Although hypothesized to confer more advantageous viscoelastic properties on the migrating keratinocytes (36), there is, at this time, no evidence to support this claim. Recent work has, however, uncovered a mechanistic link

between K17 and one aspect of re-epithelialization: the increase in protein synthesis and cell growth seen in murine keratinocytes in the wound bed. K17 can bind to an adaptor protein member of the 14-3-3 family, which in turn activates the mTOR kinase signaling pathway to result in stimulation of cell growth (37). Since the mTOR kinase pathway is activated in many cancers (38), this non-mechanical role of K17 may have implications not only in wound healing, but in control of carcinogenesis, cell growth and tissue homeostasis.

5. GROWTH FACTORS AND CYTOKINES

Growth factors and cytokines help regulate wound healing events. They orchestrate a complex and dynamic balance of signals that induce cellular migration, proliferation, and differentiation, underscoring the complex integration of signals in the wound healing environment. Recent excellent reviews have detailed the interactions of growth factors and cytokines with the various cells within the healing wound (39-45), and this review will, therefore, only focus on those that specifically interact with keratinocytes to modulate the re-epithelialization process.

5.1. EGF family

The role of the epidermal growth factor (EGF) family of growth factors that signals through the EGF receptor (EGFR), is perhaps the best characterized. These ligands induce dimerization of their tyrosine kinase transmembrane receptor, which results in autophosphorylation of the receptor, tyrosine phosphorylation of other proteins and subsequent activation of a number of pathways that converge to promote cell migration. Numerous *in vitro* studies have demonstrated that activation of the EGFR, by the addition of exogenous ligands, increases keratinocyte proliferation (5, 46), and cell migration (47, 48) (reviewed in (5, 39, 40)). Early wound studies demonstrated a correlation in the increased expression of the EGFR with increased thickness of the epidermis of either tape-stripped wounds or burn wounds (49, 50). On the other hand, loss of expression of the EGFR was correlated with a decrease in epidermal thickness in healing wounds (49). Members of EGF family that have been shown specifically to modulate keratinocyte migration include EGF, HB-EGF, and TGF-alpha, as well as ligands for other receptors, angiotensin II (51) and the keratinocyte-released antimicrobial hCAP-18 (52), that stimulate keratinocyte migration by transactivation of the EGFR. Although there is agreement that the addition of exogenous EGFR ligands improves wound re-epithelialization and wound closure rates in a number of different types of human skin wounds (53-57), it is not clear if the improvement is clinically significant enough to warrant the addition of exogenous EGFR ligand as a therapeutic approach (58). The pro-migratory effects of EGFR ligands in keratinocytes are well-documented (59), and a recent study suggests that of all the EGRF ligands, TGF-alpha is the most potent in terms of its ability to increase keratinocyte migration (47).

In vivo wound healing studies with knock-out mice have yielded conflicting results and reveal the complexity in analyzing the wound environment wherein multiple growth factors may serve redundant functions. Few wound healing studies have been performed with EGFR deficient animal models, although abnormal epidermal proliferation was noted in EGFR null skin grafts onto nude mice (60). Two early studies with TGF-alpha deficient mice found no decrease in wound healing rates (61, 62), while a later, more detailed study found that partial-thickness murine ear wounds re-epithelialized at a lower rate and epidermal thickness was reduced in TGF-alpha deficient mice (63). Interestingly, this study found that early re-epithelialization rates were impaired but by day 8 re-epithelialization rates were not altered. Another study looking at HB-EGF deficient mice found that re-epithelialization of full-thickness punch wounds was unaltered early but was reduced at day 7 (64). Furthermore, this study found cell proliferation to be unaltered at 48 hours and later time points, in contrast to previous cell culture studies that had shown that HB-EGF increased keratinocyte proliferation (65). These contrasting results may be attributed to the differing time course of when certain EGF family growth factors are expressed, the differences in the models used, or the ability of one EGF ligand to compensate for the absence of another. Wound fluid studies have found increased levels of EGF, TGF-alpha, and HB-EGF (65-67) in the early wound and these factors may be able to compensate for each other. However, since TGF-alpha is the most potent activator of keratinocyte migration (47), it would follow that early wound re-epithelialization rates would be impaired in its absence. It is unknown if TGF-alpha is also the most potent activator of epidermal proliferation although this is suggested from the TGF-alpha deficient mice studies (63). HB-EGF has been found to be secreted by keratinocytes at the leading edge of re-epithelialization in an autocrine mechanism, (68, 69) and this may play an important role in later stages of the healing wound as is suggested by the finding in HB-EGF deficient mice. Thus, there is strong evidence to support the notion that EGFR ligands in the wound work to promote its re-epithelialization by directly activating keratinocyte migration and inducing keratinocyte proliferation. Investigations continue with clinical trials to optimize the potential us of this growth factor to enhance healing in hard-to- heal wounds (70).

5.2. FGF family

The fibroblast growth factor (FGF) family, comprised of 23 members, is mitogenic for a broad range of cell types. Cutaneous wound healing studies have focused on four members of this family: FGF-2, FGF-7, FGF-10, and FGF-22. FGF-2, also known as bFGF, is released from damaged endothelial cells and has been found to be increased in wounds (5, 71-73). Cell culture studies have shown that FGF-2 is capable of increasing keratinocyte motility (47) and re-epithelialization in human skin explants (74). Similar results have also been found in animals studies where exogenous administration of FGF-2 increased wound re-epithelialization (75, 76). Knock-out studies have further confirmed these findings, demonstrating that mice deficient in FGF-2 have impaired re-epithelialization after wounding (77, 78).

FGF-7, also known as keratinocyte growth factor 1 (KGF-1), is increased in murine (71) and human skin wounds (79), and is secreted by dermal fibroblasts and gamma-delta T cells (5, 80-83). FGF-10, also known as KGF-2, is a close homologue to FGF-7 (84) that is expressed in normal and wounded skin (85). FGF-10 is also produced by dermal fibroblasts and gamma-delta T cells (81). The receptor to FGF-7 and FGF-10 is the FGFR2IIIb splice variant of the FGFR2 receptor and is found on epithelial cells (86). Thus, in the skin, only keratinocytes express this receptor. FGF-7 and FGF-10 act on the keratinocyte to increase proliferation and migration (reviewed in (80, 82)). In addition, FGF-7 and FGF-10 increase transcription of factors involved in the detoxification of reactive oxygen species (ROS), thereby reducing ROS-induced apoptosis of keratinocytes in the wound bed. This presumably allows wound keratinocytes to survive and carry out the functions necessary for re-epithelialization. Studies using transgenic animals reveal the complex balance between these factors. Mice deficient in the FGFR2IIIb receptor have significant impairment in wound re-epithelialization (87), suggesting the importance of this signaling cascade in wound re-epithelialization. However, mice deficient in FGF-7 do not suffer from impaired wound healing (88), and this was hypothesized to be due to the compensatory effect of FGF-10. FGF-10 knock-out mice are not suitable for study since these mice do not survive long after birth due to severe developmental abnormalities (89), but mice deficient in the T-cell delta receptor had decreased keratinocyte proliferation and decreased wound closure that was restored with the addition of FGF-7 or wild-type gamma-delta T cells (81). Because the gamma-delta T cells are an important source of FGF-7 and FGF-10, the mice deficient in the T-cell delta receptor had an absence of both FGF-7 and FGF-10 in their wounds. Interestingly, FGF-10 was present in the wounded skin of FGF-7 deficient mice (81), lending support to the notion that FGF-10 can compensate for the lack of FGF-7 in animals that have this gene deleted.

FGF-22 has been recently identified as a factor with homology to both FGF-7 and FGF-10 and has thus been hypothesized to also act through the FGFR2IIIb receptor (90, 91). Unlike FGF-7 and FGF-10, FGF-22 is expressed in an autocrine fashion by hair follicle keratinocytes and by the keratinocytes of the hypertrophic epidermis of the wound bed in the late stages of wound healing (90, 91). FGF-22 has been measured in unwounded skin (90, 92) but conflicting results have been found regarding post-wound levels of FGF-22. One investigation found that FGF-22 levels were increased from day 7 to day 13 after wounding (90), but another study found FGF-22 levels to be decreased to half the pre-wound levels at 21 days after wounding (92). This discrepancy may be due to the different strains of mice used in each study. More studies will be needed to better identify whether the keratinocyte is the target of FGF-22 and what role it plays in keratinocyte and wound healing physiology.

5.3. TGF-beta family

The transforming growth factor-beta (TGF-beta) superfamily consists of TGF-beta1-3, bone morphogenic proteins (BMPs), activins, nodals, growth and differentiation factor (GDF), and Muellerian inhibiting substance (93). This review will primarily focus on the effects of the TGF-beta1-3 subfamily, the activin subfamily, and the BMP subfamily, since these factors have been found to have effects on wound keratinocytes (reviewed in (40, 45)). TGF-beta has been found in wound fluid and TGF-beta1 and TGF-beta2 are secreted by keratinocytes in a paracrine and autocrine fashion (94).

Studies into the role of TGF-beta in the wound are conflicting and further studies will be necessary to better define the effects of TGF-beta on re-epithelialization. TGF-beta has been shown to modulate keratinocyte integrin expression toward a more migratory phenotype (95, 96), suggesting a supportive role in re-epithelialization. On the other hand, TGF-beta was found to inhibit keratinocyte proliferation *in vitro* (97) and *in vivo* (98, 99), suggesting that TGF-beta would decrease the proliferative phase of the re-epithelialization process. However, other studies in animals that overexpressTGF-beta1 either through the K5 promoter (100) or as a response to overexpression of Smad2 via the K14 promoter (101) found an increase in the proliferative phenotype to the epidermis. A recent study that compared proliferation with different post-wound time points in mice overexpressing TGF-beta found that proliferation was decreased at early wound times but this trend reversed to an increase in keratinocyte proliferation at late wound healing time points (102). Exogenous TGF-beta has been shown to increase *in vitro* keratinocyte migration (96, 103) and partial thickness porcine skin explant wound re-epithelialization (104), lending support that TGF-beta increases keratinocyte motility and re-epithelialization. On the other hand, TGF-beta antagonists have been shown to increase porcine full-thickness wound re-epithelialization (105). TGF-beta overexpressing mice have decreased re-epithelialization of partial thickness burn wounds (99), and Smad 2 overexpressing mice exhibit delayed re-epithelialization of full-thickness excision wounds. Full thickness incisional wounds re-epithelialize faster in both TGF-beta null (106) and Smad 3 null mice (107). These apparently contradictory findings may be better understood in light of a recent study that compared full-thickness to partial thickness wound healing in mice overexpressing TGF-beta (102) and found that TGF-beta overexpression resulted in more rapid re-epithelialization of partial thickness wounds, yet a delay in re-epithelialization of full thickness wounds. The authors of this study suggest that in partial thickness wounds, a dermal substrate exists to allow for efficient keratinocyte migration once TGF-beta induces changes to a migratory integrin expression. However, in full thickness wounds, no such dermal substrate exists and they hypothesize that TGF-beta may be inhibiting re-epithelialization through a mechanism independent of keratinocyte migration. In the study that showed delayed re-epithelialization in Smad 2 overexpressing animals (99) with partial-thickness burn wounds, it should be noted that burn wounds undergo a period of dermal necrosis after wounding (108), making it likely that keratinocytes adjacent to thermally damaged dermis are in an environment similar to that of a full-thickness wound.

Finally, TGF-beta3, present in serum which is a component of the wound, but absent from plasma (present in unwounded skin) may also play a role by selectively stimulating keratinocyte, but not fibroblast migration (103).

Activin, another member of the TGF-beta superfamily of ligands, is expressed in wound tissue and is produced by granulation tissue, suprabasal keratinocytes at the hyperproliferative epidermal wound margin, and in keratinocytes of the re-epithelializing tongue (109). *In vitro* studies have found that activin reduces cell proliferation and induces terminal differentiation in cultured keratinocytes (110). Studies with mice that overexpress activin under the control of a K14 promoter in the epidermis demonstrate a disorganized epidermal architecture with an increase in its thickness and altered differentiation (111). These activin-mediated changes were dose-dependent (112). In contrast to the hypertrophy seen in the epidermis of activin overexpressing animals, atrophy of the epidermis was found in mice overexpressing the activin antagonist, follistatin (113). Yet, no significant difference in re-epithelialization rates was found after wounding either of these transgenic mice. Since studies in mice deficient in the TGF-beta downstream signaling molecule, Smad 3, demonstrate acceleration of re-epithelialization after wounding (107), it is likely that activin does not utilize this signaling pathway (113). *In vitro* studies show that the addition of exogenous activin inhibits keratinocyte proliferation (110), yet the aforementioned *in vivo* studies show that activin overexpression leads to increased epidermal thickening. A recent study (114) sheds more light on this discrepancy: overexpression of the Id1 gene, a downstream regulator of activin, only alters keratinocyte biology when cells are in a three-dimensional environment, and has no effect in monolayer culture. Thus, additional studies using *in vivo* wounds or three dimensional *in vitro* wounds are needed to fully clarify the role of the activin cascade in wound re-epithelialization.

The BMPs are another subfamily within the TGF-beta superfamily and many BMPs are involved in the formation of skin (reviewed in (115)). In particular, the effects of BMP-6 have been studied in keratinocytes. *In vitro* studies have shown that BMP-6 inhibits proliferation and induces differentiation of keratinocytes (116, 117). This finding is supported by *in vivo* studies that demonstrate that BMP-6 accumulates in suprabasal keratinocytes following wound re-epithelialization (118). *In vivo* studies with mice overexpressing BMP-6 in suprabasal keratinocytes have shown differential effects on keratinocytes based on the level of BMP-6 expression (119). High expression of BMP-6 inhibited keratinocyte proliferation without affecting differentiation while low, patchy expression of BMP-6 resulted in a hyperproliferative epidermis, suggesting that BMP-6 was important in controlling hyperproliferation of the epidermis. Indeed, transgenic animals that overexpress BMP-6 suffer from delayed re-epithelialization (118). Taken together, these results suggest that BMP-6 plays an important role in maturation and stratification of the epidermis after wound re-epithelialization, and early pathological expression of this protein can lead to impairment in wound healing.

5.4. Other growth factors

Less studied growth factors in keratinocyte physiology include granulocyte macrophage colony stimulating factor (GM-CSF) and insulin growth factor (IGF). GM-CSF has been shown to be increased in the epidermis of full thickness excisional wounds in mice (120). Studies utilizing transgenic mice that expressed GM-CSF in their suprabasal layers found that these animals had increased keratinocyte proliferation at the wound edge and enhanced re-epithelialization of full-thickness excision wounds (120). Since IFN-gamma and IL-6, known to be important in re-epithelialization (121), were also temporally elevated compared to controls, the authors hypothesized (120) that GM-CSF expression increases re-epithelialization through the upregulation of these cytokines in addition to its direct action on keratinocytes. IGF has also been measured in wounds (65, 122, 123), and has been shown to increase keratinocyte motility (47, 124). Studies of keratinocyte monolayer scratch wounds revealed that IGF-1 is able to increase wound closure alone and can also act in a synergistic manner with EGF (60).

5.5. Dependence on extracellular matrix

It should be mentioned that the growth factors and cytokines provide motogenic stimulation for keratinocytes that are adherent to matrix, and thus the nature of the matrix on which they are adhered modulates the motogenic response. Numerous studies have demonstrated that the composition of the underlying extracellular matrix can modify the speed of keratinocyte migration in the presence of growth factors (103, 125-131). Mechanisms by which this occurs include synergistic activation of kinase cascades that converge upon migration (132), and upregulation of specific integrins that contribute to a motile phenotype by the growth factors (95). A marked change in the composition of the matrix underlying the keratinocytes accompanies wounding. Post-wounding, keratinocytes are in contact with the provisional matrix composed of fibronectin and fibrinogen rather than the components of the basement membrane (133), and this can alter the phenotype of the overlying keratinocytes (reviewed in (134)). This underscores the complex and synergistic interaction of keratinocytes with its matrix, and wound-expressed growth factors and cytokines.

5.6. Cytokines

Cytokines are small protein hormones primarily secreted by immune cells and are important mediators of host defense, post-injury repair, cell growth and maturation (135). Inflammatory cytokines are felt to have roles in wound healing including stimulation of keratinocytes and proliferation of fibroblasts. Although these cytokines have many important actions in the actively healing wound, this review will focus on the cytokines that are able to modulate keratinocyte migration and wound re-epithelialization. IL-1, IL-6, and TNF-alpha are upregulated during the inflammatory phase of wound healing (reviewed in (40)) and are important in modulating the migratory phenotype of the keratinocyte.

IL-1 has been measured in the active wound and is produced by monocytes, macrophages, and also by keratinocytes (136-138). Keratinocytes contain both the unprocessed forms of both alpha and beta forms of IL-1 in the cytoplasm, and these are further processed and released after injury (reviewed in (139)). Besides serving as a paracrine signal to surrounding cells of the wound, IL-1 also acts as an autocrine signal that increases keratinocyte migration and proliferation (139-141). IL-1 induces the expression of K6 and K16 in keratinocytes migrating into the wound (34, 139). Indeed, exogenously administered IL-1 had been previously found to increase healing in porcine partial thickness wounds (136). It was later shown that IL-1 released by keratinocytes activates nearby fibroblasts and increases the secretion of FGF-7 (142-146), which was noted to increase keratinocyte survival, motility, and proliferation (as discussed earlier in this review). In fact, keratinocyte released IL-1 has recently been shown to increase transcription of genes in dermal fibroblasts whose protein products have important roles in augmenting keratinocyte re-epithelialization in the wound (147).

TNF-alpha mRNA and protein were detected in murine (148, 149) and human skin wounds (150). Although this proinflammatory cytokine is produced by many cell types in the healing wound, IL-1 also induces keratinocyte production of TNF-alpha (151-153). Similar to IL-1, TNF-alpha can induce the production of FGF-7 (146), suggesting that TNF-alpha can promote keratinocyte wound re-epithelialization. However, mice deficient in the TNF-alpha receptor, p55, were paradoxically found to have increased wound re-epithelialization (154) and keratinocytes are known to normally express this receptor (155). This suggests that TNF-alpha may indirectly augment re-epithelialization though the expression of FGF-7 while directly inhibiting re-epithelialization through the keratinocyte p55 receptor. The direct effects seem to play a more dominant role based on the p55 knock-out mice wound healing studies (154), but the mechanisms involved are still unknown.

Like IL-1 and TNF-alpha, IL-6 is also increased locally after wounding (138, 150, 156), and interestingly IL-6 was found to persist in older wounds. IL-6 has been shown to both increase keratinocyte proliferation (157, 158) and migration (159), and indeed, IL-6 deficient mice have a significant delay in wound re-epithelialization (121). Whether IL-6 affects keratinocytes through a direct or indirect mechanism is still under study and there is evidence that possibly both occur. IL-6 seems to increase keratinocyte migration through an indirect stimulatory pathway involving the release of factors from dermal fibroblasts that have yet to be identified (159). IL-6 is hypothesized to act through a STAT3-dependent pathway since phosphorylation of STAT3 increased in dermal fibroblasts treated with exogenous IL-6 (159). Targeted deletion of keratinocyte STAT3 results in an animal with impaired wound re-epithelialization, and keratinocytes derived from these animals fail to respond to recognized motogenic factors such as EGF, TGF-alpha, or IL-6 with a migratory response (160). Interestingly, IL-22 is another activator of STAT3 that has also been shown to promote keratinocyte migration (161, 162). Therefore, it is still unclear how important of a role IL-6 plays in the direct regulation of keratinocyte migration in comparison to other cytokines and growth factors readily available in the wounded environment. Taken together, it seems reasonable to propose that IL-6 plays an important role in regulating keratinocyte motility and wound re-epithelialization and seems to act through both indirect and direct signaling pathways.

6. CHEMOKINES

Chemokines are family of cytokines that stimulate chemotaxis in a number of different cell types, and play an important role in lymphocyte trafficking and in recruiting inflammatory cells into cutaneous wounds. In this review, however, we focus on the chemokines that have been shown to alter keratinocyte migration and/or wound re-epithelialization.

There are four subfamilies in this family, based on their conserved cysteine residues: CXC, CC, CX3C and C, (where X represents any amino acid residue) and of these, the CXC, CC and C families appear to contribute to the keratinocyte's role in wound repair. These ligands bind to G protein coupled receptors expressed on resident target cell membranes. Specific receptors for these two members of the family, the CXCR (previously called the receptor for IL-8) and CCR (and their subtypes) have been identified in keratinocytes (163-166) and account for the functional migratory responses of these cells to locally generated chemokines.

Macrophage chemoattractant protein (MCP-1 or CCL2) is a CC family chemokine, which is a strong chemoattractant for macrophages and monocytes (reviewed in (40)). In both human and murine wounds, upregulation of either MCP-1 mRNA or the protein has been observed (167) (168, 169). The temporal pattern of MCP-1 expression suggests its role is to recruit macrophages to the wound, although in transgenic animals in which this gene has been knocked out demonstrate wound macrophage numbers equivalent to wild type animal (170). Nevertheless, the MCP-1 deficient animals display delayed wound re-epithelialization, suggesting that this chemokine is involved in regulating keratinocyte migration.

The GRO-alpha /CXCL1 chemokine, a recognized regulator of neutrophil chemotaxis, (reviewed in(40)) is upregulated in the epidermis of regenerating human burn wounds (166), and its murine homlog, MIP-2, is upregulated in keratinocytes adjacent to incisional wounds (171). GRO-alpha binds to the CXCR2 receptor on keratinocytes, and this receptor is upregulated in human burn wound epidermis in the early postburn phase (166). Other evidence to support a role for this chemokine in re-epithelialization comes from studies using CXCR2 knock-out animals, in which re-epithelialization of an excisional wound is delayed (163). In addition to the co-associated defect in neutrophil recruitment to the wound, loss of the CXCR2 receptor on the keratinocyte imparts a direct effect on its ability to migrate into the wound. *In vitro* studies,

with keratinocytes isolated from these animals, demonstrate that their migration and ability to heal an *in vitro* wound is also impaired (163).

C10, a CC family chemokine also called macrophage inflammatory protein-related prtoein1 (MRP-1) or CCL6 (172), is also implicated in wound re-epithelialization. Both C10 protein and mRNA are increased in the epidermis adjacent to healing excisional murine wounds. However, functional studies for this chemokine to support its role in modulating keratinocyte migration are not yet available.

Two chemokines of the CXC family, in which a Glu-Leu-Arg sequence critical for neutrophil activation is missing, hence named the ELR-negative CXC chemokines, that bind to a common CXCR3 receptor (173) have been recently found to specifically modulate keratinocyte migration. One member of this group, interferon gamma-inducible protein (IP-10, or CXCL10) is highly expressed in inflammatory dermatoses, including psoriasis (reviewed in (40)). It is actively synthesized and secreted by human keratinocytes (174, 175), and is present in the wound site (167). It appears however to have a negative impact on wound healing. In transgenic animals that constitutively over-express 1P-10 in their keratinocytes, wound healing is delayed (176). Part of the delay may derive from its effects on dermal fibroblasts, where it decreases their migratory rate by inhibiting the release of the rear of the cell from the substratum, though a protein kinase A- calpain inhibitory cascade (177). Evidence for specific migratory effects on keratinocytes is lacking. A second member of this ELR-negative CXC chemokine family, interferon-inducible protein 9 (IP-9 or CXCL11) is also expressed in keratinocytes (178) and displays similar inhibition of fibroblast migration (173). Interestingly, however, it has the opposite effect on keratinocytes, since it increases their motility (179). This is accomplished by the different signaling pathway the keratinocyte uses to transduce the IP-10 signal, through phospholipase C-beta / Ca+2 resulting in activation of a distinct calpain isoform (179). Although wounding monolayers of cultured keratinocytes increases their expression of IP-10 (173), to date, there is no evidence that the synthesis of this chemokine is modulated in wounded skin in vivo.

Levels of the chemokine interleukin 8 (IL-8, or CXCL8) are increased in the dermis of acute surgical human wounds as well (167). Since in vitro studies show that IL-8 can stimulate both human keratinocyte migration (180) and proliferation (181), it is proposed to play a positive role in wound re-epithelialization (167, 182). Yet, paradoxically, high levels of this chemokine accumulate in non-healing human burn wounds, although the source of the generated chemokine is not known (183). Further, addition of IL-8 to cultured keratinocytes at the high level found in burn wounds decreases, rather than increases, their proliferation (183). Thus, not only is wound healing regulated by the temporo-spatial expression of multiple chemokines, but also the balance of production of the individual players.

The study of the expression of other chemokines in keratinocytes is an area that is rapidly evolving, and chemokines other than the ones discussed above have been noted to be expressed in keratinocytes (e.g. CX3CL1 (184)), as have other chemokine receptors (e.g CCR4 (164)). However, no evidence connecting them functionally to keratinocyte re-epithelialization of wound has yet been presented. These, and other chemokines and their receptors may yet prove to be important in this process, and further investigations will likely supply answers regarding their roles.

7. EICOSANOIDS

Eicosanoids are biologically active lipid mediators and second messengers, derived from the precursor 20 carbon polyunsaturated arachidonic acid (AA) (*eicosa* = Greek word for 20). AA is normally esterified within cell membrane phospholipids, where it can be released by a number of stimuli that activate the appropriate phospholipase. Once released, AA is metabolized by one of three distinct enzyme systems: the cyclooxygenase (COX) pathway to generate prostaglandin derivatives, the lipoxygenase (LOX), to generate HETEs and leukotrienes, or the cytochrome P450 dependent epoxidases that can generate either HETEs or EETs. The repertoire of metabolites generated is highly dependent on cell type and the initiating stimulus. Thus a keratinocyte can be highly plastic in its ability to modulate its response via metabolism through one of the three pathways.

Human keratinocytes possess active COX and LOX pathways and synthesize a number of eicosanoid products (reviewed in (185)). Two isoforms of cyclooxygenase, COX-1 and COX-2 are expressed in human keratinocytes, although it is the COX-2 isozymes that is constitutively expressed in the in the more differentiated layers of the epidermis (186). The major COX product expressed in the epidermis is PGE2, and the major LOX product is 15-HETE. Notably absent from normal epidermis or from cultured keratinocytes are the leukotrienes (187, 188). While keratinocytes do not appear to be able to synthesize LTs de novo, they do have an active LTA4 hydrolase, and can convert preformed LTA4, either exogenously added, or provided by leukocytes (via trans-cellular metabolism) into LTB4 (188-191). Thus, in the absence of leukocytes, the epidermis does not synthesize LTs. Since wound re- epithelialization can and does proceed in the experimental absence of PMNs (192), this suggests that leukotrienes are not required for normal wound re-epithelialization.

The lipoxygenases are a structurally related family of non-heme iron dioxygenases that function in the production of fatty acid hydroperoxides. Their substrates are polyunsaturated fatty acids containing a series of cis double bonds, i.e. essential fatty acids in humans. The nomenclature for the family is based on the specificity of each enzyme's action on its substrate. So, for example, 12-LOX oxygenates AA at carbon-12, generating 12-HETE, 5-LOX at the carbon-5 position, and so on. The immediate, unstable oxygenated intermediate is the corresponding

hydroperoxy-eicosatetraenoic acid, HPETE, which is rapidly transformed to the corresponding HETE. The products of 5-LOX, include 5-HETE and LTA4 which may be further metabolized to generate leukotriene mediators of inflammation and allergy. Additionally, LOXs may be identified by their stereo-specificity, for example 12*R*-LOX or 12*S*-LOX have both been described in keratinocytes (193). There is an additional level of nomenclature, where the enzymes are named after the tissue in which they where they were first observed: e.g. platelet, leukocyte, and epidermal 12-LOXs (194).

Despite the widespread use of non-steroidal anti-inflammatory drugs that inhibit eicosanoid generation, their effect on cutaneous wound healing has only recently been investigated. These drugs exert their anti-inflammatory effects by inhibiting cyclooxygenase (COX) enzymes, and subsequent prostaglandin synthesis. One might reasonably expect COX metabolites to regulate wound repair. In fibroblasts, LOX metabolism is required for the first stage of cell adhesion, and the COX pathway is required for the subsequent formation of actin bundles that promote cell motility (195-197). Thus, the expectation is that inhibition of the COX pathway would result in decreased cell motility, and therefore delayed wound repair. Indeed, the ability of COX-1 and COX-2 inhibitors to negatively impact upon gastric ulcer healing is well documented. Yet, remarkably, COX products do not appear to play a role in cutaneous wound repair. Both incisional and excisional wounds, in either rats or mice, appear to heal normally despite pharmacologic inhibition of either COX-1 or COX-2 with appropriate systemically delivered inhibitors (198-200). Although wounding skin results in a transient upregulation of COX-2, particularly in the epidermal wound margin (201), animals in whom either COX-1 or -2 has been knocked-out demonstrate no difference in the rate of wound re-epithelialization (202). A decrease in wound tensile strength has been reported in these animals suggesting that COX enzymes may contribute to the later remodeling phase, rather than the re-epithelialization phase of wound healing.

Even less is known about the roles of LOX enzymes with respect to wound healing. In normal human skin and keratinocytes, the major HETEs identified are products of 12 and 15-LOX enzymes (203-205)(reviewed in(185)). However, at least nine lipoxygenases have been identified and cloned in human or murine skin, including three isoforms of 12*S* LOX, 12*R* LOX, two 15-LOXs, 5 LOX, epidermis-type LOX-3, and 8-LOX (identified in murine skin only)(193, 206-208). HETEs in the epidermis may also be generated by cytochrome P450 isoforms, including a novel cytochrome P450 enzyme, CYP2B19, that generates 11*S*-, 12*R*- and 15*R*-HETEs in murine skin (209). LOX products have long been implicated in skin physiology, starting with the observation of the abnormal permeability in the skin of patients whose diets are deficient in essential fatty acid eicosanoid precursors (210). However, a physiologic role has only been identified for two of the possible HETE products of the epidermis. The previously presumed role of 12-HETE in maintaining the percutaneous barrier was convincingly demonstrated in mice in whom the 12-LOX gene has been inactivated. These animals demonstrate increased transepidermal water loss (211). 12-HETE has also been implicated in inflammatory processes in the skin, most notably in psoriasis where expression of the 12-LOX enzyme and its HETE product are increased in the hyperproliferative epidermis (204). Very recently, mutations in the genes for LOX-3 and 12*R*-LOX have been reported in kindreds with the autosomal recessive, non-bullous congenital ichthyosiform erythroderma (208), consistent with the proposed role for LOX in maintaining epidermal homeostasis. Only two studies, both by Ruzicka (212, 213), however, have hinted at a role for 12-HETE in wound healing. High affinity binding of 12-HETE on cultured keratinocytes was described, along with keratinocyte chemotaxis to this eicosanoid, implicating 12-HETE in wound healing. Curiously, no other studies have followed up on this original observation.

8. OXYGEN

Anoxia is a contributor to non-healing wounds, and increasing delivery of oxygen to tissue is one clinical approach to enhancing wound repair. The changes in oxygen tension induced by hyperbaric oxygen may, by rapidly increasing and then decreasing oxygen concentrations in the tissue, initiate as yet unclear signaling cascades that culminate in proliferation and increased collagen production (214, 215). Although increases in oxygen tension indeed to improve many aspects of the dermal component of wound healing, the effects on keratinocyte and re-epithelialization, the subject of this review, are quite different.

Interestingly, human keratinocyte proliferation and clonogenicity in culture are enhanced by anoxic (<1% oxygen tension) as compared to normoxic conditions (21% oxygen tension) (216). Higher oxygen tensions, consistent with hyperbaric oxygen clinical treatments, decrease proliferative capacity further (217). Oxidative stress induced by the increased concentrations may play a role in decreasing keratinocyte proliferation, since this is diminished in the presence of antioxidants (218). Hypoxemic conditions (2%) can also suppress the genetic differentiation program in cultured keratinocytes (219), and thus sustain the cellular phenotype that is more able to participate in wound repair. Hypoxia also appears to be beneficial to the re-epithelialization process by increasing keratinocyte motility. A number of studies have demonstrated that keratinocyte migration *in vitro* is enhanced under hypoxic conditions (216, 220, 221). The mechanism for the increase in motility seem to be related to an increase in lamellipodial extrusion, with an associated redistribution of lamellipodia-associated proteins (erkin, radexin, moesin) (220) as well as an increased expression of matrix metalloproteinases (221). Hypoxemic culture conditions also upregulate the alphaVbeta6 integrin (222) associated with a more motile keratinocyte phenotype(23). How, or if, these *in vitro* findings translate to *in vivo* wound re-epithelialization is not yet clear and awaits further investigation.

9. ANTIMICROBIAL PEPTIDES

Keratinocytes play an important role in the innate immune response (223) and produce antimicrobial peptides that are important for both homeostatic and wound healing purposes. Human keratinocytes are known to produce four such peptides: human beta-defensin 1 (hBD-1), hBD-2, hBD-3, and hCAP-18 (and its biologically active proteolytic product LL-37) (224). hCAP-18 is a member of the cathelicidin family of antimicrobials (224). The release of these factors by keratinocytes can be induced by either the presence of microbial components or by wounding, in the absence of bacteria, through a mechanism that involves the release of HB-EGF and the subsequent transactivation of the EGF receptor (225, 226). Several different mechanisms may be involved in the release of keratinocyte hBDs: 1) activation of the EGF receptor that leads to an increase in hBD-3, 2) IL-1 activation of Toll-like receptors (TLRs) leading to the release of hBD-2, 3) increased release of hBD-1 when exposed to microbial components through an undetermined mechanism (reviewed in (225)). The cathelicidin hCAP-18 is normally processed and stored in the lamellar bodies of the keratinocytes and may be released as a result of injury or exposure to microbial components (227). After secretion, hCAMP-18 is processed into LL-37 and various other peptides which are important in killing the skin pathogens *S. aureus* and *C. albicans* (228). Interestingly, however, it has also been reported that hCAP-18 increases keratinocyte motility and therefore increases the wound re-epithelialization rate by HB-EGF mediated transactivation of the EGF receptor (52). This suggests that the antimicrobials can also play a role in increasing wound closure to help seal off the wound from microbes.

Upregulated expression of various antimicrobial peptides has been observed after wounding. Beta-defensin-2 is expressed at very low levels in normal skin, but expression is increased in the epidermis of both surgically wounded skin and chronic wounds (venous ulcers) (229). LL-37 is also upregulated in the epidermis of surgically wounded human skin within 24 hours of wounding (230), reaching highest levels at 48 hours post-wounding, and returning to basal levels when the wound is re-epithelialized (231). LL-37 immunoreactivity is also localized to the advancing edge of the epithelial tongue in acute wounds. Interestingly, although expression of the antimicrobial peptide beta-defensin-2 is increased in the epidermis surrounding chronic venous stasis wounds, LL-37 is notably absent in this area (231). It is likely that additional investigations will uncover specific roles for the individual antimicrobial peptides in the process of wound re-epithelialization. Evidence available at this point suggests that they are active participants in this process.

10. MATRIX METALLOPROTEINASES

Important in the re-epithelialization process is the ability of keratinocytes to be able to detach from the underlying basal lamina and migrate through the fibrin and ECM meshwork of the wound. Matrix metalloproteinases play a crucial role in this process and are secreted by various cells of the wound, and there are many subtypes that have identified (reviewed in (232)). After cutaneous injury, MMP expression is temporally and spatially regulated in the wound and has been the subject of other reviews (233-238). This review will focus on MMPs that help initiate and maintain keratinocyte migration necessary for wound re-epithelialization. MMPs that have been implicated in the modulation of keratinocyte motility include MMP-1, MMP-2, MMP-3, MMP-10, MMP-14, MMP-19, MMP-28 (232, 239-245).

MMPs levels increase in the early stages of wound healing (246, 247), and several signals in the wound environment are responsible for this. In unwounded skin, keratinocytes directly contact the basal lamina and do not contact the underlying type 1 collagen of the dermis. Once the skin is wounded, keratinocytes are exposed to and bind type 1 collagen and this was found to stimulate an EGF receptor-dependent cascade leading to keratinocyte expression of MMP-1, associated with increased motility (235, 242, 247, 248). Interestingly, reformation of the basement membrane and keratinocyte contact with laminin-111 (also known as laminin-1), but not laminin-332, serves as a signal for the down-regulation of MMP-1 (247). Cytokines and growth factors normally found in the wound also stimulate MMP production (59, 249-253). In fact, exogenous addition of TGF-beta antisense oligonucleotides was shown to reduce MMP production in keratinocytes (253). Other molecules, such as activated protein C, have been shown to be inducers of MMP-2 and modulate keratinocyte migration (244).

The importance of MMPs in the wound is underscored by experiments that have shown that exogenous treatment with MMP inhibitors delay wound healing and re-epithelialization (241, 254). On the other hand, overexpression of the MMPs has been shown to be detrimental to the wound healing process and have been shown to be elevated in chronic wounds (237, 255). For example transgenic animals overexpressing MMP-1 in their epidermal layers had impaired wound re-epithelialization, (256). Balancing the actions of the MMPs are the tissue inhibitors of metalloproteinases (TIMPs) (234, 238, 257, 258), which inhibit the effects of MMPs. However, transgenic mice overexpressing TIMP-1 have impaired wound re-epithelialization (245). Therefore, a balance must be maintained between MMPs and TIMPs. In fact, chronic wounds have been shown to express elevated levels of MMPs and decreased levels of TIMPs (reviewed in (232)). Taken together, successful re-epithelialization hinges on the proper balance of MMP and TIMP expression and their levels are under tight regulation for optimal keratinocyte motility.

11. OTHER MEDIATORS

Novel potential mediators of wound re-epithelialization have recently been reported. Here a few of these are reviewed.

11.1. Acetylcholine and nicotinic or muscarinic receptors

Keratinocytes can synthesize acetylcholine and its receptors, and thereby generate an autocrine system, which has recently been shown to regulate cell motility (reviewed in (259, 260)). This chemotactic pathway in keratinocytes is modulated by the alpha3 and alpha7 subtypes of nicotinic acetylcholine receptors, and transduced by a calcium-dependent pathway (261). Acetylcholine generated by keratinocytes, or neuronally released during the wound repair process (262) can also activate muscarinic receptors in keratinocytes, and depending on which subset are activated, either inhibition of migration (M3 subset) or activation of migration (M4 subset) has been observed in cultured human keratinocytes (263). Animals in whom the M4 receptor subtype has been genetically deleted also exhibit delayed wound epithelialization (263). These pro-migratory responses are mediated in part by activation of the Ras/Raf-1/MEK1/ERK pathway, and in part by modulation of the repertoire of integrins expressed in keratinocytes (259). Since the nicotinic and muscarinic receptor subtype expression on keratinocytes can be modulated by multiple culture conditions *in vitro*, it is likely that individual variations exist *in vivo* and factors including age and environmental conditions may confound the analysis of the contribution of nicotinic and muscarinic agents to wound healing.

11.2. Electric Fields

The unwounded epidermis displays a transepidermal potential voltage difference ranging from 10-60 mV, generated by the basolateral pumping of sodium ions in the epidermis (264). When wounded, this potential is short-circuited and the epidermis generates a lateral electric field with magnitudes measured in the range of 50-100 mV/mm (reviewed in (265, 266), and electric fields in this range can guide the directional migration of cultured keratinocytes *in vitro* (267). Thus, it has been proposed that endogenous wound –generated electric fields modulate keratinocyte re-epithelialization by guiding the cells' migratory paths. Multiple clinical studies have reported that application of electric fields to non-healing wounds hastens their repair, but these studies have suffered from technical shortcomings that limit their interpretation (266). Nevertheless, the signaling pathways activated by the exposure of keratinocytes and other epithelial cells to electric fields have been studied in detail, and the EGF receptor kinase (268), alpha6-beta4 integrin/ rac-1(21), and phosphatidylinositol-3-OH kinase-gamma (PI(3)K) gamma/ phosphatase and tensin homolog (PTEN) (269) signaling cascades have been identified as mediators of the cell polarization and directional migratory response to electric fields. Although direct evidence of electric field regulation of epithelial cell migration in the cornea has been documented (269), no such direct evidence in the skin is available yet.

11.3. Catecholamines and beta-adrenergic receptors

The epidermis has the capacity to generate catecholamine mediators, including epinephrine (reviewed in (259)) and these catecholamines can activate adrenergic receptors present on keratinocytes to modulate their migratory behavior. Beta2 adrenergic receptor agonists decrease keratinocyte migratory speed *in vitro* (270), and prevent wound re-epithelialization of skin both ex vivo and *in vivo* (271). On the other hand, beta2 adrenergic receptor antagonists increase keratinocyte migratory speed, and the ability of a human skin wound to re-epithelialize (272). The beta2 adrenergic receptor is a G protein coupled receptor, and both the classical cAMP- mediated as well as cAMP-independent signaling pathways mediate the migratory responses to receptor activation in keratinocytes (273, 274). Like the acetylcholine autocrine hormonal regulatory system of the epidermis, the catecholamine/ beta2 adrenergic receptor autocrine hormonal system likely also contributes to epidermal homeostasis and wound re-epithelialization.

12. CONCLUSION AND PERSPECTIVE

Multiple regulators impact on the keratinocytes' ability to migrate from the wound edge or other epidermal reservoirs to efficiently re-epithelialize a breach in the integrity of the epidermis. These signals must coordinate in a precise spatio-temporal manner to accomplish this task. The complexity of the interactions continues to be unraveled and in some ways we are just "beginning to scratch the surface" of this complicated and interactive cascade of events. Undoubtedly, new discoveries will continue to uncover the elegant network of events that result in restoration of epidermal integrity and complete the wound repair process.

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Abbreviations: BMP: bone morphogenic protein, COX: cyclooxygenase EGF: epidermal growth factor, FGF: fibroblast growth factor, GM-CSF: granulocyte macrophage colony stimulating factor, HB-EGF: heparin binding epidermal growth factor, hBD: human beta defensin, hCAP: human cathelicidin antimicrobial protein, IL: interleukin, KGF: keratinocyte growth factor, LT: leukotriene, LOX: lipoxygenase, MCP: macrophage chemosttractant protein, MMP: matrix metalloproteinase, mTOR: mammalian target of rapamycin, PKC: protein kinase C, ROS: reactive oxygen species, TGF: transforming growth factor, TNF: tumor necrosis factor

Key Words: Keratinocyte, Migration, Motility, Epithelialization, Re-Epithelialization, Wound, Healing, Desmosome, Hemidesmosome, Integrin, Keratin, Epidermal Growth Factor, Fibroblast Growth Factor, Cytokine, Chemokine, Eicosanoid, Oxygen, Antimicrobial Peptide, And Matrix Metalloproteinase, Acetylcholine, Nicotinic Receptor, Muscarinic Receptor, Catecholamine, Beta Adrenergic Receptor, Catecholamines, Review

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