# Integrin Regulation of Epidermal Functions in Wounds

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**Significance:** Integrins are bidirectional signaling receptors for extracellular matrix that regulate both inside-out signaling that controls keratinocyte-mediated changes to the wound microenvironment and outside-in signaling that controls keratinocyte responses to microenvironmental changes. As such, integrins represent attractive therapeutic targets for treatment of chronic wounds or general promotion of wound healing. Advances in wound management are particularly important as the elderly and diabetic populations within the United States continue to grow.

**Recent Advances:** Although integrins are best known for mediating cell adhesion and migration, integrins in wound epidermis also control cell survival, proliferation, matrix remodeling, and paracrine crosstalk to other cellular compartments of the wound. Importantly, the concept of targeting integrins in the clinic has been established for treatment of certain cancers and other diseases, laying the groundwork for similar exploitation of integrins as targets to treat chronic wounds.

**Critical Issues:** Despite their attractiveness as therapeutic targets, integrins have complex roles in wound healing that are impacted by both their own expression and a highly dynamic wound microenvironment that determines ligand availability. Therefore, identifying relevant integrin ligands in the wound and understanding both distinct and overlapping functions that different integrins play in the epidermis will be critical to determine their precise roles in wound healing.

**Future Directions:** Future research should focus on gaining a thorough understanding of the highly coordinated functions of different integrins in wound epidermis, and on determining which of these functions go awry in pathological wounds. This focus should facilitate development of integrin-targeting therapeutics for treating chronic wounds.

## SCOPE AND SIGNIFICANCE

Integrins are bidirectional signaling receptors that serve as an interface between extracellular matrix and the intracellular milieu. While it is well known that integrins are the major receptors for keratinocyte adhesion to the basement membrane that underlies the epidermis in skin, roles for epidermal integrins in wound healing expand far beyond adhesion. This review will address autonomous keratinocyte functions that are regulated by integrins during wound healing, including survival and proliferation. We will also discuss roles for epidermal integrins in modulating the wound microenvironment through local matrix remodeling, as well as paracrine crosstalk to other cells within the wound.



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## TRANSLATIONAL RELEVANCE

Chronic wounds are characterized by defects in skin, including impaired re-epithelialization. In their roles as signaling receptors that regulate keratinocyte functions including proliferation and migration, integrins are attractive targets for more efficacious drug therapies to treat chronic wounds. However, exploiting integrins as therapeutic targets will require a better understanding of how they coordinately regulate epidermal functions during wound healing and how these functions are altered in pathological wounds. Recent studies shed light on the complexity of integrin signaling and extracellular matrix ligation and on the various and far-reaching roles that epidermal integrins play in wound healing.

## CLINICAL RELEVANCE

The ever-growing elderly and diabetic populations in the United States create a demand for more effective wound healing therapies. For these groups in particular, chronic wounds are a major impediment to overall patient quality of life. Moreover, chronic wounds are a major clinical problem that translates to a substantial financial burden to the United States as healthcare costs associated with wound management continue to rise. The development of novel therapies to restore normal epidermal functions to chronic wounds, including those that target integrins expressed on keratinocytes, is crucial to enhance patient quality of life and reduce healthcare costs.

## INTRODUCTION

Basal keratinocytes in the stratified epidermis of skin are adherent to a specialized form of extracellular matrix (ECM) known as the basement membrane (BM), which physically separates the epidermis from the underlying connective tissue of the dermis. Regeneration of the BM is essential during wound re-epithelialization to restore tissue compartmentalization and provide structural support to the neo-epidermis. In addition, newly deposited BM proteins, together with ECM and matricellular proteins that appear in the wound bed, provide signals to the regenerating epidermis that are essential for normal wound healing. Importantly, altered mechanical properties or composition of the ECM are well known to contribute to the pathogenesis of chronic wounds.[1] Integrins are the major cell surface receptors for adhesion to the ECM, and they can mediate both inside-out and outside-in signal transduction pathways that control a wide variety of cell functions that contribute to both normal and pathological tissue remodeling processes, including proliferation, survival, ECM remodeling, migration, and gene expression.[2–4] In this review, we will provide an overview of what is currently known about the regulatory roles that epidermal integrins play in cutaneous wound healing. We will briefly cover what might be considered as "classical" roles that integrins play in the regulation of cell adhesion, migration, survival, and proliferation. In addition, we will discuss recently discovered roles for certain integrins in controlling the ability of the epidermis to modulate the wound microenvironment, through either effects on ECM or intercellular crosstalk to other cellular compartments. As space limitations preclude an exhaustive discussion of the numerous publications in this field, we direct the reader to several excellent reviews for further coverage of relevant topics.[5–8]

## DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

### Integrins: regulators of cell adhesion and signal transduction

All members of the integrin family are heterodimeric, transmembrane glycoproteins that consist of an $\alpha$ and a $\beta$ subunit, each with a large extracellular domain, a single-pass transmembrane domain, and a cytoplasmic domain. Most $\alpha$ and $\beta$ subunit cytoplasmic domains are relatively short ($\sim$20–70 amino acids), with the exception of the $\beta_4$ cytoplasmic domain (over 1,000 amino acids).[3] Eighteen $\alpha$ subunits and eight $\beta$ subunits can dimerize in different combinations to form 24 different integrins with distinct, although often overlapping ligand-binding specificities. As a group, integrins can interact with a wide variety of ECM proteins, to which they bind via their extracellular domains.[3] Simultaneously, integrins interact via their cytoplasmic domains with cytoskeletal proteins to mediate a transmembrane linkage of the ECM to the cytoskeleton, which is critical for controlling cell shape, polarization, and motility.[3,4,9–11]

Although integrins lack intrinsic enzymatic activity, they can interact directly or indirectly with a wide variety of signaling effectors, thereby functioning as conduits of bidirectional signal transduction across the cell membrane.[3,9,12,13] Cytoplasmic interactions can regulate the activation state of an integrin, thereby modulating its affinity for extracellular ligands during "inside-out" signaling.[3,14] In addition, integrins can

regulate numerous intracellular pathways in response to ECM binding or other extracellular cues during "outside-in" signaling.[12] Indeed, as integrins are clustered at sites of cell adhesion, their cytoplasmic domains function as docking sites to recruit signaling and adaptor proteins that regulate a wide range of downstream pathways. In addition, some integrins undergo lateral interactions with other cell surface proteins, such as growth factor receptors, tetraspanins, urokinase receptor (uPAR), syndecan, or caveolin, which may occur at sites of cell-ECM adhesion or from within specialized membrane microdomains to modulate integrin signaling or adhesive functions.[2,15–23] Thus, signaling functions of an individual integrin may be regulated through subcellular localization that controls its association with distinct binding partners, or vice-versa.

The full range of signal transduction pathways that can be regulated by integrins is too extensive to discuss here and has been reviewed extensively elsewhere.[2–4,12] However, the adhesion-dependent activation of focal adhesion kinase (FAK) serves as an instructive example of outside-in integrin signaling.[24,25] Following integrin-mediated cell adhesion, FAK is auto-phosphorylated on Y397, creating a high-affinity binding site for the SRC-homology 2 (SH2) domain of SRC (or another SRC-family kinase). Once bound, SRC phosphorylates additional FAK tyrosines to create docking sites for other kinases or adaptors, such as GRB2, phosphatidylinositol 3-kinase (PI3-K), and p130CAS. In this way, the FAK/SRC complex can link integrins to downstream signaling effectors that include mitogen-activated protein kinases (*e.g.*, ERK, JNK, and p38), certain Rho family guanosine triphophatases (GTPases; *e.g.*, CDC42, Rho, RAC1), and the serine/threonine kinase AKT.[2,24,25]

FAK activation often occurs as a result of cooperative signaling from integrins and growth factors and has been linked to several cell functions with relevance to wound healing, including formation of polarized lamellipodia, migration, proliferation, survival, and expression or activity of ECM-degrading proteases.[26–31] These functional linkages suggest that some epidermal wound functions might be controlled by the temporal appearance during wound healing of ligands for integrins that are known to activate FAK (for example, $\alpha_3\beta_1$ and $\alpha_5\beta_1$). Interestingly, FAK, RAC1, and integrin $\alpha_3\beta_1$ have each been be linked to the maintenance of the epidermal stem cell compartment that resides in the hair follicle bulge,[30,32,33] and $\alpha_3\beta_1$ binding to laminin-332 can activate FAK/SRC-to-RAC1 signaling in cultured keratinocytes,[26,34] suggesting that this signaling axis might control expansion of stem cells that contribute to skin tumorigenesis or wound re-epithelialization. However, keratinocyte-specific knockout studies revealed that while $\alpha_3\beta_1$ and FAK each contribute to development of squamous cell carcinoma (SCC),[29,33] neither is essential for wound re-epithelialization,[29,30,35,36] indicating that elements of the wound microenvironment that are absent from cell culture may compensate for FAK signaling deficiencies *in vivo*. Indeed, it should be noted that many other integrin-linked signaling molecules, such as integrin-linked kinase (ILK) and phospholipase C (PLC), have also been shown to play important roles in the epidermis, as reviewed elsewhere.[28]

## Epidermal integrins

The stratified epidermis of the skin is a continually regenerating tissue wherein the loss of dead keratinocytes from the outermost layer is balanced by keratinocyte proliferation in the basal layer.[37–39] In normal resting epidermis, integrin expression is restricted to the basal keratinocytes that are attached to the BM, and it is down-regulated as differentiating keratinocytes detach from the BM and are displaced upwards into the suprabasal layers.[37] In the epidermis of a healing wound, keratinocytes acquire new adhesion/migration properties as a result of either changes in integrin expression or altered adhesion/signaling functions of integrins that were already expressed prior to wounding. Constitutively expressed integrins that persist or are upregulated in wound epidermis include $\alpha_3\beta_1$ and $\alpha_6\beta_4$ (both laminin-332 receptors), $\alpha_2\beta_1$ (a collagen receptor), $\alpha_9\beta_1$ (a receptor for cellular fibronectin, tenascin, and other ligands), and $\alpha_v\beta_5$ (a vitronectin receptor).[37,40] Integrins that are expressed *de novo* in healing wounds include $\alpha_5\beta_1$ (a fibronectin receptor) and $\alpha_v\beta_6$ (a fibronectin and tenascin receptor). These epidermal integrins can bind numerous ligands present in the provisional wound ECM, including fibronectin ($\alpha_5\beta_1$, $\alpha_9\beta_1$, $\alpha_v\beta_6$), vitronectin ($\alpha_v\beta_5$), and tenascin ($\alpha_9\beta_1$, $\alpha_v\beta_6$), as well as laminin-332 ($\alpha_3\beta_1$, $\alpha_6\beta_4$) that is deposited by migrating keratinocytes.[8,37,40,41]

Numerous studies in both human and rodent models have documented the various expression patterns and functions of individual integrins in the epidermis during skin development, in adult skin, and during wound repair.[8,37] Of course, there is a need for caution when extrapolating results obtained in murine models to human wound healing, as there are species-specific differences that include hair follicle density, skin thickness, and relative importance of wound contraction.

Nevertheless, many aspects of human wound healing are recapitulated in murine models.[42] Moreover, many integrin functions have been evolutionarily conserved in mice and humans, and genetic studies in global or epidermis-specific knockout mice have been instructive in determining the relative importance of individual integrins for specific epidermal functions in both resting skin and during wound healing.[8] The general importance of $\beta_1$ integrins is illustrated by the phenotypes of mice that harbor epidermis-specific null mutations in the *Itgb1* gene (encoding the integrin $\beta_1$ subunit), which display an array of defects that includes impaired proliferation, loss of sebaceous glands and hair follicles, organizational defects in the BM, and impaired wound re-epithelialization.[43–45] Importantly, knockout of individual $\beta_1$ integrins through null mutation of specific $\alpha$ subunits leads to only a subset of the $\beta_1$-null phenotypes in each case, indicating unique functions for different integrins. Nevertheless, some integrins have overlapping functions, such as in the regulation of epidermal migration (see *Epidermal migration*). Interestingly, while early studies in cultured keratinocytes implicated $\beta_1$ integrins in the control of cell differentiation, the effects of either epidermis-specific $\beta_1$ subunit deletion or knockout of individual integrins ($\alpha_3\beta_1$, $\alpha_6\beta_4$, $\alpha_2\beta_1$, $\alpha_9\beta_1$, or $\alpha_v\beta_5$) on epidermal stratification and differentiation were mild or absent,[43,44,46–51] indicating that adhesion-dependent regulation of keratinocyte differentiation *in vivo* is not dependent on any particular integrin(s).

In the sections that follow, we will discuss what is currently known about the roles of individual integrins in the regulation of distinct epidermal/keratinocyte functions, many of which have been elucidated through studies utilizing both cell culture and genetic models. Expression patterns, potential ligands, and known functions of individual integrins in the unwounded and wounded epidermis are summarized in Table 1 and described in detail in the corresponding subsections. However, it is important to consider that the repertoire of integrins expressed on wound epidermis suggests complex interactions, where interplay between different integrins with synergistic or opposing effects may be necessary for proper regulation of keratinocyte wound functions. This concept is supported by observations that different integrin ligands present in the wound bed can have either cumulative or dominant effects on keratinocyte function.[34,41,52–56] Thus, the precise temporal and spatial control of integrin–ECM engagement, perhaps dictated by the temporal appearance or accessibility of ECM ligands in the wound bed, is likely to be critically important for coordinate regulation of different integrins during wound healing.

Finally, it is worth noting there are compelling similarities between wound healing and development of squamous cell carcinoma (SCC),[57] and that

**Table 1.** *Epidermal integrins, their known ligands, and summary of their known functions in unwounded or wounded skin*

| *Integrin* | *ECM Ligands in Skin* | *Known Functions in Unwounded Epidermis* | *Known Functions in Wound Epidermis* |
|---|---|---|---|
| $\alpha_3\beta_1$ | Laminins (LN-332 and LN-511) | Expressed constitutively. Required for BM assembly during skin development and epidermal-dermal adhesion in neonates. | Required for stability of nascent BM and epidermal-dermal adhesion. Promotes wound angiogenesis through crosstalk to vasculature. May negatively control epidermal migration. |
| $\alpha_6\beta_4$ | Laminins (mainly LN-332 in hemidesmosomes) | Expressed constitutively. Function within hemidesmosomes is essential for epidermal-dermal adhesion. | Presumably required for hemidesmosome assembly and epidermal adhesion following re-epithelialization. Roles in epidermal migration are unclear. |
| $\alpha_9\beta_1$ | FN, TN, OPN, VEGF, TSP, ADAMs, EMILIN1, and others | Expressed constitutively at low levels. Nonessential for epidermal development or adhesion. | Required for normal keratinocyte proliferation during wound re-epithelialization. |
| $\alpha_2\beta_1$ | Collagens | Expressed constitutively. Nonessential for epidermal development or adhesion. | No essential roles identified. May contribute to epidermal migration over collagen. |
| $\alpha_5\beta_1$ | FN (via RGD) | Expressed at very low levels. Nonessential for epidermal development or adhesion. | No essential roles reported. May contribute to epidermal migration over fibronectin. |
| $\alpha_v\beta_6$ | FN, TN, LAP of TGF$\beta$-1 and -3 (via RGD) | Not expressed in interfollicular epidermis. Nonessential for epidermal development or adhesion, although required in juvenile mice for normal hair growth. | No essential roles identified, although may promote keratinocyte-mediated activation of latent TGF$\beta$. Also implicated in ECM proteolysis and keratinocyte survival. |
| $\alpha_v\beta_5$ | VN (via RGD) | Expressed at very low levels. Nonessential for epidermal development or adhesion. | No essential roles identified; may contribute weakly to keratinocyte-mediated activation of latent TGF$\beta$. |

See text for expanded discussions and supporting literature.

LN, laminin; BM, basement membrane; FN, fibronectin; TN, tenascin, OPN, osteopontin; VEGF, vascular endothelial cell growth factor; TSP, thrombospondin; ADAM, a disintegrin and metalloproteinase; EMILIN, elastin microfibril interfacer; RGD, arginylglycylaspartic acid; LAP, latency-associated proteins; TGF$\beta$, transforming growth factor beta; ECM, extracellular matrix; VN, vitronectin.

some integrin expression patterns in SCC mirror those that occur in cutaneous wound healing.[37,40] Consistently, integrins regulate a number of epithelial cell functions that are important in both processes, including migration, proliferation, survival, matrix remodeling, and the production of pro-angiogenic factors. Therefore, we will occasionally refer to studies of certain integrins in SCC or other carcinomas, where results from these models may offer clues regarding integrin expression or function during wound healing.

*The laminin-binding integrins ($\alpha_3\beta_1$ and $\alpha_6\beta_4$)*

Laminin-332, the main adhesive ligand in the resting epidermis, is composed of three distinct chains designated $\alpha_3$, $\beta_3$, and $\gamma_2$.[58,59] The effects of laminin-332 on keratinocyte behavior are mediated mainly through its two integrin receptors, $\alpha_3\beta_1$ and $\alpha_6\beta_4$,[41,54–56] although other receptors such as syndecan-1 also contribute.[60] Integrin $\alpha_6\beta_4$ is a major transmembrane component of hemidesmosomes, which are the intermediate filament-associated structures on the basal surfaces of keratinocytes that anchor the epidermis to the dermis.[10,61] In this context, $\alpha_6\beta_4$ is thought to play primarily a structural role with little or no signal transduction function.[8] In contrast, $\alpha_3\beta_1$-mediated adhesions are associated with the actin cytoskeleton and manifest as focal adhesions in cultured keratinocytes.[10,61] Moreover, $\alpha_3\beta_1$ can initiate adhesion-dependent signal transduction in keratinocytes through activation of FAK, SRC, and other signaling molecules.[26,34,62] As receptors for a common BM ligand, it seems likely that $\alpha_3\beta_1$ and $\alpha_6\beta_4$ function coordinately (either cooperatively or in opposition) with regard to some keratinocyte processes. However, since these two integrins differ considerably with regard to both cytoskeletal interactions and signaling capacity, their combined effects on keratinocyte function are likely to occur through convergence of their distinct functions, rather than as redundant functions. Correspondingly, there is evidence that distinct proteolysis of laminin-332 (LN-332) may differentiate between $\alpha_3\beta_1$ and $\alpha_6\beta_4$ binding. For example, an N-terminal proteolytic cleavage fragment of the full-length laminin $\alpha_3$B chain can preferentially ligate integrin $\alpha_3\beta_1$.[63] Moreover, laminin-$\alpha_3$ chain processing within the C-terminal globular domains appears to modulate whether LN-332 localizes to $\alpha_6\beta_4$-containing hemidesmosomes or $\alpha_3\beta_1$-mediated adhesions, suggesting differential integrin interaction.[64]

Although the epidermis stratifies normally in mice that lack $\alpha_3\beta_1$ and $\alpha_6\beta_4$, either alone or in combination, these integrins play essential but distinguishable roles in maintaining the epidermal–dermal junction through their interactions with laminin-332 in the BM.[50,51,65–67] Indeed, human gene mutations in the relevant integrin subunits, or in the individual subunits of laminin-332 itself, lead to variants of the human blistering skin disease junctional epidermolysis bullosa (JEB). However, differences in the details of these JEB variants, which are mirrored in the corresponding knockout mice, illustrate the very different roles that $\alpha_3\beta_1$ and $\alpha_6\beta_4$ play in epidermal–dermal adhesion. Indeed, the essential adhesion role for $\alpha_6\beta_4$ in hemidesmosomes is revealed by the extensive epidermal blistering that occurs in mice that harbor a null mutation in either the *Itga6* or *Itgb4* gene (encoding the $\alpha_6$ or $\beta_4$ subunit, respectively),[65–67] and in human patients with loss-of-function mutations in $\alpha_6\beta_4$.[68,69] In contrast, the absence of $\alpha_3\beta_1$ (through null, or loss-of-function, mutation of the *Itga3* gene encoding the $\alpha_3$ subunit) causes much less severe epidermal blistering in newborn mice[50] or young patients,[70] and hemidesmosomes are left intact in non-blistered regions of $\alpha_3\beta_1$-deficient skin. Moreover, blisters in $\alpha_3\beta_1$-deficient skin form via a mechanism of BM rupture that is distinct from loss of epidermal attachment to laminin-332 *per se*, which persists in these mice via $\alpha_6\beta_4$.[50,51]

$\alpha_6\beta_4$. Hemidesmosomes are essential for stable epidermal adhesion, and their dissolution is necessary for epidermal migration during wound healing.[10] Hemidesmosome disassembly involves the SRC-mediated phosphorylation of the $\beta_4$ cytoplasmic domain, possibly triggered by endothelial growth factor (EGF) or other signals from the wound microenvironment.[71] In this sense, the function of $\alpha_6\beta_4$ from within hemidesmosomes can be viewed as a brake to epidermal migration that must be released for wound re-epithelialization to proceed. Following wound healing, hemidesmosomes are re-assembled to restore stable adhesion of the neo-epidermis.[10]

While the hemidesmosomal role for $\alpha_6\beta_4$ in stable epidermal adhesion is well established, whether this integrin also regulates signal transduction pathways that control keratinocyte functions (*e.g.*, survival, proliferation, migration) is less clear.[8] Indeed, pro-migratory/pro-invasive roles for integrin $\alpha_6\beta_4$ have been described for carcinoma cells, where this integrin may cooperate with receptor tyrosine kinases such as EGFR, RON, and MET to activate migration-promoting signals, as reviewed elsewhere.[5] However, the extent to which these roles extend to keratinocyte migration during wound re-epithelialization remains uncertain.

Noteably, some studies do support a pro-migratory role for $\alpha_6\beta_4$ in keratinocytes, possibly through antagonism of $\alpha_3\beta_1$[72] and/or through regulation of laminin-332 matrix deposition that directs keratinocyte migration.[73]

$\alpha_3\beta_1$. *In vivo* and *in vitro* studies have identified several roles for integrin $\alpha_3\beta_1$ in regulating keratinocyte functions that may contribute to wound healing, including ECM deposition and organization,[74,75] cell polarization and migration,[34,53] cell survival,[62] cell proliferation,[76] and secretion of ECM proteases and pro-angiogenic factors.[36,77–79] Many of these processes depend on $\alpha_3\beta_1$ binding to laminin-332, although some may involve ECM-independent functions of $\alpha_3\beta_1$ that are modulated by lateral interactions (direct or indirect) with other cell membrane proteins, such as tetraspanins,[77,80] uPAR,[81] or adherens junction proteins.[82,83] For example, $\alpha_3\beta_1$ binding to the tetraspanin CD151[84] can regulate $\alpha_3\beta_1$-mediated signaling[85] and epithelial cell motility.[86] Interestingly, CD151 is upregulated in wound epidermis, and deletion of CD151 leads to wound healing defects,[87] suggesting that CD151 might modulate $\alpha_3\beta_1$ function during wound repair.

Roles for $\alpha_3\beta_1$ in keratinocyte migration have been controversial. On one hand, studies using either human or murine keratinocytes support a role for $\alpha_3\beta_1$ in regulating front–back polarization and promoting processive migration on laminin-332,[34,53] which occurs in part through regulation of a FAK/SRC–RAC1 signaling axis.[26,34] In contrast, other studies report that $\alpha_3\beta_1$-deficient keratinocytes display enhanced motility and directional migration, although in some cases this appears to involve compensatory upregulation of other integrins such as $\alpha_6\beta_1$.[35,74] These discordant results may stem in part from differences in the cell culture models used, including species from which cells were derived and the differential deposition of ECM ligands for other integrins.[41,52,54–56] Indeed, $\alpha_3\beta_1$ may been have *trans*-dominant inhibitory effects on other keratinocyte integrins.[35,36,88,89] In any case, *in vivo* studies performed by separate groups using similar epidermis-specific $\alpha_3$-knockout models have shown that $\alpha_3\beta_1$ is not essential for efficient wound closure[35,36] and may even slightly inhibit re-epithelialization, at least in adult mice.[35,36] Interestingly, absence of $\alpha_3\beta_1$ reduced efficient wound closure in skin grafts from neonatal $\alpha_3$-null mice,[90] which might suggest a role in wound re-epithelialization at earlier developmental stages, or that compensatory mechanisms present in adult skin are absent from neonatal skin. Alternatively, absence of $\alpha_3\beta_1$ from other cellular compartments may have caused reduced wound closure in the latter study, since skin grafts were derived from mice with a global $\alpha_3$-null mutation.[90] These questions might be resolved in future studies using mice with compound, epidermis-specific deletion of multiple integrins.

While the dispensability of $\alpha_3\beta_1$ for epidermal migration was unexpected, *in vivo* and cell culture studies have revealed other roles for this integrin in allowing the epidermis to modulate the tissue microenvironment; for example, through alterations of the ECM or paracrine crosstalk to other cellular compartments. Indeed, epidermis-specific deletion of $\alpha_3\beta_1$ was associated with reduced wound angiogenesis, and $\alpha_3$-null keratinocytes showed reduced secretion of factors that stimulate endothelial cell migration.[36] In addition, our group recently identified a novel role for $\alpha_3\beta_1$ in promoting the stability of nascent BM that forms during wound healing, as we discuss later (see *Local basement membrane deposition and assembly*).[91] Moreover, $\alpha_3\beta_1$ has been shown to control laminin-332 deposition and organization in cultured keratinocytes,[74] and we recently observed that proteolytic processing of the laminin-$\gamma_2$ chain is delayed in wounds of $\alpha_3\beta_1$-deficient epidermis and impaired in $\alpha_3$-null keratinocytes.[91]

#### *Other $\beta_1$ integrins ($\alpha_9\beta_1$, $\alpha_2\beta_1$, and $\alpha_5\beta_1$)*

Integrin $\alpha_9\beta_1$ is expressed constitutively in the epidermis and is upregulated during wound healing, where it may bind to several ECM proteins or other ligands in the wound, including the EIIIA/EDA segment in cellular fibronectin, tenascin, EMILIN1, osteopontin, thrombospondin, certain ADAM (a disintegrin and metalloprotease domain) family members, and VEGF (vascular endothelial growth factor).[8,92,93] Distinct binding motifs for $\alpha_9\beta_1$ have been identified, including the AEIDGIEL in tenascin-C, the PEDGIHE motif that occurs within an exposed loop of the fibronectin EIIIA domain, and distinct motifs within ADAM family members.[93,94] While $\alpha_9\beta_1$ has been studied less extensively than other integrins, its roles are likely to be complex given the wide range of potential ligands for this integrin that are present in wounds. Genetic studies have revealed an important role for $\alpha_9\beta_1$ in wound re-epithelialization. Indeed, keratinocyte proliferation was significantly impaired in wounds of mice with epidermis-specific deletion of $\alpha_9\beta_1$, while the rate of re-epithelialization was unaffected, resulting in diminished thickness of the neo-epidermis that was presumably due to a reduced number of migrating keratinocytes.[49] Thus,

$\alpha_9\beta_1$ contributes to establishing integrity of the neoepidermis by promoting proliferation of wound keratinocytes, rather than by driving epidermal migration *per se*.

The collagen-binding integrin, $\alpha_2\beta_1$, is also expressed constitutively in epidermis and upregulated in wounds. Remarkably, however, mice with a global $\alpha_2$-null mutation display normal skin development with no obvious defects in wound reepithelialization or contraction, indicating that this integrin is not essential for wound closure.[47,95] However, $\alpha_2\beta_1$-deficient mice did display enhanced angiogenesis, reduced infiltration of mast cells, and (in one study) reduced tensile strength of healed wounds, indicating complex roles in wound healing.[47,48] While many of the wound healing defects in the $\alpha_2$-null mice are probably caused by absence of $\alpha_2\beta_1$ from the involved cells, it remains to be determined whether some of these defects reflect the loss of integrin-dependent crosstalk between different cellular compartments of the wound, as mentioned above for $\alpha_3\beta_1$. Moreover, there is the possibility that loss of $\alpha_2\beta_1$–collagen interactions that promote keratinocyte migration are compensated by other integrin–ECM interactions. Future studies in which $\alpha_2\beta_1$ is deleted from epidermis, alone or in combination with other integrins, may address these questions.

Integrin $\alpha_5\beta_1$ is largely absent from resting epidermis and expressed *de novo* in wound epidermis, where it presumably contributes to epidermal migration over fibronectin in the provisional matrix of the wound.[96] However, *in vivo* roles for epidermal $\alpha_5\beta_1$ during wound healing are not defined, since the $\alpha_5$-null mutation is embryonic lethal,[97] and studies in epidermis-specific $\alpha_5$ knockout mice have not been reported. Thus, it remains possible that loss of $\alpha_5\beta_1$ accounts for much of the epidermal migration defect that was reported in mice lacking $\beta_1$,[45] since other $\alpha$ subunit knockouts did not phenocopy this defect.

### *The $\alpha_v$ integrins ($\alpha_v\beta_5$ and $\alpha_v\beta_6$)*

The integrins $\alpha_v\beta_5$ and $\alpha_v\beta_8$ are expressed at low levels in resting epidermis, with the latter being expressed suprabasally, while $\alpha_v\beta_6$ is restricted to hair follicle stem cells and is not normally expressed in the interfollicular epidermis of unwounded skin.[8,98–100] However, both $\alpha_v\beta_6$ and $\alpha_v\beta_5$ are upregulated during wound healing,[8,98–100] where each may bind to several ECM ligands through the arginine-glycine-aspartate (RGD) motif (*e.g.*, fibronectin, tenascin, vitronectin).[3,40] Given the diversity of ligands that $\alpha_v$ integrins can bind, their upregulation in wound epidermis may be important for modulating keratinocyte motility over the complex matrix of the wound bed. Moreover, $\alpha_v\beta_6$ has been shown to regulate the expression of extracellular proteases, including matrix metalloprotease (MMP)-9,[101] MMP-3,[102] and uPA,[103] suggesting roles in ECM remodeling. Finally, as discussed below (in epidermal survival section), $\alpha_v\beta_6$ might promote cell survival and prevent keratinocytes from differentiating.

The *de novo* induction of $\alpha_v\beta_6$ during wound healing might also provide temporal control over local transforming growth factor-$\beta$ (TGF$\beta$) activation, since this integrin can activate the ECM-bound pool of latent TGF$\beta$.[40,104,105] Each of the mammalian TGF$\beta$ isoforms (TGF$\beta$-1, 2, and 3) is secreted as an inactive complex consisting of the latency-associated protein (LAP) and the latent TGF$\beta$ binding protein (LTBP), which is covalently linked to the ECM through fibronectin.[104,106] Integrin $\alpha_v\beta_6$ (and perhaps to a lesser extent $\alpha_v\beta_5$) can bind to the RGD motif within the LAP, resulting in a conformational change in the complex that activates latent TGF$\beta_1$ or TGF$\beta_3$.[104,105,107] Although this mechanism has been best characterized in carcinoma cells, it also occurs in keratinocytes and may initiate $\alpha_v\beta_6$-dependent TGF$\beta$ signaling pathways that are important for wound healing.[105]

### *Functions of wound epidermis that are regulated by integrins*

A number of diverse keratinocyte functions are regulated by adhesion to the ECM, and epidermis-specific gene knockout studies have revealed roles for individual integrins in distinct keratinocyte functions that are important for normal wound healing, including migration, proliferation, ECM remodeling/BM regeneration, stable attachment of the neo-epidermis, and induction of angiogenesis. In some cases, however, there is considerable discordance between results from cell culture studies and those from *in vivo* studies. For example, studies in cultured keratinocytes have clearly implicated certain integrins in the control of cell migration, while studies in knockout mice have so far failed to identify individual integrin–ECM interactions that are essential for wound reepithelialization in adult skin (discussed below).

The complex process of wound healing is further regulated by growth factors, some of which work in concert or collaboration with integrins to control epidermal functions. Two growth factors of particular importance in wound healing, VEGF and TGF$\beta$,[108] have each been functionally linked to integrins that are expressed in wound epidermis. For instance, $\alpha_6\beta_4$ was shown to enhance VEGF

translation in carcinoma cells,[109] and VEGF expression can upregulate $\alpha_v\beta_6$ in some carcinoma cells,[110] raising the intriguing possibility of dynamic integrin–growth factor feedback loops that might extend to wound keratinocytes. Additionally, $\alpha_v\beta_6$-mediated activation of latent TGF$\beta$ (see previous section) might stimulate the expression of $\alpha_5\beta_1$ and $\alpha_v\beta_5$ in keratinocytes.[111] However, while TGF$\beta$ stimulates pro-migratory integrins, it has also been shown to inhibit keratinocyte proliferation, so there are conflicting reports with regard to the net effect of TGF$\beta$ signaling on re-epithelialization.[112] In any case, it is clear that the repertoire of integrins expressed in wound epidermis, in combination with growth factors present in the wound microenvironment, is important for coordinating diverse keratinocyte functions that collectively ensure efficient wound repair and epidermal regeneration.

In the following sections, we will discuss diverse functions of the wound epidermis that are regulated by keratinocyte integrins (summarized in Fig. 1). We will begin with a discussion of the roles that integrins play in autonomous keratinocyte functions that promote wound healing, followed by a discussion of more recently appreciated roles for certain epidermal integrins in the regulation of paracrine crosstalk to other cell types in the wound.

### *Autonomous keratinocyte functions*

Epidermal migration. Given their global importance in cell adhesion and motility, it seems intuitive that a major function of integrins during wound healing is the regulation of epidermal migration. Consistently, deletion of $\beta_1$ integrins from the epidermis resulted in severely compromised wound re-epithelialization,[45] and numerous cell culture studies have shown that individual integrins can regulate keratinocyte adhesion and migration on their respective ECM ligands.[34,45,53,61,73,113,114] Nevertheless, results from *in vivo* wound healing studies in integrin knockout mice are surprisingly discordant with findings from cell culture models, and the roles for individual integrins in epidermal migration remain unclear. For example, $\alpha_v\beta_6$ and $\alpha_v\beta_5$ can each mediate keratinocyte migration on their respective ligands *in vitro*, yet absence of either or both integrins *in vivo* did not cause impaired wound healing in young adult mice (although deletion of $\alpha_v\beta_6$ caused delayed wound healing in older mice).[46,115,116] Similarly, integrins $\alpha_3\beta_1$, $\alpha_2\beta_1$, and $\alpha_9\beta_1$ can each mediate keratinocyte migration on relevant ligands *in vitro*, but their ablation *in vivo* had remarkably mild or no effects on wound re-epithelialization in adult mice.[35,47–49] As already mentioned, in a striking example of discordance,

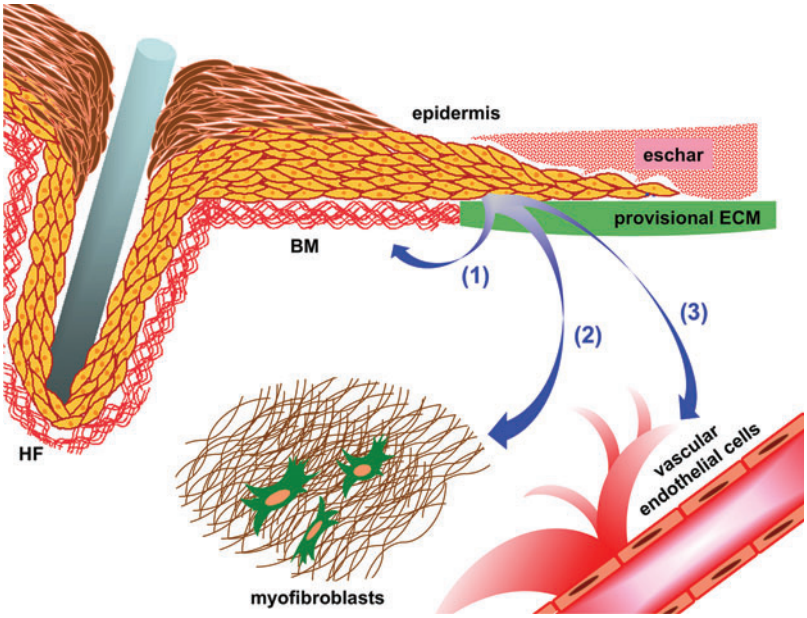


**Figure 1.** A model depicting functions of wound epidermis that may be controlled by keratinocyte integrins. Arrow (1) indicates autonomous regulation of keratinocyte functions such as migration, proliferation, local matrix remodeling, and stable adhesion of the neo-epidermis. Arrows (2) and (3) indicate regulation of paracrine crosstalk from the epidermis to other cellular compartments, including myofibroblasts to control wound contraction (2) and vascular endothelial cells to promote wound angiogenesis (3). Components of the wound microenvironment are indicated. BM, basement membrane; HF, hair follicle; ECM, extracellular matrix. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

$\alpha_3\beta_1$ has been reported to promote migration of cultured keratinocytes on laminin-332,[34,53] yet it is not essential for wound closure *in vivo*, at least in adult mice.[35] In fact, deletion of $\alpha_3\beta_1$ from the epidermis led to a slightly increased rate of wound closure,[35] consistent with other studies using anti-$\alpha_3\beta_1$ blocking antibodies that indicated a suppressive role in migration.[89] Together, these disparate findings indicate a complex role for $\alpha_3\beta_1$ in epidermal migration that is sensitive to ECM composition and may include modulation of the rate of re-epithelialization.

In some cases, these discrepancies between cell culture models and *in vivo* wound models may reflect the greater complexity of ECM *in vivo*, where multiple ligands in the wound bed might provide opportunity for compensation between integrins with overlapping migration functions, as discussed at length elsewhere.[8] Such overlap makes sense from an evolutionary standpoint, given the importance of rapidly restoring epidermal barrier function after wounding. Consistent with this idea, mice with epidermis-specific deletion of all $\beta_1$ integrins (*i.e.*, $\beta_1$-null) showed impaired wound re-epithelialization,[45] while deletion of individual integrins did not,[8,35,47–49] suggesting that there is considerable overlap in the ability of distinct $\beta_1$ integrins to promote epidermal migration.

Although $\alpha_6\beta_4$ can regulate the motility of cultured keratinocytes,[72,73] it remains uncertain whether this integrin has important pro-migratory roles in wound re-epithelialization. It is clear that $\alpha_6\beta_4$ is not sufficient for normal wound re-epithelialization in the absence of $\beta_1$ integrins, since $\beta_1$-null epidermis shows severe migration defects.[45] Nevertheless, it remains possible that some pro-migratory/pro-invasive roles for $\alpha_6\beta_4$ that have been described in carcinoma cells (mentioned previously) may extend to wound keratinocytes. In any case, a role for $\alpha_6\beta_4$ in epidermal migration cannot yet be ruled out, since the severe epidermal blistering that occurs in $\alpha_6\beta_4$-deficient mice presents a formidable challenge to wound healing experiments. More detailed discussions of potential roles for $\alpha_6\beta_4$ in keratinocyte migration can be found in separate reviews[8] (also, see the review by Hopkinson *et al.*[117] in this issue).

Epidermal proliferation. Epidermal homeostasis is maintained by a resident population of stem cells within the basal cell layer that gives rise to committed progenitor cells, or transit-amplifying cells, which in turn replenish the differentiated keratinocytes that are eventually shed from the outer layer of the skin.[37–39] While most of the proliferative cells that maintain homeostasis are thought to reside in the interfollicular epidermis, stem cells in the bulge of the hair follicle make the most substantial contribution to the replenishment of keratinocytes that accompanies wound re-epithelialization.[118,119] $\beta_1$ integrins as well as $\alpha_6\beta_4$ are expressed at higher levels in epidermal stem cells, where they are thought to help control the balance between stem cell renewal and terminal differentiation.[5,37,39,120–123] It follows that changes in integrin signaling or adhesion functions that shift this balance are likely to be important for wound healing. Consistently, integrin signaling through mitogen-activated protein kinases (MAPKs) or the Rho family GTPase, RAC1, has been linked to the maintenance of epidermal stem cells.[32,33,123] As discussed in the section on other $\beta_1$ integrins, integrin $\alpha_9\beta_1$ is essential to maintain proper levels of keratinocyte proliferation during wound healing.[49] Interestingly, a recent study in an SCC model revealed a novel role for $\alpha_3\beta_1$ in the retention of the slow-cycling cells in the hair follicle bulge, where deletion of this integrin allowed these cells to detach from the BM and emigrate into the suprabasal layers where they terminally differentiated. This loss of slow-cycling cells resulted in reduced skin tumor formation, presumably because these are the same cell that would otherwise accumulate oncogenic or tumor suppressor mutations that eventually give rise to tumors.[124] These findings might reflect similar functions for $\alpha_3\beta_1$ or other integrins within the hair follicle stem cells that participate in epidermal regeneration during wound healing.

Epidermal survival. Presumably, wound keratinocytes must somehow maintain appropriate survival signals that prevent them from undergoing anoikis (*i.e.*, apoptosis that is induced by reduced or inappropriate adhesion) as they lose contact with damaged BM and encounter the dramatically altered ECM of the wound bed. As mentioned above, the *de novo* expression of $\alpha_v\beta_6$ may prevent wound keratinocytes from undergoing anoikis, as this integrin is known to activate AKT survival pathways in SCC cells.[125] In addition, $\alpha_3\beta_1$-mediated adhesion has been shown to promote the survival of keratinocyte cell lines through pathways that involve FAK and ERK, suggesting a possible role in survival of wound keratinocytes.[62] Adhesion through $\alpha_6\beta_4$ has also been linked to epidermal cell survival,[67] and it is conceivable that this integrin contributes to adhesion-dependent survival of the neo-epidermis when hemidesmosomes are assembled following wound closure.

However, mice lacking both $\alpha_3\beta_1$ and $\alpha_6\beta_4$ (*e.g.*, through combined $\alpha_3$-null and $\beta_4$-null mutations) displayed apoptotic basal keratinocytes only in detached regions of epidermis, but not in non-blistered epidermis,[51] indicating that other adhesion mechanisms are sufficient to protect from anoikis.

Local basement membrane deposition and assembly. The cutaneous BM not only provides a physical separation between the epidermal and dermal compartments of the skin, but it also provides cues for signaling pathways that regulate a variety of epidermal cell functions including polarization, differentiation, survival, tissue structure, and migration. Indeed, many of the keratinocyte functions discussed above are regulated by keratinocyte-mediated changes to the local ECM, including the BM, which as discussed here may occur through either deposition of ECM proteins or matrix proteolysis. Early studies in $\beta_1$ integrin-deficient mice or embryoid bodies have revealed essential roles for certain $\beta_1$integrins in proper BM formation in embryonic or adult tissues.[50,126,127] *In vivo* and *in vitro* studies from our group and others collectively support roles for integrin $\alpha_3\beta_1$ in several steps that may be important for the regeneration of a stable BM during wound healing, providing an example of how some integrins may regulate the ability of the epidermis to regulate ECM organization and assembly (Fig. 2). Indeed, as already mentioned, $\alpha_3\beta_1$ regulates laminin-332 organization in cultured keratinocytes.[74] Moreover, we recently demonstrated that mice lacking epidermal $\alpha_3\beta_1$ display blisters in newly regenerated wound epidermis,[91] which are characterized by BM rupture that phenocopies the epidermal blisters in neonatal $\alpha_3$-null mice ,[50] indicating that the developmental role for $\alpha_3\beta_1$ in maintaining BM integrity is recapitulated during adult wound healing. Interestingly, this blistering phenotype was linked to reduced fibulin-2 levels in both neonatal skin and early wounds, and genetic deletion of fibulin-2 (*i.e.*, in the presence of $\alpha_3\beta_1$) was sufficient to generate neonatal skin blisters.[91] Since fibulin-2 binds to the laminin-$\gamma_2$ chain within the L4 module near the N-terminus,[128] and this interaction has been implicated in stable incorporation of laminin-332 into the developing BM,[129] these findings suggest an important role for $\alpha_3\beta_1$-dependent fibulin-2 expression in BM maturation (Fig. 2).

Integrins can also control matrix proteolysis by the epidermis, as supported by the ability of several

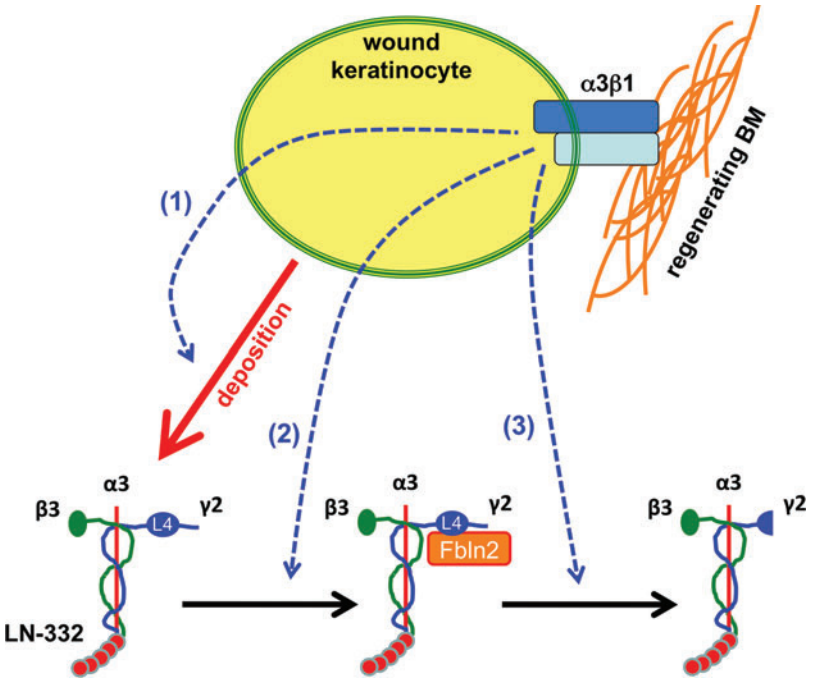


**Figure 2.** A model depicting possible roles for epidermal integrin $\alpha_3\beta_1$ in regeneration of BM during wound healing. *In vivo* and cell culture studies have implicated $\alpha_3\beta_1$ in several different steps that may impact the stable assembly of laminin-332 (LN-332) into BM, including (1) LN-332 deposition, (2) expression of the LN-332-binding protein fibulin-2 (Fbln2), and (3) proteolytic processing of the LN-332 $\gamma_2$ chain (see text for detailed discussion and supporting references). Other epidermal integrins may have similar roles in the incorporation of LN-332 or other BM proteins. The $\alpha_3$, $\beta_3$, and $\gamma_2$ chains of LN-332 trimer, as well as the L4 module on the $\gamma_2$ chain, are indicated. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

integrins to control the expression or functions of MMPs or other ECM-degrading extracellular proteases (discussed below). While a variety of ECM proteins present in the provisional wound matrix undergo proteolysis, we will focus on laminin-332 as an example since both its $\alpha_3$ and $\gamma_2$ chains undergo extensive proteolytic processing.[130–133] Although the importance of these processing events *in vivo* are not yet fully understood, some have been shown to influence the migratory behaviors of cultured keratinocytes. For example, differential processing of the laminin $\alpha_3$ chain, regulated by tissue-type plasminogen activator and plasmin, was shown to alter epithelial cell motility.[64,134] Laminin-332 proteolysis is also likely to be an important mode of regulating BM architecture through modulation of key linkages with other ECM proteins, such as the interaction of fibulin-2 with laminin-332 described above.[58] Indeed, the L4 module to which fibulin-2 binds is lost upon proteolytic processing of $\gamma_2$, which might be an important step for BM maturation.[128,129,132] Interestingly, recent data from our laboratory indicates a role for epidermal $\alpha_3\beta_1$ in the regulation of laminin-$\gamma_2$ chain processing, both *in vivo* during wound healing and in keratinocytes cultured under conditions of high calcium.[91] Thus, $\alpha_3\beta_1$ may have a dual role in BM assembly that involves both matrix protein deposition and proteolytic processing of matrix proteins (Fig. 2).

### *Epidermis-mediated modulation of the wound microenvironment*

Most studies of keratinocyte integrins have focused on their "classical" roles in regulating cell-autonomous processes such as motility, proliferation, local matrix assembly/remodeling, and survival, as discussed above. However, epidermal integrins may have far-reaching effects on other aspects of the wound microenvironment, including modification of the ECM in distal parts of the wound, and crosstalk to distal cells with essential roles in wound healing (*i.e.*, endothelial cells, fibroblasts). Consistently, some keratinocyte integrins can modulate the expression of genes that are implicated in paracrine crosstalk to other cells.[36,47,135,136] Indeed, our own studies have identified $\alpha_3\beta_1$-dependent regulation of a number of genes in keratinocyte cell lines that encode growth factors, extracellular proteases, or ECM/matricellular proteins with known roles in modulating the microenvironment of wounds or tumors[36,79,137] (Missan and DiPersio, unpublished observation). In this section, we will discuss these more recently appreciated roles for certain epidermal integrins in modification of the wound microenvironment, including paracrine functions that influence extra-epidermal compartments of the wound.

**Proteolysis and remodeling of the wound extracellular matrix.** While the effects of local matrix remodeling on epidermal functions are clear, long-range effects of altered wound ECM are also important. Indeed, controlled ECM remodeling is an essential feature of normal wound healing,[138] and defects in ECM organization are associated with chronic wounds.[139] It has long been known that changes in matrix composition or structure can act upstream of integrins through ligation that turns on intracellular signaling pathways. However, integrin–ECM signals should more accurately be considered as bidirectional, or even as signaling loops, as many integrins have been shown to alter ECM composition/structure at the levels of both gene expression and matrix assembly. For example, integrin $\alpha_2\beta_1$ has been shown to be a positive regulator of type-1 collagen gene expression,[140] while $\alpha_1\beta_1$ suppresses collagen synthesis in the dermis.[141] Furthermore, both fibronectin-binding integrins and ILK have been shown to support fibronectin matrix assembly and fibrillogenesis.[142,143]

The dynamic regulation of MMPs and other extracellular proteases can also contribute to changes in ECM that are crucial for successful wound healing. Indeed, MMPs are involved in all stages of wound resolution, from early removal of damaged ECM to late stage scar remodeling,[144] and defects in MMP expression or function contribute to the pathogenesis of chronic wounds.[139,145] Furthermore, MMPs are increasingly credited with the proteolytic release of important growth factors from the ECM or cell surface,[144,146] thereby supplying the wound microenvironment with the appropriate effector molecules to promote healing. Not surprisingly, several integrins that are expressed during wound healing (Table 1) have been implicated in modulating MMP expression in keratinocytes or other cells, suggesting similar roles in the wound microenvironment. Examples include $\alpha_3\beta_1$-mediated induction of MMP-9 or uPA in keratinocytes,[79,137] $\alpha_2\beta_1$-mediated induction of MMP-1 in osteogenic cells,[140] and $\alpha_5\beta_1$-mediated regulation of MMP-3 and MMP-9 in some cells.[147]

**Paracrine crosstalk to other cellular compartments.** It is well known that a complex network of communication exists between the various cell types that reside in the wound microenvironment. The epidermis can send paracrine signals to other cellular compartments that contribute to wound healing, including the vasculature and the stroma

(as depicted in Fig. 1), and recent evidence suggests that some of these signals are regulated by keratinocyte integrins.[32,62,122,123] Such intercellular signals may be propagated from the epidermis to other cells through physical changes in the ECM (*i.e.*, mechanical signaling), through diffusible growth factors that are secreted by the epidermis, or through generation of bioactive fragments following ECM proteolysis.

An important mode of intercellular crosstalk within the wound microenvironment is growth factor–mediated communication. Although keratinocytes reside in the epidermal compartment of skin and are spatially separated from the stromal compartment, it has long been known that growth factors and cytokines produced *de novo* by the epidermis can diffuse to other cellular compartments of the wound.[148] Thus, the epidermis is able to influence other cell types (*i.e.*, endothelial cells, fibroblasts, immune cells) by regulating the availability of growth factors such as VEGF, TGF$\beta$, and KGF. Some integrins can regulate the expression of growth factors by keratinocytes, which can then mediate paracrine stimulation of other cell types. For instance, deletion of $\alpha_3\beta_1$ from cultured keratinocytes or epidermis reduced the expression of the pro-angiogenic factor mitogen-regulated protein 3 (MRP-3), which contributed to reduced stimulation of endothelial cell migration *in vitro* and impaired wound angiogenesis *in vivo*.[36] Conversely, it has been shown in $\alpha_2$-null mice that ablation of $\alpha_2\beta_1$ (albeit in all cell types at once) results in neovascular enhancement in both wounds and sponge implants, indicating an anti-angiogenic role for this integrin that might involve intercellular crosstalk.[47] These findings demonstrate that epidermal integrins may regulate keratinocyte-to-endothelial cell crosstalk to modulate wound angiogenesis, and that coordinated activities of different integrins may be important for proper outcome. As integrin–ECM interactions are likely to be regulated temporally during wound healing, we speculate that integrins might regulate the ability of epidermis to influence not only angiogenic growth, but also blood vessel regression and vascular normalization at later stages of wound healing.

There is also published evidence to support a role for crosstalk from keratinocytes to mesenchymal fibroblasts during wound healing. Indeed, delays in wound re-epithelialization were associated with enhanced wound fibrosis, possibly due to a lack of signaling from epidermis, which may play a role in hypertrophic scar development.[135,149] Studies in co-culture models have demonstrated that many fibroblast genes are regulated by keratinocyte-derived factors, including genes coding for growth factors, ECM components, and MMPs,[136] all vital constituents for successful wound healing. Integrin $\alpha_3\beta_1$ is an intriguing candidate for influencing keratinocyte-to-fibroblast crosstalk in cutaneous wounds, given its above described role in promoting keratinocyte-to-endothelial cell crosstalk.[36] For example, in a murine model of lung fibrosis, ablation of $\alpha_3\beta_1$ in lung epithelial cells resulted in reduced $\beta$-catenin/Smad signaling, accompanied by decreased accumulation of lung myofibroblasts.[83]

Some epidermal integrins may control the bioavailability of ECM-bound growth factors or bioactive ECM fragments, either directly or indirectly, that influence the behaviors of other cell types. A direct role for certain $\alpha_v$ integrins in the local activation of the ECM-associated latent TGF$\beta$ complex has already been discussed in an earlier section.[104] While the extent to which the latter mechanism might render an activated growth factor available to a distal cellular compartment remains unclear, there are also examples of integrin-mediated liberation and diffusion of ECM-bound growth factors. For example, certain integrins (*i.e.*, $\alpha_v\beta_6$ and $\alpha_3\beta_1$) can induce expression of MMP-9, uPA, or other extracellular proteases[79,101,137,150] that can degrade ECM and release reservoirs of ECM-associated growth factors (*i.e.*, VEGF) to promote angiogenesis.[151,152] In addition, degradation of laminins, collagens, or other ECM proteins by some of these extracellular proteases may lead to the generation of bioactive matrix fragments that can directly stimulate cell growth or motility.[152,153]

Finally, changes in ECM composition or structure that alter the physical properties (*i.e.*, stiffness) of a tissue can influence cell function through mechanical signals. It follows that integrin-dependent changes in ECM, brought about by deposition of matrix proteins or matrix proteolysis, might mediate intercellular crosstalk from the epidermis to other wound cells through mechanical signaling. Mechanisms whereby different cell types use mechanical signals to communicate with one another are still poorly understood. However, mounting evidence supports an important role for alterations in matrix compliance and mechanical stress in the pathogenesis of chronic wound healing,[1] and the current use of topical negative pressure devices as an adjuvant therapy for chronic wounds may promote healing through increased mechanical tension in the wound.[154,155] Moreover, defects in ECM deposition or proteolytic processing that are likely to abberantly alter the mechanical properties of the ECM are thought to contribute to the pathogenesis of chronic wounds.[156,157]

## FUTURE DIRECTIONS

### Exploiting integrins as therapeutic targets in wound healing

The ever-growing elderly and diabetic populations in the United States create a continuously increasing demand for effective wound healing therapies, which have yet to be identified. Impaired re-epithelialization is a hallmark of chronic wounds and poses a major clinical concern due to increased susceptibility of patients to infection. Prolonged treatment of chronic wounds, as well as infection due to slow healing, places a substantial financial burden on the U.S., and negatively impacts quality of life for the patient. Mounting evidence supports the idea that changes or defects in integrin-dependent keratinocyte functions contribute to the pathogenesis of chronic wounds, providing a strong rationale for exploiting epidermal integrins as therapeutic targets for their treatment.

**TAKE-HOME MESSAGES**

- Integrins are bidirectional signaling receptors that regulate both keratinocyte-mediated changes to the wound microenvironment and keratinocyte responses to microenvironmental changes.
- In addition to controlling many cell-autonomous keratinocyte functions such as migration, proliferation, and survival, some integrins can regulate the ability of epidermis to cross-talk to other cellular compartments, such as endothelial cells and fibroblasts, in a paracrine fashion.
- The dynamic regulation of ECM composition during wound healing is critical to coordinate integrin activity in a spatiotemporal manner in order to successfully promote wound healing. Alternatively, inappropriate ECM composition/mechanics are known to contribute to the pathogenesis of chronic wounds.
- Chronic wounds display altered integrin expression, as well as changes in the extracellular milieu, which can both be caused by, and contribute to, inappropriate integrin signaling or aberrant MMP activity.
- Integrins expressed on wound epidermis represent potential therapeutic targets for the treatment of chronic wounds, although some formidable challenges lie ahead before we can fully exploit integrins as clinical targets.

Chronic wounds are often characterized by persistent inflammation, unhealthy granulation tissue, and impaired re-epithelialization.[139] The mechanisms that prevent the healing of chronic wounds are unknown. However, it is clear that the ECM is compromised in chronic wounds, and aberrant changes in ECM or its receptors are implicated in the pathology of chronic wounds, as reviewed elsewhere.[139] For example, it has been postulated that elevated levels of pro-inflammatory cytokines in chronic wounds results in atypical secretion/activation of extracellular proteases, which in turn may lead to "suboptimal ECM composition" and reduced integrin ligation that does not support normal growth factor activity.[139] In addition, diabetic foot ulcers often display upregulation of MMPs 1, 2, 8, and 9, and concurrently reduced levels of tissue inhibitor of metalloprotease-2 (TIMP-2) compared to traumatic wounds.[139,145] Moreover, deregulation of syndecan-1 and syndecan-4 have been detected in venous leg ulcers.[158] Fibronectin deficiency, most likely resulting from enhanced ECM degradation, has been reported to occur in chronic wounds where it may impair cell migration.[139,159,160] Moreover, fibronectin fragments (generated by fibronectin degradation) have been shown to modulate levels of MMPs and TIMPs in some models,[139,161] and have been reported to occur in chronic wound fluids.[162–164]

As bidirectional signaling receptors that regulate both keratinocyte-mediated changes to the wound microenvironment, and keratinocyte responses to those microenvironmental changes, integrins are attractive targets for therapeutic strategies to promote wound healing or to treat chronic wounds. The general concept of therapeutically targeting integrin function is already well established. For example, the integrin-blocking agent Cilengitide, an RGD mimetic, has shown promise in clinical trials involving the treatment of recurrent glioma.[165,166] It is well known that wound healing and skin carcinogenesis share similarities regarding both epidermal cell function and microenvironmental factors that drive each process,[57] and integrin-targeting therapies are relevant to the treatment of chronic wounds for many of the same reasons that they are relevant to the treatment of cancer. For example, we have already discussed how roles for some integrins within the stem cell compartment may be important during both wound re-epithelialization and skin carcinogenesis (see *Epidermal proliferation*).

Integrins that are upregulated in chronic wounds might serve as particularly attractive therapeutic targets. For example, integrin $\alpha_v\beta_6$ is not normally expressed in epidermis but is induced during normal wound healing and has been shown to be strongly upregulated in chronic wounds of human patients.[167] In the same study, transgenic mice that constitutively over-expressed $\beta_6$ integrin in the epithelium showed no change in wound closure rate but spontaneously developed chronic fibrotic ulcers,[167] while a separate study showed that diabetic $\beta_6$-null mice showed delays in early

wound closure.[168] These findings indicate complex roles for integrin $\alpha_v\beta_6$ in wound healing that are likely to be precisely timed and may require the correct extracellular milieu. More work in this area must be done to tease apart and fully understand the precise functions of particular integrins in normal wound healing, then identify which of these functions are deficient in chronic wounds, in order to fully exploit integrins as therapeutic targets.

In addition to direct targeting of integrins, rational wound healing therapies might exploit integrins as a means of targeted therapeutic delivery. For instance, one group recently generated an injectable complex containing platelet derived growth factor B (PDGF-B) plasmid DNA and an integrin-selective RGDK-lipopeptide, towards the goal of delivering PDGF-B directly to relevant integrin-decorated cells within the wound bed, thereby circumventing the modest efficacy achieved through repeated topical application.[169] A single, subcutaneous injection of this complex was demonstrated to promote wound healing in a streptozotocin-induced diabetic rat model of chronic wounds, resulting in enhanced re-epithelialization, collagen fibrillogenesis, and blood vessel formation.[169] Thus, it is possible that similar strategies can be developed to direct therapeutic agents to wound epidermis using lipopeptides that recognize keratinocyte integrins.

In summary, while the field has made good progress in identifying functions of individual keratinocyte integrins, and understanding how their coordinated activities might control the range of epidermal functions that are required for normal wound healing, some formidable challenges lie ahead as we attempt to translate this knowledge into therapeutic approaches for wound healing deficiencies in human patients. Considering that several epidermal integrins are required for different epidermal functions, one challenge will be to develop multicombinatorial strategies to target several integrins. An additional challenge is that the relevant ligands in the wound microenvironment have not yet been identified for all epidermal integrins. Finally, as already mentioned, it is likely that the control of diverse epidermal functions by individual integrins is precisely timed, in order to maintain properly coordinated regulation of epidermal wound functions.

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## AUTHOR DISCLOSURE AND GHOSTWRITING STATEMENT

The authors declare no competing financial interests or other conflicts of interest. No ghostwriters were involved in the preparation of this manuscript.

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### Abbreviations and Acronyms

BM = basement membrane
ECM = extracellular matrix
EGF = epidermal growth factor
FAK = focal adhesion kinase
GTPase = guanosine triphosphatase
ILK = integrin-linked kinase
JEB = junctional epidermolysis bullosa
LAP = latency-associated protein
LTBP = latent TGF$\beta$ binding protein
MAPK = mitogen-activated protein kinases
MMP = matrix metalloprotease
PDGF-B = platelet derived growth factor B
PI3-K = phosphatidylinositol 3-kinase
PLC = phospholipase C
SCC = squamous cell carcinoma
SH2 = SRC-homolog 2
TGF$\beta$ = transforming growth factor beta
TIMP = tissue inhibitor of metalloprotease
uPAR = urokinase receptor
VEGF = vascular endothelial growth factor