The Yin and Yang of Integrin Function in Re-Epithelialization During Wound Healing

Manuela Martins-Green*

Department of Cell Biology and Neuroscience, University of California, Riverside, California.

Background: Integrins are transmembrane proteins that are present in the plasma membrane of basal keratinocytes and connect them to the underlying basement membrane and to the dermis. There are primarily two types of interactions between the epidermis and the dermis—via focal adhesion plaques and hemidesmosomes. It is critical that these interactions form properly to confer the skin strong mechanical properties. Integrins are also critical during wound healing, particularly in closure of the wound.

The Problem: Margadant *et al.* (2009) address proper closure of cutaneous wounds. They developed a conditional knockout mouse for integrin α_3 and showed that the absence of the α_3 integrin resulted in faster migration of the keratinocytes during wound healing. However, its absence also led to inflammation, hair loss, basement membrane duplication, and loss of dermal epidermal interactions with blister formation. The latter has important consequences for the ability of the skin to withstand mechanical challenges.

Basic/Clinical Science Advances: Models such as the conditional model developed by Margadant *et al.* (2009) will provide the opportunity for making major advances in understanding the complex function of integrins during healing.

Clinical Care Relevance: The model and the findings provide an opportunity to decipher mechanisms of disease and for potential development of treatments for human skin disorders and impaired healing, including chronic ulcers.

Conclusion: This work provides knowledge that leads to the understanding of delayed re-epithelialization during wound healing and dermal epidermal defects, blistering, and chronic skin diseases, hence providing the opportunity to understand the basic cellular and molecular mechanisms involved in these situations.

BACKGROUND

EPITHELIALIZATION OF WOUNDS IS critical for proper healing. Indeed, in most impaired cutaneous wounds, the keratinocytes do not function properly and do not migrate over the wound, leaving the wound open. This situation is problematic, including the possibility of systemic infection. In general, epithelia interact with the underlying connective tissue through transmembrane proteins called integrins.^{1–3} These proteins contain two subunits denoted α and β . When one α and one β subunit come together in the

plasma membrane, an integrin receptor forms. Some are very specific for one extracellular matrix (ECM) molecule, whereas others can bind more than one molecule, providing for plasticity in function. There are at least 18 α and 8 β conserved subunits that combine in various ways, giving rise to a large family of receptors (Fig. 1).⁴ Keratinocytes of the basal layer of the epidermis express integrins $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_4$, and $\alpha_v\beta_5$, which interact with various components of the basement membrane, an ECM structure that underlies the epidermal layer and

Manuela Martins-Green, PhD

Submitted for publication August 22, 2011.

*Correspondence: Department of Cell Biology and Neuroscience, Biological Sciences Building, University of California–Riverside, 900 University Ave., Riverside, CA 92521 (e-mail: manuela.martins@ucr.edu).

Abbreviations and Acronyms

ECM = extracellular matrix

FN = fibronectin

LN = laminin

Figure 1. Integrin family members: members of the integrin family of ECM receptors and their respective ligands are shown. These heterodimeric receptors are composed of one α and one β subunit and are capable of binding a variety of ligands, including Ig superfamily cell adhesion molecules, complement factors, and clotting factors, in addition to ECM molecules. Cell-cell adhesion is largely mediated through integrin heterodimers containing the β_2 subunits, whereas cell-matrix adhesion is primarily mediated via integrin heterodimers containing the β_1 and β_3 subunits. In general, the β_1 integrins interact with ligands found in the connective tissue matrix, including LN, FN, and collagen, whereas the β_3 integrins interact with vascular ligands, including thrombospondin, vitronectin, fibrinogen, and von Willebrand factor. *RGD mediated binding. CO, collagens; C3bi, complement component; FG, fibrinogen; FN, fibronectin; FX, factor X; ICAM, intercellular adhesion molecule; LN, laminin; OSP, osteopontin; TN, tenascin; TSP, thrombospondin; VCAM, vascular cell adhesion molecule; VN, vitronectin; vWF, von Willebrand factor. Reprinted with permission from Dueck-Petreaca and Martins-Green (2007).⁴ Color images available online at www.liebertpub.com/wound

is critical for integrity, function, stability, and resistance to mechanical forces. The subject of the article discussed here is the integrin $\alpha_3\beta_1$ and the ECM molecule laminin (LN) 332 (also called LN 5).

CLINICAL RELEVANCE

The clinical problem addressed by these investigators is re-epithelialization of wounds. Proper closure of wounds is necessary, because the epithelium protects the organism from local and potential systemic infections and additional damage to the underlying tissue. Further, as the epithelium closes the wound, it forms strong interactions with the dermis, which provide strength to the skin and prevent reopening of the wound. Complete and proper closure is critical to avoid blister formation. The latter problem occurs in major skin diseases such as the genetic disease epidermolysis bullosa and the acquired disease bullous pemphigoid, for which there is still no cure.^{5,6}

RELEVANT BASIC SCIENCE CONTEXT

Integrin expression is primarily restricted to the basal keratinocytes of the epidermis. As these cells differentiate, they stop proliferating, lose integrin expression, move to the suprabasal layers of the epidermis, and differentiate. Change in integrin expression also occurs when the keratinocytes proliferate and migrate to close wounds, in skin diseases such as psoriasis, and also when carcinomas develop.^{1,2,7} Two integrins are critical in these processes: $\alpha_3\beta_1$ and $\alpha_6\beta_4$. Both bind LN332, a specific, short form of LN that has the important ability to form different types of attachment structures when it interacts with these integrins. Interactions with $\alpha_3\beta_1$ result in focal adhesion formation involving talin, vinculin, and α -actinin, which link LN332 to the microfilament cytoskeleton. This ensemble is involved in short-term adhesions. In the case of $\alpha_6\beta_4$, binding of LN332 contributes to formation of hemidesmosomes, structures that firmly anchor the keratinocytes to the underlying basal lamina (Fig. 2).

The term basal lamina is often confused with the term basement membrane. Basal lamina is primarily deposited by the epithelium and is only visible at the electron microscope level. It is composed of a lamina lucida (contains LN, entactin, and dystroglycans) and a lamina densa (contains primarily Coll IV, nidogen, and perlecan). The basal lamina is in immediate contact with the reticular lamina (contains Coll III, fibronectin [FN], fibrillin, Coll VII, and proteoglycans), which is deposited by the cells of the connective tissue. Coll VII and fibrillin attach the reticular lamina to the basal lamina. The basal lamina plus the reticular lamina form the basement membrane, which is the structure we see with the light microscope when appropriate labels are used (Fig. 2).

The major function of the basement membrane molecules is to anchor the epidermis to the dermis. However, these molecules also convey signals to the epidermal cells via the integrins and mediate communication with the cells in the connective tissue of the dermis to ensure strong dermal/epidermal connections that are instrumental for conferring mechanical strength to the skin.^{1,2,8}

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

The mouse model used by Margadant *et al.*⁹ involves deletion of the α_3 integrin subunit in the basal keratinocytes. The authors generated this model using the integrin α_3 gene floxed (flanked) by 2 loxP sites, sequences of 34 base pairs that can be recognized by the enzyme Cre recombinase. This

Figure 2. Schematic representation of the basal keratinocyte interactions with the basement membrane and the dermis: two types of interactions are seen. One represents a focal adhesion type interaction and the other an anchoring-type interaction through hemidesmosomes. The focal adhesion involves $\alpha_3\beta_1$, LN332, and critical proteins that link the integrin intracellular domains to the microfilament cytoskeleton. The hemidesmosome interaction involves $\alpha_6\beta_4$, LN332, Coll XVII, and BP230 as well as plectin, which helps in the association of the intracellular domain of the integrin and the keratin cytoskeleton. Both adhesion complexes interact with Coll IV, perlecan, and nidogen in the lamina densa of the basal lamina and in turn interact with Coll VII and Coll I and III in the reticular lamina. V, vinculin; T, talin; α_A , alpha actinin; BP, Bullous Pemphigoid. Color images available online at www.liebertpub.com/wound

arrangement allows for the development of conditional knockout mice in which the gene of interest can be deleted from a specific tissue by the Cre enzyme. The floxed α_3 gene was introduced in embryonic stem cells in culture, and the cells containing the floxed gene selected for and introduced into mouse blastocysts were implanted into surrogate mouse mothers. The resulting chimeric mice were then crossed to produce mice carrying the floxed α_3 gene in all tissues. To knockout the α_3 integrin in the keratinocytes only, the mice with the floxed gene were crossed with mice carrying the Cre recombinase enzyme that targets the loxP sites. Expression of the Cre enzyme was under the control of the keratin 14 promoter, which is only functional in basal keratinocytes. The result is a

mouse with normal expression of α_3 except that when the Cre enzyme is expressed the integrin is deleted in keratinocytes only. This is a very powerful technique, because it zeros in on exactly the function one wants to investigate. The probability of success is great, although it is conceivable that redundancies in function from other molecules could make up for the deletion.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

The studies presented in this publication were designed to investigate the function of $\alpha_3\beta_1$ /LN332 interactions during wound re-epithelialization. Using the mouse model described above,

the investigators found that mice lacking α_3 integrin in the basal keratinocytes showed local inflammation, hair loss, basement membrane duplication, and loss of dermal–epidermal interactions with blister formation. They also found that the lack of α_3 subunit only led to alterations in the $\alpha_3\beta_1$ integrin but not in other epidermal integrins and that the keratinocytes migrated faster in these conditional knockout mice. They conclude that keratinocyte migration during wound healing is inhibited by the presence of the $\alpha_3\beta_1$ integrin and that this effect is dependent on integrin binding to LN332 that is newly deposited in the wound bed.

Analysis of the defects in the proteins involved in these studies, $\alpha_3\beta_1$ and LN332, is important, because it reveals a number of biological and anatomical problems that affect skin function. It has been known for some time that this integrin is importantly involved in preference of adhesion of foreskin fibroblasts to LN over FN or collagen.¹⁰ It was then shown that this integrin is important in organizing the basement membrane, because $\alpha_3\beta_1$ -knockout mice exhibit blistering at the dermal–epidermal junction in which the basement membrane was ruptured rather than detached, as is the case in epidermolysis bullosa.¹¹ These and other findings suggest that $\alpha_3\beta_1$ is important for maintenance of basement membrane integrity, whereas $\alpha_6\beta_4$ is responsible for stable adhesion of the dermis to the epidermis through hemidesmosomes,^{11,12} but neither integrin is required for epidermal morphogenesis during skin development.¹³ It is also becoming clear that when α_3 pairs with β_1 it contributes to determining the organization of LN332 into higher-order molecular structures within the ECM of keratinocytes. This contributes to LN332 function, particularly in providing strong adhesion between the dermis and the basement membrane.¹⁴ Indeed, when LN332 is first deposited in the basal lamina of keratinocytes, it is arranged in arrays in the absence of $\alpha_3\beta_1$. However, in the presence of $\alpha_3\beta_1$, LN332 reorganizes and is able to bind $\alpha_6\beta_4$, providing stabilization of adhesion and increasing attachment through formation of hemidesmosomes.¹⁴ In addition, in the absence of the α_3 subunit, LN332 does not undergo reorganization from an array structure to a ring structure and the keratinocytes become more migratory.¹⁴ Moreover, $\alpha_3\beta_1$ may also serve as a receptor that functions in the organization of basement membrane; hence, when the α_3 subunit is lacking, specific bridging interactions will be missing, which compromise the integrity of the basement

membrane, a structure critical for dermal–epidermal stability.^{15,16} Indeed, it has been shown that inhibition of α_3 or LN332 during insulin-induced healing results in a less-differentiated epidermis in which the number of keratinocytes is higher, the basal surface is not well defined, the basal lamina is not deposited properly, and there is poor differentiation of the epidermis as judged by keratin 10 labeling.¹⁷

$\alpha_3\beta_1$ may be a modulator of keratinocyte migration that would pace the speed at which they cover the wound to allow for more effective interactions with the basement membrane and dermis. Indeed, it has been proposed that when $\alpha_3\beta_1$ binds LN332, this leads to inhibition of integrins $\alpha_5\beta_1$, which only binds FN, and $\alpha_2\beta_1$, which binds collagen and FN. This confers an adherent phenotype to the keratinocytes, which modulates their migration. When the skin is wounded, the interactions of $\alpha_3\beta_1$ with LN332 are changed by alterations in either of these molecules leading to a phenotype that is non-adherent, releasing its transdominant effects on $\alpha_2\beta_1$ and $\alpha_5\beta_1$, which promote keratinocyte migration.^{12,13}

As indicated later, the paper by Mardagant *et al.* (2009) discussed here provides value to the field by having developed a conditional mouse model for a very important integrin in wound closure and dermal epidermal interactions. This mouse model has broad biological implications, because it also can be used to study processes other than wound healing, which involve $\alpha_3\beta_1$, such as inflammation, blood vessel development, and carcinogenesis.^{1–3,18–20}

INNOVATION

Most of the basic findings in this study had been shown in previous systems using α_3 -null mice.^{11–13} However, these mice have a problem that diminishes their value for application to humans; they die during the neonatal stage because of defects in the kidneys and lungs. As a consequence, investigations cannot be performed on adult animals, leading to the need for conditional knockouts of this integrins. Although the concept for the development of conditional knockout animal models is not new, the authors have used this technology to develop a new mouse model that is very useful to study the function of the integrin $\alpha_3\beta_1$ in epidermal function during wound healing in adults. Prior to this study, the role of integrin $\alpha_3\beta_1$ on keratinocyte migration in adult wound healing was not clear. Mardagant *et al.*,⁹ using this very powerful con-

ditional mouse mutant, showed that $\alpha_3\beta_1$ delays keratinocyte migration during wound healing.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

Although conditional mouse models can be very powerful, they also may have drawbacks. It is important to make sure that there is no leakage in expression, that the expression occurs in the tissue in question, and that it does not result in death of the animal. Further, like any loss-of-function system, it must be confirmed with a gain-of-function approach. Therefore, these mice must be designed carefully and precisely. Regarding the integrin function, it is critical to be aware of reagents that inhibit these receptors, because they may instead activate them by ligating the subunits. In addition, because of redundancy in integrin function, the relevance of the specific integrin being studied can be obscured. To avoid some of the drawbacks, it is wise to use a variety of other approaches *in vivo* and *in vitro* that help confirm the results obtained with these conditional mutant animals.

FUTURE DEVELOPMENT OF INTEREST

It is quite evident by now that the $\alpha_3\beta_1$ integrin and LN332 are not only important in dermal-epidermal interactions but also involved in wound closure and promotion of angiogenesis during wound healing, and that the lack of this integrin can contribute to inflammation, hair loss, and kidney and lung abnormalities during embryonic development. These molecules also play a role in carcinogenesis of the skin and other neoplastic conditions. Therefore, it is important to determine whether their functions are similar in all of these processes or if they are microenvironment dependent. The conditional mouse developed by Margadant *et al.*⁹ will be very useful to pursue such investigations. *In vitro* approaches should be used with caution to decipher mechanism when it is not possible to do it *in vivo*, because *in vitro* conditions do not mimic the complexity of the wound environment.

TAKE-HOME MESSAGES

Basic science advances

- Integrins are a very important family of receptors that bind to ECM molecules and lead to signaling cascades that translate in a variety of cellular effects including keratinocyte migration and other functions of the epidermis.^{1-3,17}
- Integrins have also been implicated in various stages of carcinogenesis.¹⁹
- LN332 has not only been implicated in wound healing¹⁷ but also in squamous-cell carcinoma.⁷ In this cancer, it works through signaling cascades that promote tumor invasion and metastasis.⁷
- Because of the multiple combinations of pairing between α and β subunits, the ability of each receptor to bind several different ECM molecules, and the influence each integrin has on others, it is very difficult to decipher function and to identify redundancies.
- Models such as the conditional model developed by Margadant *et al.*⁹ will provide the opportunity for making major advances in understanding the complex function of these receptors during healing.

Clinical science advances

- This article contributes to clinical advances in integrin biology, because the model and the findings provide an opportunity to decipher mechanisms of disease and for potential development of treatments for human skin disorders and impaired healing, including chronic ulcers.
- This knowledge could lead to better understanding of the role of integrins in cancers for which the α_3 subunit is important.
- Not much is being done to target integrins in therapies for wound healing, but that is not the case for cancer. At least six integrin inhibitors are currently being used in clinical trials, particularly for melanoma.²¹

Relevant clinical care

- The work presented in this article provides knowledge that leads to the understanding of delayed re-epithelialization during wound healing and dermal epidermal defects, blistering, and chronic skin diseases.
- This knowledge can potentially inform physicians on how to approach treatment, in general, but, in particular, allow for a systems biology approach that will benefit each individual being treated.

ACKNOWLEDGMENTS AND FUNDING SOURCES

The author thanks Drs. Vandewater and Green for helpful discussions and Allen Wang for help with the formatting of the references and posting of the article. The author also thanks the University of California at Riverside for support during the preparation of this article.

AUTHOR DISCLOSURE AND GHOSTWRITING

The author has nothing to disclose. No ghostwriters were used to write this article.

REFERENCES

1. Margadant C, Charafeddine RA, and Sonnenberg A: Unique and redundant functions of integrins in the epidermis. *FASEB J* 2010; **24**: 4133.
2. Watt FM: Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO* 2002; **21**: 3919.
3. Truong H and Danen EH: Integrin switching modulates adhesion dynamics and cell migration. *Cell Adh Migr* 2009; **3**: 179.
4. Dueck-Petreaca M and Martins-Green M: Cell-extracellular matrix interactions with implications for tissue engineering. In: *Principles of Tissue Engineering*, 3rd edition, edited by Lanza R, Langer R, and Vacanti J. Academic Press, 2007, pp 81–99.
5. Ludwig RJ and Zillikens D: Pathogenesis of epidermolysis bullosa acquisita. *Dermatol Clin* 2011; **29**: 493.
6. Parker SR and McKelfresh J: Autoimmune blistering diseases in the elderly. *Clin Dermatol* 2011; **29**: 69.
7. Marinkovich MP: Laminin 332 in squamous-cell carcinoma. *Nat Rev Cancer* 2007; **7**: 370.
8. Dueck-Petreaca M and Martins-Green M: Biologic and molecular basis of regenerative medicine: cell-extracellular matrix interactions in repair and regeneration. In: *Principles of Regenerative Medicine*, edited by Atala A, Lanza R, Thomson J, and Nerem R. Academic Press, 2008, pp. 66–99.
9. Margadant C, Raymond K, Kreft M, Sachs N, Janssen H, and Sonnenberg A: Integrin $\alpha_3\beta_1$ inhibits directional migration and wound re-epithelialization in the skin. *J Cell Sci* 2009; **122**: 278.
10. Carter RT: The role of integrins in corneal wound healing. *Vet Ophthalmol* 2009; **12 (Suppl 1)**: 2.
11. DiPersio CM, Hodivala-Dilke KM, Jaenisch R, Kreidberg JA, and Hynes RO: Alpha-3-beta-1 integrin is required for normal development of the epidermal basement membrane. *J Cell Biol* 1997; **137**: 729.
12. Hodivala-Dilke KM, DiPersio CM, Kreidberg JA, and Hynes RO: Novel roles for $\alpha_3\beta_1$ integrin as a regulator of cytoskeletal assembly and as a trans-dominant inhibitor of integrin receptor function in mouse keratinocytes. *J Cell Biol* 1998; **142**: 1357.
13. DiPersio CM, van der Neut R, Georges-Labouesse E, Kreidberg JA, Sonnenberg A, and Hynes RO: $\alpha_3\beta_1$ and $\alpha_6\beta_4$ integrin receptors for laminin-5 are not essential for epidermal morphogenesis and homeostasis during skin development. *J Cell Sci* 2000; **113 (Pt 17)**: 3051.
14. de Hart GW, Healy KE, and Jones JCR: The role of $\alpha_3\beta_1$ integrin in determining the supramolecular organization of laminin-5 in the extracellular matrix of keratinocytes. *Exp Cell Res* 2003; **283**: 67.
15. Wu C, Chung AE, and McDonald JA: A novel role for $\alpha_3\beta_1$ integrins in extracellular matrix assembly. *J Cell Sci* 1995; **108**: 2511.
16. DiPersio CM, Shah S, and Hynes RO: $\alpha_3\beta_1$ integrin localizes to focal contacts in response to diverse extracellular matrix proteins. *J Cell Sci* 1995; **108**: 2321.
17. Liu Y, Petreaca M, Yao M, and Martins-Green M: Cell and molecular mechanisms of keratinocyte function stimulated by insulin during wound healing. *BMC Cell Biology* 2009; **10**: Article No.: 1.
18. Mitchell K, Szekeres C, Milano V, Svenson KB, Nilsen-Hamilton M, Kreidberg JA, *et al.*: $\alpha_3\beta_1$ integrin in epidermis promotes wound angiogenesis and keratinocyte-to-endothelial-cell crosstalk through the induction of MRP3. *J Cell Sci* 2009; **122**: 1778.
19. Moschos SJ, Drogowski LM, Reppert SL, and Kirkwood JM: Integrins and cancer. *Oncology* 2007; **21 (9 Suppl 3)**: 13.
20. Subramani D and Alahari SK: Integrin-mediated function of Rab GTPases in cancer progression. *Mol Cancer* 2010; **9**: Article No.: 312.
21. Tucker GC: Integrins: molecular targets in cancer therapy. *Curr Oncol Rep* 2006; **8**: 96.