Dynamic Regulation of Integrin $\alpha_6\beta_4$ During Angiogenesis: Potential Implications for Pathogenic Wound Healing

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Objective: Angiogenesis is an essential component of normal cutaneous wound repair, but is altered in pathogenic forms of wound healing, such as chronic wounds and fibrosis. We previously reported that endothelial expression of integrin $\alpha_6\beta_4$ is developmentally regulated, with $\alpha_6\beta_4$ expression correlating with tissue maturation and further showed that endothelial $\alpha_6\beta_4$ is down-regulated in explant angiogenesis assays. These data support the hypothesis that dynamic regulation of $\alpha_6\beta_4$ may play an important role during new vessel formation in healing wounds.

Approach: To test this hypothesis, we examined the endothelial expression of $\alpha_6\beta_4$ using a murine model of cutaneous wound healing and *in vitro* cultures of primary human dermal microvascular endothelial cells (HDMECs).

Results: Expression of $\alpha_6\beta_4$ is downregulated during early stages of wound healing; angiogenic vessels in day 7 wounds do not express $\alpha_6\beta_4$. Endothelial expression of $\alpha_6\beta_4$ is resumed in day 14 wounds. Moreover, explanted HDMECs do not express $\alpha_6\beta_4$, but expression is induced by treatment with histone deacetylase inhibitors.

Innovation: We provide *in vivo* data supporting a role for the dynamic regulation of $\alpha_6\beta_4$ during vessel formation and remodeling during cutaneous wound repair and *in vitro* findings that suggest endothelial β_4 expression is regulated transcriptionally, providing an important foundation for future studies to understand the transcriptional mechanisms involved in endothelial cell maturation during normal wound repair.

Conclusion: Our data indicate that $\alpha_6\beta_4$ is dynamically regulated during angiogenesis and vessel maturation and suggest that disruption of this regulation may contribute to defective angiogenesis associated with diabetic wounds or cutaneous fibrosis.

INTRODUCTION

THE VASCULATURE DELIVERS oxygen and nutrients to meet the metabolic needs of all the tissues in the body. The vasculature is remodeled in the adult; new vessels are formed from pre-existing ones by a process referred to as angiogenesis, which is critical to many normal physiological processes, including wound repair.^{1,2}

However, misregulation of angiogenesis, both inadequate and excessive, contributes to a wide range of diseases, including skin-associated pathologies.^{3,4} For example, reduced neovascularization is a cardinal feature of chronic, insufficient wound healing such as diabetic ulcers.^{5,6} The balance between angiogenic and angiostatic mechanisms during ves-

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Abbreviations and Acronyms

CD31 = cluster differentiation antigen 31

ECM = extracellular matrix

FBS = fetal bovine serum

HDAC = histone deacetylase

HDMECs = human dermal microvascular endothelial cells

IgG = immunoglobulin G

RNA = ribonucleic acid

RT-PCR = reverse-transcription polymerase chain reaction

SD = standard deviation

TSA = trichostatin A

sel remodeling is thought to impact fibrosis as has been reported for idiopathic pulmonary fibrosis, scleroderma, and fibrosis in the eye.^{7–10} Thus, angiogenesis remains an important area of investigation for the identification of new targets for the development of therapeutic interventions in chronic and fibrotic wound healing.

Angiogenesis requires a complex temporal and spatial regulation of numerous extracellular signaling molecules, their receptors, downstream signaling cascades, cell–cell and cell–matrix adhesion receptors, in particular integrins, as well as the remodeling of extracellular matrices and basement membranes.^{3,11} Angiogenesis is initiated when quiescent endothelial cells receive angiogenic signals from growth factors, chemokines, and extracellular matrix (ECM) molecules.^{3,11} In cutaneous wound, these signals are released from platelets, macrophages, keratinocytes, and myofibroblasts at the wound site.^{2,12} These signals trigger a cascade of events, leading to the activation and the sprouting of endothelial cells and their invasion into the neighboring interstitial matrix, or provisional matrix in the case of cutaneous wound. Endothelial cells proliferate and invade as stalks, with neighboring stalks fusing with one another to form an immature tubular network. Pericytes are recruited and a basement membrane is assembled. The neovasculature is then remodeled: some vessels regress as others mature to meet the needs of the healing tissue.²

Several integrins regulate the formation of new vessels.^{13–16} Integrins are α/β heterodimeric receptors that mediate the adhesion of cells to components of the ECM and basement membranes.¹⁷ Integrins are also signaling receptors, collaborating with other surface receptors to regulate cell migration, proliferation, survival, and differentiation.^{17,18} Although integrin $\alpha_6\beta_4$ is mostly known for its role in simple and stratified epithelia, where it mediates strong adhesion to laminin in the basement membrane,¹⁹ $\alpha_6\beta_4$ is also expressed by other cell types. We previously demonstrated that $\alpha_6\beta_4$ is expressed in the human dermal microvasculature.²⁰ Analysis of individual cells isolated from trypsin-disrupted foreskin tissue indicated that $\alpha_6\beta_4$ is expressed by a subset of epithelial cells and endothelial cells.²⁰ Notably, cells expressing smooth muscle actin, such as vascular smooth muscle cells, did not express the β_4 subunit.²⁰ We also examined endothelial expression of $\alpha_6\beta_4$ in murine tissue and found that both small and medium size vessels express $\alpha_6\beta_4$ and that the endothelial expression of $\alpha_6\beta_4$ coincided with tissue

differentiation, and therefore may be associated with vessel maturation.²⁰ Endothelial expression of $\alpha_6\beta_4$ is downregulated in explant angiogenesis assays using human saphenous vein explants cultured in fibrin gels. The $\alpha_6\beta_4$ integrin was not expressed in outgrowing endothelial cells consistent with the notion that $\alpha_6\beta_4$ is expressed only in mature vessels.²⁰ Interestingly, the expression of $\alpha_6\beta_4$ in Schwann cells, thymocytes, and monocytes also correlates with the differentiated and quiescent phenotype.^{21–24}

In the current study, we sought to determine whether or not the $\alpha_6\beta_4$ integrin is dynamically regulated during angiogenesis associated with wound repair. We show that the endothelial expression of $\alpha_6\beta_4$ is downregulated in angiogenic vessels in granulation tissue at day 7 following wounding and re-expressed as the neovasculature matures and the wound resolves. We also found that an inhibitor of histone deacetylases (HDACs) derepressed $\alpha_6\beta_4$ expression in cultured dermal microvascular endothelial cells. We suggest that inhibition of the dynamic regulation of $\alpha_6\beta_4$ in endothelial cells may lead to defects in the formation of the neovasculature that could contribute to pathological wound healing.

CLINICAL PROBLEM ADDRESSED

The current study determined the pattern of expression of $\alpha_6\beta_4$ in angiogenesis and vessel remodeling associated with cutaneous wound healing. It has clinical relevance, as abnormal angiogenesis and vessel remodeling is associated with chronic wounds, such as diabetic ulcers, and overexuberant fibrotic wounds.

MATERIALS AND METHODS

Wounding

Four full-thickness wounds (4 mm in diameter) were placed on the dorsum of anesthetized female adult BALB/C mice (triplicate mice, 5–6 weeks old) as described previously.²⁵ Mice were euthanized 7 or 14 days after wounding. Skin and wound tissue were harvested and processed for frozen sections as previously described.^{20,25} All procedures for animal care and handling had the approval of the Albany Medical College Institutional Animal Care and Use Committee.

Immunofluorescence microscopy

Tissue sections were fixed and processed for immunofluorescence microscopy as previously described.^{20,25} The following antibodies were used. Rabbit polyclonal antibodies to mouse cluster

differentiation antigen 31 (CD31)²⁶ were a generous gift from Dr. J. Madri (Yale University). The rat monoclonal antibody GoH3 to the α_6 subunit and the rat monoclonal antibody 345-11A to the β_4 subunit were from BD Biosciences. Goat anti-rat immunoglobulin G (IgG) AlexaFluor[®]594 and goat anti-rabbit IgG AlexaFluor[®]488 were from Molecular Probes.

Immunostaining was analyzed using a Nikon TE-2000-E inverted microscopy equipped with phase contrast and epifluorescence, a CoolSNAP HQ digital camera (Roper Scientific), a Ludl rotary encoded stage and Metavue[™] acquisition and image analysis software (Molecular Devices). Images were acquired using the same exposure conditions and processed similarly for quantitative analysis. CD31-positive structures that were also positive for either β_4 or α_6 were identified by overlayed images. Manual counting was performed using a feature of Metavue analysis software that both tallied and marked counted structures to prevent duplicate sampling. The percentage of vessels, identified by CD31 staining, that expressed the α_6 or β_4 subunit was determined per section. The mean percentage of vessels from sections of two wounds from each of these mice was calculated together with the standard deviation. The signifi-

cance of differences observed between normal skin and 7- and 14-day wounds was determined as indicated in the legend to Fig. 3.

Cell culture

Human dermal microvascular endothelial cells (HDMECs) were purchased from Vec Technologies and were cultured on gelatin-coated tissue culture plates in MCDB-131 (Invitrogen) containing 20% fetal bovine serum (FBS; Hyclone), 2 mM L-glutamine (Invitrogen), 10 μ g/mL heparin (Sigma Aldrich), and Egm-2mv Quot supplements (Fisher Scientific). HaCAT cells were generously provided by Dr. P. Higgins (Albany Medical College) and were cultured in Dulbecco's modified Eagle's medium containing 10% FBS, 2 mM L-glutamine, 10 mg/mL streptomycin, and 10 U/mL penicillin (Invitrogen).

Reverse-transcription polymerase chain reaction

HDMECs were treated with trichostatin A (TSA; Sigma-Aldrich) and 5-azacytidine (Sigma-Aldrich) as indicated in the legend to Fig. 4. Ribonucleic acid (RNA) was purified from HDMECs and HaCAT cells using TRIzol Reagent (Invitrogen) following the manufacturer's instructions. Reverse-transcription (RT-) polymerase chain reaction (PCR)

Figure 1. Expression of the integrin α_6 and β_4 subunits in the murine dermis. Shown are representative images of normal skin co-immunostained with antibodies to CD31 (green) together with antibodies to either the α_6 or β_4 integrin subunit (red). Asterisks mark the basal keratinocyte layer and hair follicles. Arrowheads indicate examples of CD31-positive vessels expressing the α_6 subunit (upper panels) or the β_4 subunit (lower panels). A group of CD31-positive vessels expressing the α_6 subunit is circled (upper panels). Bar, 100 μ m. CD31, cluster differentiation antigen 31. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

was performed using total RNA, random primers from Promega, and Superscript Reverse Transcriptase from Invitrogen. Reactions for each RNA sample were also incubated without reverse transcriptase to use for control PCR. Primers for α_6 and β_4 were purchased from Sigma-Aldrich; primers for β -actin were generously provided by Dr. D. Avram (Albany Medical College). Forward (F) and reverse (R) primers used for PCR are as follows: integrin β_4 -F (TGT GAG GAA TGC AAC TTC AAG) and integrin β_4 -R (GAG CCA CCA GAA GGA GCC); integrin α_6 -F (AAA TCC TGT TTT GAA TAT ACT GC) and integrin α_6 -R (AGG GTT TCC TCC ATG CAC AC); and actin-F (TAC CTC ATG AAG ATC CTC ACC) and actin-R (TTT CGT GGA TGC CAC AGG AC). PCR was performed using Hot Master

Taq polymerase (VWR International) following the manufacturer's protocol. After an initial incubation at 95°C for 2 min, reactions were incubated for 25 cycles: 95°C for 40 s; 55°C (β_4), 53°C (α_6), or 55°C (actin) for 40 s; and 68°C for 40 s. PCR products were analyzed on 1.5% agarose gels and visualized under UV light after staining with ethidium bromide.

RESULTS

Because we previously found that endothelial expression of $\alpha_6\beta_4$ is developmentally regulated, and because $\alpha_6\beta_4$ is not expressed at the early stages of angiogenesis as determined in explant assays,²⁰ we were interested to determine whether

Figure 2. Endothelial expression of the α_6 subunit during wound healing. **(A)** Shown are representative images of the expression of α_6 (red) and the endothelial marker CD31 (green) in 7- and 14-day wounds. Integrin α_6 is expressed by keratinocytes of the basal layer of the epidermis, hair follicles (normal skin), hyperproliferative keratinocytes of the wound, in addition to endothelial cells. **(B)** Quantification of endothelial expression of α_6 in normal skin (NS), and in 7- and 14-day wound tissue. Plotted is the mean percentage of vessels ($\pm$ SD) identified by CD31 staining that are also positive for α_6 . Sections of two wounds from each of the three mice were quantified per condition. Asterisks indicate proliferative keratinocytes in day 7 wounds and the keratinocyte layer in day 14 wounds. The majority of CD31-positive vessels in day 7 and 14 wounds express the α_6 subunit. Bar, 100 μ m. SD, standard deviation. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

$\alpha_6\beta_4$ is expressed during angiogenesis associated with cutaneous wound healing. For these studies, we used a well-accepted, murine model of cutaneous healing in which, full-thickness, replicate excisional wounds at defined intervals were prepared. We used antibodies to the β_4 subunit to examine the expression of the $\alpha_6\beta_4$ integrin. This is sufficient to track $\alpha_6\beta_4$ expression, because the β_4 subunit only heterodimerizes with the α_6 subunit.¹⁷ It is also important to note that the α_6 subunit also heterodimerizes with the β_1 ; however, when both β_1 and β_4 subunits are expressed, α_6 preferentially heterodimerizes with β_4 .¹⁷

Our previous published studies demonstrated that $\alpha_6\beta_4$ is expressed by a subset of small- and medium-sized vessels in both human and mouse dermis.²⁰ As a control and for the purpose of quantification, we re-examined the expression of the α_6 and β_4 subunits in the unwounded murine dermis. Figure 1 shows representative micrographs of normal murine skin costained with antibodies either to the integrin β_4 subunit and CD31 or to the integrin α_6 subunit and CD31. As expected, the integrin β_4 subunit, and thus $\alpha_6\beta_4$, is expressed in a subset of vessels in the mouse dermis. This is also true of the expression of $\alpha_6\beta_4$ in the human dermis.²⁰

Figure 3. Endothelial expression of the β_4 subunit during wound healing. **(A)** Shown are representative images of the expression of β_4 (red) and the endothelial marker CD31 (green) in 7- and 14-day wounds. Like the α_6 subunit, the β_4 subunit is expressed in proliferative keratinocytes of the wound (*); however, unlike the α_6 subunit, the β_4 subunit is not expressed by CD31-positive vessels in 7-day wounds. *Asterisks in the images indicate the keratinocyte layer in day 14 wounds. The β_4 subunit is expressed by the majority of CD31-positive vessels at day 14. A few examples are indicated by arrowheads. Bar, 100 μ m. **(B)** Quantification of endothelial expression of β_4 in normal skin (NS), and in 7- and 14-day wound tissue. Plotted is the mean percentage of vessels ($\pm$ SD) identified by CD31 staining that are also positive of β_4 . Sections of two wounds from each of the three mice were quantified per condition. * $p < 0.05$. Significance was determined using a one-way analysis of variance with the Dunnett's multiple comparison test for *post hoc* analysis. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

To determine whether $\alpha_6\beta_4$ is expressed by angiogenic vessels associated with cutaneous wound repair, we analyzed tissue from 7-day wounds; at this time, granulation tissue has formed and contains many angiogenic vessels. The results indicate that although angiogenic vessels at day 7 express the α_6 subunit (Fig. 2A), they do not express the β_4 subunit (Fig. 3A). In the absence of β_4 , the α_6 subunit heterodimerizes with the β_1 subunit. Thus, these vessels express the $\alpha_6\beta_1$ integrin. Importantly, the lack of expression of the β_4 subunit in endothelial cells in day 7 wounds indicates that the $\alpha_6\beta_4$ integrin is downregulated during wound angiogenesis.

By day 14 after wounding, granulation tissue has begun to transform into scar tissue. The wound vasculature initiates remodeling with the regression of some capillaries and the maturation of the neovasculature. We analyzed endothelial β_4 expression at day 14 to determine whether the $\alpha_6\beta_4$ integrin would be re-expressed as the neovasculature matures. The data indicate that similar to α_6 expression at day 14, the majority of vessels expressed the β_4 subunit, and thus, the $\alpha_6\beta_4$ integrin (Figs. 2A and 3A). The results indicate that $\alpha_6\beta_4$ is re-expressed in endothelial cells of vessels as the wound resolves.

We also quantitatively compared the number of CD31-positive vessels that expressed the α_6 subunit in normal skin, and in day 7 and 14 wounds (Fig. 2B). We similarly examined the expression of the β_4 subunit (Fig. 3B). The vast majority of CD31-positive vessels express the α_6 subunit in normal skin as well as in 7- and 14-day wound tissue (Fig. 2B). In contrast, slightly less than half the CD31-positive vessels in normal skin express the β_4 subunit (Fig. 3B). Seven days after wounding this number drops to about 20%. By 14 days after wounding, the percentage of CD31-positive vessels that express the β_4 subunit is up to ~70% (Fig. 3B). Thus, the endothelial expression of $\alpha_6\beta_4$ integrin is dynamically regulated during cutaneous wound healing.

Growing evidence suggests that the epigenetic control of gene expression plays an important role in regulating both normal and pathological wound repair.^{27,28} The inhibition of histone deacetylases in fibroblasts with TSA suppresses fibrosis, and thus, promotes wound resolution.^{29,30} Epigenetic regulation of gene expression is likely critical to the behavior of other cellular components of the healing wound. Our published studies demonstrate that endothelial β_4 expression is downregulated when we place saphenous explants in fibrin gels in culture.²⁰ Others have reported the loss of $\alpha_6\beta_4$

expression in cultured murine lung endothelial cells.³¹ Because HDMECs express $\alpha_6\beta_4$ *in situ*, we wanted to examine the expression of β_4 and its regulation in cultured primary HDMECs. Using an RT-PCR approach, we found that β_4 mRNA was not easily detected in cultured HDMECs, although it was observed in the HaCAT keratinocyte cell line as expected (Fig. 4). Given the known effects of TSA on wound fibroblasts,^{29,30} we were interested to determine whether the treatment of primary HDMECs with TSA promoted the expression of β_4 mRNA, since the β_4 subunit is expressed by mature endothelial cells. The data indicate that treatment with the HDAC inhibitor, TSA, and the DNA methylation inhibitor 5-azacytidine promoted the expression of integrin β_4 mRNA (Fig. 4), suggesting that the inhibition of β_4 expression in HDMECs occurs by a mechanism involving transcriptional repression by HDACs and DNA methylation. Thus, β_4 expression is actively repressed in cultured primary dermal endothelial cells, but can be derepressed experimentally. This data suggest that the endothelial expression of β_4 may be regulated epigenetically during wound repair, with its expression repressed during angiogenesis, perhaps, by mechanisms involving HDACs and DNA methylation, which are reversed during the maturation of the vasculature and resolution of the wound.

Figure 4. β_4 expression is inhibited by the HDAC activity in primary HDMECs when placed in culture. Total RNA was isolated from HaCAT (C), a human keratinocyte line, and from cultured HDMECs, which were either left untreated (–) or treated with 300 mM TSA for 24 h, with 2 mM 5-azacytidine for 48 h, or with 2 mM 5-azacytidine for 48 h together with 300 mM TSA for the last 24 h. Primer pairs were designed to hybridize with the coding sequences of β_4 , α_6 , or actin that were interrupted by large introns in genomic DNA, but would generate reverse-transcription polymerase chain reaction products ~200 bp in length from the corresponding mRNA. Reactions were incubated with (+rt) or without (–rt) reverse transcriptase and analyzed by electrophoresis together with size markers (M). HDAC, histone deacetylases; HDMECs, human dermal microvascular endothelial cells; RNA, ribonucleic acid; TSA, trichostatin A.

DISCUSSION

Wound repair is a complex process that is influenced by many physiological and environmental factors, which can impact both angiogenesis and vascular remodeling.³² Published data from many laboratories suggest that the interaction of endothelial cells with components of the ECM and basement membranes regulates new vessel formation, remodeling, and homeostasis.¹⁵ In this article, we showed that integrin $\alpha_6\beta_4$ is not expressed by endothelial cells during angiogenesis in granulation tissue, but its expression is induced later, presumably as vessels mature. These data together with our published studies^{20,31} suggest that $\alpha_6\beta_4$ does not function during endothelial migration, proliferation, or tube formation, but is required later for vessel maturation and function. The loss of endothelial expression of $\alpha_6\beta_4$ during angiogenesis and its upregulation in vessels as the wound resolves is the first example of such dynamic regulation of integrin expression during angiogenesis. The α_v and β_1 integrins have been shown to be important regulators of angiogenesis in a number of different *in vivo* models.^{15,16} Integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, and $\alpha_v\beta_3$ are upregulated in angiogenic endothelial cells and antagonists of these receptors inhibit angiogenesis. Moreover, a number of keratinocyte integrins, including $\alpha_6\beta_4$ are upregulated following cutaneous injury.^{25,33}

In simple and stratified epithelia, $\alpha_6\beta_4$ mediates strong adhesion to laminin in the basement membrane due to the unique ability of the β_4 cytoplasmic domain to connect the $\alpha_6\beta_4$ integrin with keratin intermediate filaments.¹⁹ Previous studies from our laboratory demonstrated that the β_4 cytoplasmic domain connects the $\alpha_6\beta_4$ integrin with the vimentin intermediate filament cytoskeleton, suggesting that endothelial $\alpha_6\beta_4$ may also function in mediating strong adhesion,^{34,35} which would be consistent with its expression in mature vessels.

Basement membranes associated with vascular endothelium contain two laminin isoforms: laminin-411, composed of the α_4 , β_1 , and γ_1 subunits, previously referred as laminin-8; and laminin-511, composed of the α_5 , β_1 , and γ_1 subunits, previously referred as laminin-10.³⁶ Interestingly, laminin-411 is expressed by all endothelial cells, regardless of the developmental stage. The $\alpha_6\beta_1$ and $\alpha_3\beta_1$ integrins are known to bind to laminin-411; however, there are no studies to date indicating that $\alpha_6\beta_4$ can do so.³⁶ Thus, angiogenic vessels lacking β_4 , but expressing the α_6 subunit could adhere to laminin-411 via $\alpha_6\beta_1$. Interestingly, current evidence indicates that laminin-511 is not expressed until after birth and expression is limited to capillaries, ve-

nules, and quiescent mature vessels.³⁶ Integrin $\alpha_6\beta_4$ can bind to laminin-511,³⁶ consistent with our observation that $\alpha_6\beta_4$ is expressed as vessels mature.

In addition to mediating cell adhesion, the $\alpha_6\beta_4$ integrin also functions in signal transduction, collaborating with other cell surface receptors.^{37,38} Nikolopoulos *et al.* used a targeted mutation of the C-terminal signaling portion of the β_4 cytoplasmic tail to examine the role of $\alpha_6\beta_4$ during angiogenesis.³¹ From these studies, the authors concluded that $\alpha_6\beta_4$ is required for the initiation of angiogenesis,³¹ suggesting that $\alpha_6\beta_4$ may be required for quiescent endothelial cells to respond to angiogenic signals.³¹ However, it is important to note that the expression of the mutant β_4 integrin was not restricted to endothelial cells, and thus, some of the observed effects on angiogenesis, could be indirect due to the lack of normal integrin $\alpha_6\beta_4$ function in other cellular compartments, such as the epidermis. In fact, others have shown that the inhibition of the $\alpha_3\beta_1$ integrin in keratinocytes suppresses wound angiogenesis.³⁹ Our data are not inconsistent with the findings of Nikolopoulos *et al.*, however, we suggest that endothelial expression of $\alpha_6\beta_4$ may need to be downregulated to allow endothelial proliferation and tube formation during early stages of angiogenesis. Thus, the $\alpha_6\beta_4$ integrin may be a negative regulator of some stages of the angiogenic process. Moreover, it is intriguing that $\alpha_6\beta_4$ is expressed by the majority of vessels in 14-day wounds, suggesting that most vessels temporarily express $\alpha_6\beta_4$ before the remodeling and maturation of the neovasculature is complete. These findings are consistent with published studies from Jarvinen and Ruoslahti who demonstrated temporal changes in phage-derived peptide binding as the wound vasculature matured.⁴⁰ Importantly, an understanding of the function of $\alpha_6\beta_4$ during angiogenesis and the remodeling of the neovasculature will require both the inducible, targeted endothelial deletion of the β_4 subunit, as well as the inducible, forced endothelial expression of the β_4 subunit from a heterologous promoter.

In summary, we demonstrated that dynamic regulation of the endothelial expression of the $\alpha_6\beta_4$ integrin occurs during cutaneous wound healing. We also observed in dermal endothelial cells that inhibitors of HDACs relieve the transcriptional repression of $\alpha_6\beta_4$ integrin. Given our observations that the endothelial expression of $\alpha_6\beta_4$ is temporally regulated at key junctures during wound repair, it will be very interesting to examine the endothelial expression of the $\alpha_6\beta_4$ in chronic and fibrotic wounds. Targeting angiogenesis may pro-

KEY FINDINGS

- Endothelial expression of the $\alpha_6\beta_4$ integrin is downregulated during angiogenesis associated in the granulation tissue stage of cutaneous wound repair.
- The $\alpha_6\beta_4$ integrin is re-expressed coincident with the cessation of granulation tissue.
- The expression of the β_4 subunit is repressed in cultured primary endothelial cells by a mechanism requiring the HDAC activity.

vide novel therapeutic interventions for chronic and fibrotic wound healing.

INNOVATION

There is a growing awareness that the mechanisms governing vascular maturation and remodeling are critical to the progress of normal wound healing, as well as to pathological healing as it occurs in chronic, insufficient wounds and in fibrotic, exuberant healing. We now provide foundation data, acquired *in vivo* and *in vitro*, supporting a role for the regulated expression of $\alpha_6\beta_4$ at the transcriptional level during vessel remodeling. Therapeutic manipulation of $\alpha_6\beta_4$ expression may provide a novel approach to modulating or promoting vessel maturation in pathogenic wounds.

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