Vascular Endothelial Growth Factor and Cutaneous Scarring

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Significance: The proangiogenic mediator vascular endothelial growth factor (VEGF) plays an important role in cutaneous wound repair. Most of the work on VEGF and wound healing has focused on its role in mediating angiogenesis and how this affects wound closure rates. Less is known about how VEGF affects other phases of wound healing, including scar formation.

Recent Advances: Over the last 10 years, mounting evidence suggests that VEGF plays an important role in regulating scar tissue production. Multiple studies have linked high VEGF levels with scar formation in normal, hypertrophic, and keloid scars. In addition, there is experimental evidence that VEGF inhibition can reduce scar tissue deposition.

Critical Issues: While there is evidence that VEGF can promote scar formation in the skin, there are several unanswered questions that remain. First, the mechanisms by which VEGF promotes scar formation have not been completely characterized. While both indirect and direct mechanisms could be involved, clear evidence for a specific mechanism is lacking. In addition, despite the availability of anti-VEGF drugs, the potential value in targeting VEGF to attenuate scar formation clinically is not yet known.

Future Directions: While there are a significant number of studies examining the effects of VEGF on angiogenesis and wound closure, much less attention has been paid to the contribution of VEGF to scar tissue production. Additional studies are required to learn more about how VEGF regulates scar formation and whether VEGF inhibition could be used clinically to manage scars.

Keywords: VEGF, scar, angiogenesis, hypertrophic scar, keloid

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SCOPE AND SIGNIFICANCE

WOUND HEALING IN FULLY developed skin is an imperfect process that leads to the formation of scar tissue rather than normal tissue. Scars are characterized by disorganized collagen and a lack of skin appendages. The biomechanical and architectural properties of scars are unique; as a result, scar tissue is weaker and functions at a suboptimal level compared to normal skin. Many different proteins produced during wound healing have been shown to regulate scar formation in

the skin with the most well-studied fibrogenic mediator being transforming growth factor-beta. Another protein that is produced at high levels during the repair process is vascular endothelial growth factor (VEGF or VEGF-A), and there has been mounting evidence over the last 10 years that VEGF stimulates scar tissue production. This review will discuss published data implicating VEGF in the regulation of scar formation, as well as possible underlying mechanisms and potential clinical implications.

TRANSLATIONAL RELEVANCE

VEGF has been studied extensively in the context of cutaneous wound healing. Most studies have focused on the role of VEGF as a proangiogenic mediator, which is understandable given the importance of angiogenesis in wound repair. However, recent studies have indicated that VEGF may also act as a profibrotic mediator. The mechanisms by which VEGF promotes scar formation are not entirely clear, but several have been proposed. These include indirect effects associated with the stimulation of endothelial cells or amplification of inflammation and possible direct effects on fibroblasts. Understanding more about how VEGF influences scar formation could aid in the development of new therapeutic strategies to minimize scar formation.

CLINICAL RELEVANCE

Scarring is a significant clinical problem that affects millions of patients. Unfortunately, there is currently a lack of clinically beneficial noninvasive antiscar therapies, so finding new therapeutic targets to control scar formation is important. Several studies in animal models suggest that blocking the biological activity of VEGF may be beneficial for limiting scar formation; however, the clinical utility of this approach is not yet known. There are a number of FDA-approved anti-VEGF drugs on the market for the treatment of cancer and other diseases, so there are options available to investigate clinically whether blocking the function of VEGF can prevent or treat scars. Further studies are needed to explore this possibility.

OVERVIEW

The cutaneous wound repair process is characterized by a series of distinct stages that begins with an acute inflammatory response and is followed by periods of cellular proliferation and extracellular matrix (ECM) deposition/remodeling, ultimately leading to the formation of a scar.¹⁻³ Scars are produced to quickly patch the damaged area in the dermal layer of the skin, but the deposition of scar tissue as a replacement for the original tissue is not ideal. Scars are weaker than normal skin and, depending on the location and severity, they can cause restricted tissue growth and mobility of the joints.⁴ Scars are also a cause of significant morbidity and the cosmetic issues associated with scarring can have substantial consequences. Studies have shown that scars can have psychosocial implications and can significantly affect quality of life by negatively affecting confi-

dence, mood, and personal relationships.⁵ Unfortunately, clinically beneficial antiscar therapies are limited, and there is currently no standardized, accepted method for treatment.⁶ Given that millions of people are affected by scars each year, either from traumatic events or surgical procedures, the identification of new therapeutic targets has the potential to make an impact on the lives of a significant number of patients. In the sections below, published studies suggesting that VEGF may be a beneficial target to reduce scarring will be discussed.

VASCULAR ENDOTHELIAL GROWTH FACTOR

Angiogenesis, or the generation of new blood vessels, is a prominent feature of healing wounds.⁷ New blood vessels are needed during the repair process to deliver oxygen and nutrients present in the blood to the site of injury. One of the most potent and well-studied proangiogenic mediators is VEGF (or VEGF-A).

VEGF-A (referred to as VEGF throughout this article) is a 45 kDa heterodimeric heparin-binding protein that is one of several proteins in a family of VEGF. VEGF was initially described as a vascular permeability factor,⁸ and later it was found to act as a potent proangiogenic mediator, in part, by stimulating endothelial cell proliferation, migration, differentiation, and survival.⁹⁻¹¹ Several isoforms of the VEGF protein can be produced from alternatively spliced variants of the VEGF mRNA.¹² Other members of the VEGF family include VEGF-B, VEGF-C, VEGF-D, and placental growth factor, and each family member binds to different tyrosine kinase receptors.¹³ VEGF-A binds to VEGF receptor-1 (VEGFR-1) and VEGF receptor-2 (VEGFR-2) on endothelial cells.¹⁴ These receptors are characterized by seven extracellular immunoglobulin-like domains, a transmembrane region, and an internal tyrosine kinase domain.¹⁵ When VEGF binds to its receptors on endothelial cells, autophosphorylation of tyrosine residues activates various signaling pathways that stimulate the proliferation, migration, and survival needed for a strong angiogenic response.¹⁶

VEGF AND WOUND HEALING

Numerous studies have shown that VEGF is important for wound healing in the skin. VEGF is highly upregulated in healing animal¹⁷ and human¹⁸ wounds. VEGF production has been demonstrated in epidermal keratinocytes, mast cells, monocytes/macrophages, and activated fibroblasts after injury.¹⁷⁻²³ The overall importance of VEGF in wound healing has been demonstrated by studies

in which treatment with VEGF/VEGFR inhibitors or genetic deletion of VEGF in specific cell types (*i.e.*, keratinocytes and myeloid cells) results in delayed wound closure.^{22–25} In addition, diabetic wounds, which heal more slowly than normal wounds, generally have less VEGF,^{26–28} and enhancing VEGF levels in diabetic wounds can accelerate healing or improve the quality of the healed wound.^{28–31} While most of these effects have been attributed to the stimulation of angiogenesis by VEGF, it should be noted that multiple non-endothelial cell types have been reported to contain VEGFRs (reviewed in¹³); therefore, it is possible that some of the effects of VEGF are not related to angiogenesis. While most functional studies on VEGF in wound healing have focused on its ability to accelerate epidermal closure, VEGF has also been implicated in dermal repair processes. Based on the results of multiple studies, there appears to be a strong correlation between VEGF levels or VEGF activity and the amount of granulation tissue formed in both healthy and diabetic animals.^{20,28–33} In addition, VEGF has been shown to affect wound breaking strength in some cases.^{31,34} This could result from alterations in collagen production or the organization/arrangement of the deposited collagen. The ability of VEGF to influence dermal aspects of repair suggests that VEGF may alter fibroblast behavior during wound healing, ultimately affecting the scar formation process.

VEGF AND SCAR FORMATION

During the terminal stages of wound healing, fibroblasts restore the dermal layer of the skin by depositing and remodeling collagen, eventually forming a mature scar. In most cases, a normal scar will form; however, abnormal scars such as hypertrophic scars and keloids can develop when fibroblasts produce an overabundance of collagen. Hypertrophic scars are raised red scars that are generally confined to the borders of the original wound, whereas keloids are fibroproliferative lesions characterized by the formation of scar tissue that extends beyond the boundaries of the initial wound. A number of studies have now suggested that VEGF contributes to the formation of normal scars, hypertrophic scars, and keloids.

NORMAL SCARS

Studies using both human samples and animal models have linked VEGF to normal scars. Kumar, *et al.* showed a time-dependent increase in microvessel density and VEGF levels in human surgical scars.³⁵ Vessel density and VEGF followed similar

patterns, with the highest levels observed between 2 and 12 weeks postsurgery. Although VEGF levels declined over time, they remained significantly elevated in scars out to 2 years after surgery compared to normal skin.³⁵ The relationship between VEGF and scarring has also been examined in animal studies. Using a murine model of fetal wound healing, in which incisional wounding of the skin at embryonic day 15 (E15) results in scarless healing and wounding at embryonic day 18 (E18) results in scar formation, our laboratory showed that VEGF protein levels were higher in scar-forming E18 wounds compared with scarless E15 wounds.³⁶ In contrast, a fetal wound healing study in a rat excisional wound model suggested an increase in VEGF mRNA levels in scarless wounds.³⁷ It should be noted that the murine and rat fetal wound studies differed in the type of wound (incisional vs. excisional) and how VEGF was measured (protein vs. mRNA), which could explain the differences in the reported results between the two studies. Interestingly, a study on oral mucosal wound healing demonstrated a reduction in VEGF in scarless wounds³⁸ similar to what was observed in scarless murine fetal wounds.

In addition to correlative studies, functional studies also implicate VEGF in the process of scar formation *in vivo*. Injection of recombinant VEGF into E15 fetal wounds, which normally heal scarlessly, caused them to heal with prominent scars.³⁶ In addition, in a model of incisional wound healing in adult mice, neutralization of VEGF and inhibition of angiogenesis by injection of anti-VEGF antibodies caused a decrease in the size of scars that formed and also led to a more normal structure of collagen fibrils.³⁶ Overall, data from both human and animal studies support a role for VEGF in normal scar formation.

Hypertrophic scars

With the exception of one study that did not report differences between human hypertrophic and normal scars between 4 and 7 months after burn injury,³⁹ all other studies examining VEGF in hypertrophic scars have reported that high VEGF levels are associated with hypertrophic scarring. Cao, *et al.* showed that VEGF levels are higher in proliferative hypertrophic scars from trauma or burn injury and that VEGF levels decline in more mature scars.⁴⁰ In another study looking at sternotomy incisions, microvascular density was increased in hypertrophic scars at 12 and 52 weeks postsurgery compared to normal skin and normal scars.⁴¹ Although no significant changes in VEGF mRNA levels were observed, immunostaining for

VEGF showed more intense staining in keratinocytes and blood vessels in hypertrophic scar samples.⁴¹ Wang, *et al.* showed an increase in angiogenesis and VEGF mRNA and protein expression in hypertrophic scars compared to normal skin, with VEGF localization observed primarily in keratinocytes and endothelial cells.⁴² VEGF levels have also been examined in a large animal model of hypertrophic scarring. A red Duroc pig model, in which shallow wounds form normal scars and deep wounds form scars that resemble human hypertrophic scars, VEGF protein levels were shown to be elevated in deep wounds compared with shallow wounds and normal skin.⁴³

Several studies have also suggested that VEGF may be a viable target to reduce hypertrophic scarring. In patients receiving interferon- α 2b treatment for burn injury-induced hypertrophic scars, clinical improvement was associated with a reduction in angiogenesis.⁴² In addition, cultured endothelial cells treated with interferon- α 2b displayed reduced responses to VEGF as measured by tube formation and proliferation, possibly due to inhibiting the expression of VEGFRs on endothelial cells.⁴² This suggests that the ability of interferon to improve hypertrophic scarring may depend, in part, on inhibition of VEGF. A more recent study by Kwak, *et al.* demonstrated the potential of VEGF as a therapeutic target for hypertrophic scars more directly.⁴⁴ Using a rabbit ear model of hypertrophic scarring, the authors showed that local injection of bevacizumab, a humanized anti-VEGF antibody that is used to treat various malignancies, reduced the appearance of scars.⁴⁴ The scars treated with bevacizumab had fewer blood vessels, were less red and less raised, and had a less dense collagen arrangement based on histology.⁴⁴ Taken together, the studies showing a correlation between VEGF levels and hypertrophic scarring along with studies indicating that VEGF inhibition can minimize scarring suggest that VEGF could be a viable target to treat hypertrophic scars.

Keloids. Multiple studies have linked VEGF with keloid scars. Gira, *et al.* demonstrated higher VEGF mRNA expression in keloids by *in situ* hybridization, with the keratinocytes of hyperplastic epidermis expressing higher VEGF compared with keratinocytes in normal adjacent epidermis.⁴⁵ Mogili, *et al.* found an increase in serum VEGF levels as well as increases in VEGF mRNA and protein in keloid versus normal tissue.⁴⁶ Several other studies have also reported an increase in VEGF protein levels in keloid versus normal tissue,^{47,48} and immunohistochemistry results from

one of these studies suggested that there may be a change in VEGF localization in keloid keratinocytes.⁴⁷ Nuclear staining was observed in the keratinocytes of normal skin with an increase in cytoplasmic staining in keloid keratinocytes⁴⁷; however, the functional significance of this finding is not entirely clear. In addition to higher tissue levels, VEGF gene expression and protein production have also been reported to be higher in cultured keloid fibroblasts.^{47–49}

While there are no good *in vivo* models to study the potential effects of VEGF inhibition on keloid development/growth, several human studies have hinted that VEGF levels may be connected to therapeutic efficacy. Salem, *et al.* examined VEGF levels in biopsies taken before and after treatment with either intralesional injection with a corticosteroid (triamcinolone acetonide) or with cryotherapy.⁵⁰ Immunostaining showed that there was a reduction in dermal VEGF staining after treatment compared with before treatment, and a decrease in VEGF levels were associated with resolution of nodular collagen and a favorable clinical response.⁵⁰ Wu, *et al.* showed a similar reduction in VEGF staining after intralesional steroid injection and demonstrated in *in vitro* studies that VEGF reversed the ability of dexamethasone to reduce keloid fibroblast proliferation.⁵¹ Dexamethasone also reduced VEGF mRNA and protein levels and inhibited tube formation in cultured endothelial cells,⁵¹ suggesting that the therapeutic effects of steroid treatment on keloids may be related to the drug's ability to reduce VEGF and inhibit neovascularization.

POTENTIAL MECHANISMS FOR THE PROMOTION OF SCARRING BY VEGF

As discussed in the previous section, there are several lines of evidence suggesting that VEGF plays a role in scar formation (summarized in Table 1). Despite the positive association between VEGF levels and scar formation, little is known about the mechanisms responsible. However, it is possible that VEGF regulates scar formation indirectly, through its ability to stimulate endothelial cells or by affecting inflammation, or directly by influencing fibroblasts. (Fig. 1).

Indirect mechanisms: Endothelial and inflammatory cells

Endothelial cells are likely the primary target of VEGF and the cell type able to most efficiently respond to VEGF in a healing wound. Therefore, it is possible that VEGF promotes scar tissue by stimulating endothelial cells. During the process of sprouting angiogenesis, matrix metalloproteinases

Table 1. Summary of evidence that vascular endothelial growth factor stimulates scar formation

SCAR Type	Finding
Normal scars	Higher VEGF levels in scar tissue ^{35,36} Lower VEGF levels in scarless wounds ^{36,38} VEGF inhibition reduces scarring ³⁶
Hypertrophic scars	Higher VEGF levels in hypertrophic scar tissue ^{40–43} VEGF inhibition reduces scarring ⁴⁴
Keloids	Higher VEGF levels in keloid tissue ^{45,47,48,50} Higher VEGF levels in keloid fibroblasts ^{47,49} Lower VEGF levels in treated keloid tissue ^{50,51}

VEGF, vascular endothelial growth factor.

and other proteases must be produced to degrade the ECM surrounding the original vessel so that the tip cells can migrate into the tissue and guide the formation of a new sprout. This degradation of ECM components may stimulate compensatory collagen production by neighboring fibroblasts, leading to more scar tissue deposition. VEGF could also enhance the production of profibrotic mediators (cytokines, growth factors, etc.) by endothelial cells that promote collagen production by fibroblasts. It is also known that VEGF stimulates the expression of adhesion molecules by endothelial cells that facilitate the recruitment inflammatory cells from the bloodstream into the skin.⁵² In fact, transgenic mice, in which VEGF is overexpressed in epidermal keratinocytes, display an increase in leukocyte–endothelial interactions such as rolling

and adhesion⁵³ which likely contribute to the increase in inflammation seen in these mice. Furthermore, high VEGF levels in the skin result in an increase in the presence of various inflammatory cell types in the dermis, including mast cells and macrophages,^{53,54} cell types that have been linked to granulation tissue and/or scar formation^{21,55–59} and are found in high numbers in hypertrophic scars and keloids.^{60–65} It should be noted that VEGF may also lead to direct recruitment and/or activation of some inflammatory cells, such as monocytes/macrophages, which have been shown to express VEGFRs and migrate in response to VEGF.^{66–68}

Direct mechanism: Fibroblasts. Despite the lack of a comprehensive study determining whether VEGF can directly affect fibroblasts during wound healing to enhance scar formation, several studies have suggested that VEGF can stimulate cultured dermal fibroblasts. Wu, *et al.* observed an increase in fibroblast proliferation upon treatment with VEGF.⁵¹ They also showed that VEGF treatment could reverse dexamethasone-induced suppression of keloid fibroblast proliferation.⁵¹ In another set of studies, Brem, *et al.* showed that VEGF treatment increased migration of dermal fibroblasts isolated from human wounds using a scratch assay.³¹ In cultured human fibroblasts from normal or scleroderma patients, VEGF was shown to increase collagen production.⁶⁹ In addition to stimulating proliferation, migration, and collagen production,

Figure 1. Potential mechanisms of VEGF in scar tissue production. VEGF could promote scar formation via indirect or direct mechanisms. VEGF could enhance the migration and recruitment of inflammatory cells to the site of injury, and these cells could produce factors that stimulate scar tissue formation by fibroblasts. VEGF could also stimulate endothelial cells to produce proteases, express adhesion molecules that aid in inflammatory cell recruitment, or produce mediators that stimulate scar tissue production by fibroblasts. Finally, VEGF could directly promote scar tissue production by affecting migration, proliferation, or collagen production by fibroblasts. VEGF, vascular endothelial growth factor.

VEGF treatment was shown to increase plasminogen activator inhibitor-1 levels in cultured keloid fibroblasts through ERK1/2 signaling,⁴⁸ suggesting that VEGF may stimulate the production of mediators by fibroblasts that regulate the production and degradation of collagen.

ALTERING VEGF FOR THERAPEUTIC PURPOSES

The data supporting a role for VEGF in the development of scar formation suggest that VEGF could be targeted to minimize scarring and fibrosis. In fact, several studies in animal models support the idea that treatment with anti-VEGF drugs can reduce scarring.^{36,44} These studies used anti-VEGF antibodies and showed a reduction in scar formation. While there are currently no published clinical studies evaluating anti-VEGF drugs to prevent or treat scars, there are several FDA-approved drugs for cancer therapy that could be tested. For example, bevacizumab is a humanized monoclonal anti-VEGF antibody that neutralizes the bioactivity of VEGF by preventing it from binding to its receptor, and there are a number of small molecule tyrosine kinase receptors, such as sunitinib and sorafenib, which block VEGFR signaling.

There would be several potential caveats to using a VEGF inhibitor in a wound setting to reduce scarring, as blocking the activity of VEGF could delay wound closure. The risk to benefit ratio would have to be carefully considered, and the patient would have to be willing to sacrifice any potential delays in healing rates for a favorable cosmetic outcome. Characteristics of the patient, such as age and other conditions such as diabetes that might increase risk of wound healing complications, and characteristics of the wound, such as size, type (traumatic vs. surgical wound), and location, would have to be carefully considered.

CONCLUSIONS

Multiple published studies suggest that VEGF can influence the amount of scar tissue produced in a healing wound. These include studies linking higher VEGF levels with normal, hypertrophic, and keloid scarring, studies demonstrating that VEGF

TAKE-HOME MESSAGES

- VEGF is known for its role as a proangiogenic mediator in healing skin wounds, but recent studies have suggested that it also helps mediate scar formation.
- VEGF levels correlate with scar formation in several types of scars. Reduced VEGF levels are observed in scarless wounds, and elevated VEGF levels are associated with scar formation, especially in abnormal scars such as hypertrophic scars and keloids.
- Inhibition of VEGF can reduce scar formation in animal models, suggesting that VEGF could be targeted clinically to limit scar formation.
- Further studies are needed to evaluate the clinical efficacy of VEGF inhibitors as antiscarring agents.

inhibition can limit scar formation in normal and hypertrophic scar models, and studies showing reduced VEGF levels are associated with clinical improvements in keloids after therapy. Together, the evidence suggests VEGF could be a viable target to minimize scarring clinically. More work will have to be done and clinical testing will have to be performed to determine whether VEGF inhibition would be an effective antiscarring strategy. Since there are few proven treatment options available for scars, therapies with new mechanisms of action could have a significant impact in this area.

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AUTHOR DISCLOSURE AND GHOSTWRITING

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Abbreviations and Acronyms

E15 = embryonic day 15
 E18 = embryonic day 18
 ECM = extracellular matrix
 VEGF = vascular endothelial growth factor
 VEGFR = vascular endothelial growth factor receptor