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Chronic and non-healing wounds: The story of vascular endothelial growth factor

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ABSTRACT

The pathophysiology of the chronicity and non-healing status of wounds remains unknown. This paper presents the following hypothesis: abnormal patterns of vascular endothelial growth factor receptors (VEGFRs) are the culprits of wound chronicity and non-healing. More specifically, for patients with poor circulation, the decreased VEGFR-2 level is the cause of poor wound healing; for patients with non-compromised circulation, for example, patients with concurrent chronic wounds and active autoimmune diseases, the increased VEGFR-1 level is related to the non-healing status of wounds.

The hypothesis is supported by the following facts. VEGFR-1 is the main contributor for inflammation and VEGFR-2 facilitates angiogenesis; soluble VEGFR-1 (sVEGFR-1) inactivates both VEGFR-1 and VEGFR-2. Patients with auto-immune disease have abnormally increased VEGFR-1 and decreased sVEGFR. Wounds in patients with active autoimmune diseases have poor response to electric stimulation which facilitates chronic wound healing in patients without active autoimmune diseases via increasing vascular endothelial growth factor (VEGF) secretion. Patients with chronic wounds (including diabetic foot ulcers and venous leg ulcers) but no active autoimmune diseases have decreased VEGFR-2 levels.

We thus believe that abnormal patterns of VEGFRs are the culprits of wound chronicity and non-healing. For wounds with compromised circulation, VEGFR-2 decrease contributes to its chronicity; whereas for wounds with non-compromised circulation, VEGFR-1 increase is the leading cause of the non-healing status of chronic wounds. Treatments and research in wound care should be tailored to target these changes based on circulation status of wounds. Complete elucidation of changes of VEGFRs in chronic and non-healing wounds will enhance our understandings in tissue healing and thus better our selection of appropriate treatments for chronic and non-healing wounds.

Introduction

Approximately 6.5 million patients in the United States are being affected by chronic and non-healing wounds; the economic impact of chronic wound care is estimated to be US$25 billion [1]. Although new and effective treatments, such as new collagen dressings and high voltage pulsed current therapy, are available for those suffering from open wounds, chronicity and delayed healing continue to impair lives and, in some cases, cause death [1]. Factors including poor circulation, ongoing pressure, systemic illnesses, age, and repeated trauma are known to contribute to the chronicity or non-healing status of wounds [2]. Besides the limited understandings of detrimental effects of poor circulation to wound healing, the pathophysiology of the chronicity or non-healing status of wounds, especially in patients with sufficient blood supply to wound area, remains unknown. Pathophysiology of chronic wound healing is closely related to inflammation and angiogenesis. The vascular endothelial growth factors (VEGFs), a group of signal proteins, participate in critical rate-limiting steps of physiological vasculogenesis and angiogenesis [3]. Angiogenesis lays the foundation for granulation tissue development and the epithelialization process [3]. However, the role of VEGF in inflammation deserves better attention as abnormal high levels of VEGF contributes to the development of continuous inflammation [4,5]. Pro-inflammation effects of VEGF have been summarized as the following: (1) activates endothelial cells to secrete inflammatory

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cytokines and facilitates leukocyte adhesion and migration; (2) increases the production of tumor necrosis factor-alpha and interleukin-6 from mononucleocytes; (3) induces T cell transformation toward Th1 phenotype through increasing the production of interferon-gama and decreasing interleukin-10; (4) increases vascularity at the site of inflammation leading to more severe reactions [6]. Functions of VEGF depend on the activation of its receptors: activation of VEGF receptor-1 (VEGFR-1) leads to inflammation whereas activation of VEGF receptor-2 (VEGFR-2) causes angiogenesis; soluble VEGFR-1 (sVEGFR-1) sequesters VEGF, binds and inactivates membrane-bound VEGFR-1 and -2 receptors [4–6]. Electric stimulation therapy (E-stim) has been found to increase VEGF release and thus facilitates wound healing in various types of chronic wounds [3]. Research evidence from clinical trials indicates that E-stim improves wound healing in many patients, but not in others [3]. Based on our clinical experiences, wounds with non-optimal circulation usually respond better than wounds with simultaneous active autoimmune diseases but no compromise in circulation.

The hypothesis

Thus, this paper presents the following hypothesis: abnormal patterns of VEGFRs are the culprits of wound chronicity and non-healing. More specifically, for patients with poor circulation, the decreased VEGFR-2 level is the cause of poor wound healing; for patients without compromised circulation, for example, patients with concurrent chronic wounds and active autoimmune diseases, the increased VEGFR-1 level is the leading cause of the non-healing status of wounds. Thus, VEGF receptors may be used as biomarkers for predicting wound healing. In the following, we discussed the current understanding of VEGF and its receptors together with their participation in wound healing, the therapeutic mechanism of E-stim, as well as the abnormal patterns of VEGFRs in autoimmune diseases including systemic sclerosis (SS), rheumatoid arthritis (RA), and inflammatory bowel disease (IBD).

Current understandings of VEGF

Currently, the VEGF family is known to include five human VEGF isoforms (A, B, C, D, and placental growth factor-PLGF) which are produced by differential splicing of VEGF mRNA in endothelial cells, smooth muscle cells, platelets, neutrophils, keratinocytes, mast cells, monocytes/macrophages, and fibroblasts [7–10]. Effects of VEGF are mediated through binding of its subtypes to tyrosine kinase receptors (the VEGFRs) on the cell surface, causing them to dimerize and become activated through transphosphorylation [7–9]. Three main subtypes of VEGFR, numbered 1, 2 and 3, are available in the human body.

With tissue or skin injury, VEGFR-1 induces the migration of monocytes/macrophages, recruits hematopoietic stem cells, and inducts the release of matrix metalloproteinases (MMPs) [11–13]. Additionally, VEGFR-1 is also the major contributor to inflammation [14–16] and a negative regulator for angiogenesis [17,18]. The negative effects of VEGFR-1 may be related to its inhibitory effects on VEGFR-2, even though the binding of PLGF with VEGFR-1 facilitates VEGFR-2 function [19–22]. As VEGFR-1 is a small protein that binds and sequesters VEGF-A to prevent it from binding to VEGFR-2 [23] and VEGFR-1 has at least 10 folds higher affinity in VEGF-A binding than VEGFR-2 [8,9], the negative modulation of VEGFR-2 by VEGFR-1 is likely caused by VEGFR-1 activation from VEGF-A.

Activation of VEGFR-2 is the major reason for mitogenic, angiogenic and permeability-enhancing effects, abnormal lymphangiogenesis, promoting cell adhesion and migration by VEGF-A [8,9]. It is worth noting that a large amount of research studies have found that the main contributor of angiogenesis is from the activation of VEGFR-2 with VEGF-A [6–9]. Stimulation of VEGFR-3 leads to the growth of lymphatic vessels without an influence on the blood vessel architecture [8]. Nonetheless, discussions on the function of VEGFR-3 are beyond the purposes of the present article. Thus, the function of VEGF-A depends on its activation of VEGFRs: activation of VEGFR-1 promotes inflammation; activation of VEGFR 2 mediates angiogenesis.

Inflammation and angiogenesis also involve the participation of other molecules, among which, MMPs, nitric oxide synthase, cycloxygenase-2 (COX-2), hypoxia-inducible factor-1α (HIF-1α), and prostaglandin E2 have all been found to be involved in VEGF related pathophysiologies [6,13]. Activation of VEGFR-1 with VEGF-A causes the induction and secretion of matrix metalloproteinase-9 (MMP-9) which further secrets VEGF-A [24–26]. VEGFR-2/VEGF-A activation leads to the release of nitric oxide and prostacyclin [27–32]. HIF-1α targets genes of VEGF-A and VEGFR-2 and simulates their secretion [33,34]. Hypoxia also induces COX-2 expression which up-regulate HIF-1α via increasing the formation of prostaglandin E2 [35]. Prostaglandin E2 is increased in chronic inflammatory sites and in tumor sites [36].

VEGF in chronic wound healing

Right after wounding, VEGF transcription and secretion are elevated and peak on day 3 and 7 in full thickness wounds and peak on day 2–3 on superficial wounds [37]. Although some researchers did not specify which specific subtype of VEGF is involved, the transcription and secretion of VEGF-A is more likely as the findings coincide with vascularity and granulation tissue formation and the majority of VEGF research studies in the early phase usually involve VEGF-A (VEGF165). In chronic wounds, the release of VEGF and other factors including platelet derived growth factor (PDGF), and fibroblast growth factor (FGF) is delayed or inhibited [38].

Research studies found that patients with diabetic foot ulcers have decreased VEGF and VEGFR-2 expression in tissues. VEGF mRNA levels are declined in wounds of diabetic mice as compared with normal mice [39]. Rivard et al. [40] reported that the reduced expression of VEGF in non-obese diabetic mice causes decreased angiogenesis and increasing VEGF expression could restore angiogenesis. Krishnan et al. [41] reported that epidermal and dermal blood vessel VEGFR-2 is decreased in patients with diabetic foot ulcer. However, angiopoietin-1 gene transfer improves impaired wound healing in genetically diabetic mice with augmented VEGFR-2 but without increasing VEGF expression [42].

Patients with venous insufficiency tend to have abnormal high plasma and tissue VEGF levels [43,44]. Lauer et al. [45] did biopsy on venous ulcers and found similarly increased VEGF expression in the wound epidermis area of chronic venous ulcer. Additionally, the expression of VEGFR-1 and 2 were found in papillary dermal microvessels near ulcer edge but not in capillaries distant to the ulcer edge [45]. Although increased expression of VEGF protein was detected in the epidermis, the intensity of this staining was weak in wound fluid compared with epidermis [45]. Simultaneously, sVEGFR-1 which sequesters VEGF, binds and inactivates membrane-bound VEGFR-1 and -2 receptors was reported high in wound fluid of chronic venous ulcer [46]. Although quantitative changes of tissue or plasma and tissue level VEGFR-1 and sVEGFR-1 remains unknown in venous leg ulcers, patients with non-healing venous leg ulcer are likely to have a low tissue/plasma VEGFR-2 level as sVEGFR-1 inactivates VEGFR-2.

Interestingly, researchers found that VEGF gene polymorphism is related to diabetic foot ulcer and the lower frequency of A allele in patients with diabetic foot ulcer is conferring a protective effect

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[47]. Topical single-dose VEGF has no effect on wound healing [48]. Therefore, although VEGF function is essential for optimal wound angiogenesis, it may not be required for wound closure [49]. Consequently, we further believe that as compared to VEGF, VEGF receptors are more important for wound healing. The addition of VEGF apparently did not correct the deficiency of VEGFR-2 and thus likely rendered the introduction of VEGF to wound tissue non-relevant for wound healing in the previous studies [47–49].

Insights from E-stim in wound healing

Wounds in skin creates a direct current flow at the wound of at least 40 mV mm^{-1} in cornea and up to 200 mV mm^{-1} in guinea pig skin [50]. This electric field persists until re-epithelialization is complete [50]. During normal wound healing process, this endogenous electric field guides epithelial cell migration and nerve sprouting directly towards the wound edge [50]. Wound healing becomes compromised when the electric field is disrupted [50]. Wound-edge-directed nerve sprouting and angiogenesis are guided by the wound-induced electric field [50]. E-stim directly stimulate VEGF production by endothelial cells [50,51] and osteoblasts [52]. In muscle and skin of full-thickness wounds in animals, Asadi et al. [53] also reported that sensory and motor E-stim increased VEGF secretion. These effects of E-stim were also confirmed by the increase in serum and plasma VEGF in patients with dystrophic ulcers treated with E-stim [54].

To understand the roles of VEGFRs in E-stim for wound healing, Bai et al. [55] performed a study on human endothelial cells and found that E-stim upregulates VEGF-A and IL-8 secretion in human endothelial cells, and inhibition of VEGF receptor (VEGFR-1 or VEGFR-2) signaling significantly decreased VEGF production and completely abolished IL-8 production [55]. Electrical stimulation directly induces pre-angiogenic responses in vascular endothelial cells by signaling through VEGFRs [50]. Specifically, inhibition of VEGFR-2 completely abolished the E-stim-induced directional migration of the progenitor endothelial cell, thus researchers conclude that E-stim guides endothelial progenitor cell migration through VEGF receptor-2 signaling [56].

Insights learned from autoimmune diseases

In clinical practice, we observed that patients with concomitant active autoimmune disease and chronic wounds lacked favorable response to E-stim. To better elucidate the understandings of VEGFRs in wound healing, we thus reviewed changes of VEGF and its receptors in autoimmune diseases. VEGF-A which is the most widely studied VEGF isoform has been found to be significantly increased in patients with autoimmune disease, including Reynaud's phenomena in SS [4,57], uncontrolled RA (in both synovial fluid and serum) [58–61], and IBD (Crohn's disease and ulcerative colitis) [62]. Correspondingly, decrease or blockade in VEGF-A correlates with improved or decreased signs and symptoms in these disorders [61,62]. Recently, researchers found that genetic variation in the VEGF-A gene is associated with serum VEGF-A levels and correlates with the severity in RA symptoms in patients with RA [63–65]. Increase in serum VEGFR-1 has been reported in patients with RA, and correspondingly, specific blocking of VEGFR-1 has been found effective in decreasing inflammation in animal models with RA [66–68]. Increased expression of PLGF and VEGFR-1 was found in patients with RA indicating roles in rheumatoid inflammation by triggering production of proinflammatory cytokines while PLGF facilitates angiogenesis [68].

Although increased VEGF-A andVEGFR-2 levels have been reported in both patients with IBD and colitic mice, researchers did not find increase in VEGFR-1 in intestine biopsies of patients with IBD [63]. Similar situations are true for patients with SS; no increase of VEGFR-1 was found in skin biopsy in patients with SS [69]. But, sVEGFR-1 secretions were reported to be low in tissue biopsies and inversely correlates with disease severity in patients with IBD and SS [70,71]. The artificial introduction or increase of sVEGFR-1 has been found to suppress angiogenesis and minimize inflammation damage in RA [72,73], IBD [62], and SS [71]. Interestingly, serum VEGFR-1 level was reported to be high in patients with Crohn's disease [72]. Increased serum VEGFR-1 in patients [72] seems contradictory to lack of increase in tissue level VEGFR-1 in animal studies of autoimmune diseases [68,69], but for wound healing, we tend to believe that serum level VEGFR-1 is more likely to be increased in patients with IBD and SS which is also supported with the decreased sVEGFR levels. As a result of the decreased sVEGFR-1 level in IBD and SS, these patients will likely have excessive functions of VEGFR-1 which is characterized by relentless inflammation in these patients.

To complete the loop related to the functions of VEGF and VEGFR-1 and -2, we also briefly reviewed a type of closely related molecule called vasohibin and other relevant molecules. The vasohibin family also participates in the VEGF system. Recent studies found that vasohibin-1 (VASH1) can be induced by both VEGF and FGF-2 [73]. Vasohibin-2 (VASH2) is not responsive to growth factors and cytokines and thus seems to be not inducible but constitutive [73]. Through studies on gene knockout mice, researchers identified that VASH1 inhibits angiogenesis at the termination zone and VASH2 stimulate angiogenesis in the sprout front [73]. VASH1 is up-regulated by VEGF in the retina and suppresses VEGFR-2 and retinal neovascularization [74]. Transient overexpression of VASH1 in human umbilical vein endothelial cells down-regulates VEGFR-1 and sVEGFR-1 on endothelial cells [75]. Expression of VASH1 in endothelial cells has been identified in various cancers, atherosclerotic lesions, age-dependent macular degeneration, diabetic retinopathy, RA and arterial re-endothelialization after denudation [73]. In RA, significant positive correlation exists between the expression of VASH1 and histological inflammation score ($p = 0.002$, $r = 0.842$) [76]. However, studies regarding the relationship between VASH1 and IBD or SS are lacking.

Discussion

Patients with RA tend to have simultaneous increase in VEGF-A, PLGF, VEGFR-1, and VASH1; patients with IBD and SS tend to have simultaneous increase in VEGF-A, VEGFR-1, VEGFR-2, and decreased sVEGFR. Characteristically, patients with RA, IBD, and SS all have abnormal VEGF and VEGF receptors (Table 1). VEGF-A binds VEGFR-1 leading to inflammation; VEGF-A or PLGF binds to VEGFR-2 leading to angiogenesis. VEGFR-1 inhibits VEGFR-2 (angiogenesis); sVEGFR-1 inhibits both VEGFR-1 and 2 (inflammation and angiogenesis); VASH1 inhibits sVEGFR-1 and VEGFR-2 (proinflammatory and inhibiting angiogenesis). As stated previously, VEGFR-2/VEGF-A activation leads to the release of nitric oxide and prostacyclin [28–33]. Nitric oxide generation was significantly improved with E-stim [54]. Antagonism of NO also inhibited VEGF production [77]. VEGFR-1/VEGF-A activation is involved in the induction of MMPs [14,26]. VEGFR-2/VEGF-A activation is involved in angiogenesis [6–9]. (Table 1 and Fig. 1.)

Wound healing process is closely related to angiogenesis (VEGFR-2) and inflammation (VEGFR-1). Chronic wounds can be divided into wounds with and without compromised circulation. Patients with diabetes, venous leg ulcers, and arterial wounds usually have compromised circulation. Patients with diabetic foot ulcers have decreased VEGF and VEGFR-2 expression in tissues;

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Table 1
A summary of VEGF and its receptors.

RA (serum)	IBD & SS (serum)	Diabetic foot ulcer	Venous insufficiency/ulcer	Electric therapy
↑ VEGF ↑ VEGFR1 ↑ PLGF ↑ VASH1	↑ VEGF ↑ VEGFR1, ↑ VEGFR-2 ↓ sVEGFR1	↓ VEGF in tissue ↓ VEGFR-2 in tissue	↑ VEGF in plasma and tissue of venous insufficiency ↑ sVEGFR-1 in non-healing venous wound fluid	↑ VEGF

Note: RA = rheumatoid arthritis; IBD = inflammatory bowel disease; SS = systemic sclerosis; VEGF = vascular endothelial growth factor-A; PGs = prostaglandins; NO = nitric oxide; sVEGFR1 = soluble vascular endothelial growth factor receptor 1; VASH1 = vasohibin 1; VEGFR1 and 2 = vascular endothelial growth factor receptor 1 and 2; PLGF = placental growth factor.

patients with venous insufficiency have increased VEGF in both plasma and tissue; patients with non-healing venous leg ulcers have high sVEGFR in the wound fluid which indicates possible low levels of VEGFR-2 in plasma and tissue. Consequently, for wounds with compromised circulation, the low level of VEGFR-2 is likely the cause for poor wound healing. Patients with concomitant chronic skin wounds and autoimmune disease usually have non-compromised circulation of the periwound tissue. Nonetheless, these patients also have abnormal patterns of VEGF receptors, more specifically, high VEGFR-1; non-healing skin wounds on patients with active autoimmune diseases are mainly characterized by inflammation. High VEGFR-1 level is thus likely responsible for the non-healing status of wounds without circulation compromise.

We thus believe that abnormal patterns of VEGFRs are the culprits of wound chronicity. Excessive inflammation impedes wound healing while predominant angiogenesis facilitates wound healing. VEGFR-1 is the vital molecule participating in the inflammation process whereas VEGFR-2 serves as the key factor in facilitating neovascularization. The normal physiological wound healing process requires balance between inflammation and angiogenesis. When a chronic wound is characterized by excessive inflammation which involves signs and symptoms of the heat (calor), pain (dolor), redness (rubor), and swelling (tumor), E-stim may not be beneficial but rather detrimental for wound healing. The rationales may be that increased VEGF release by E-stim will enhance the coupling of VEGF/VEGFR-1 aggravating inflammation which is common in patients with increased VEGFR-1. These patients, as exemplified by patients with concomitant autoimmune disease and chronic wounds, are likely to have relatively good circulation of the periwound tissue. To the contrary, for patients with compromised circulation who usually do not show active inflammation, they are likely to have decreased VEGFR-2. These wounds are the majority of chronic wounds typically seen in clinical practices; they include arterial wounds, diabetic ulcers, venous leg wounds, and pressure ulcers. Thus, enhancement of angiogenesis and circulation is warranted to regain the physiological balance for wound healing via regulating VEGF and its receptors. As a beneficial tool to increase VEGF and activate VEGFR-2, E-stim will likely to facilitate wound healing in these patients.

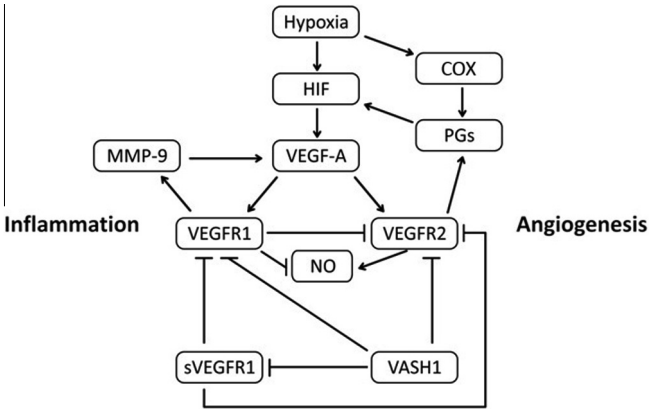

Fig. 1. Relationship among VEGF and related molecules in inflammation and angiogenesis. *Note:* VEGF-A = vascular endothelial growth factor-A; PGs = prostaglandins; NO = nitric oxide; sVEGFR1 = soluble vascular endothelial growth factor receptor 1; VASH1 = vasohibin1; VEGFR1 and 2 = vascular endothelial growth factor receptor 1 and 2. HIF = hypoxia induced factor; MMP = matrix metalloproteinases; COX = cyclooxygenase; ← represents activation; ⊥ represents inhibition.

To heal chronic wounds characterized by apparent inflammation with non-compromised circulation, therapies disrupting the VEGF-A/VEGFR-1 binding (proinflammation), more importantly decreasing VEGFR-1 activation, will be necessary. VEGFR-1 activation which is characterized by inflammation could be possibly decreased via the use of steroids. Although steroid use in chronic wound healing is a controversial topic, we believe the use of steroids in chronic and non-healing wounds with apparent inflammation are necessary and beneficial. Interestingly, this was supported by the study by Bosanquet et al. [78] in which researchers found that chronic wounds displaying abnormal inflammation could be successfully treated with topical steroids. Consequently, for the treatment of chronic and non-healing wounds with good periwound circulation, clinicians may facilitate wound healing through the following: (1) modify VEGF/VEGFR patterns; (2) modulate sVEGFR-1; (3) block inflammation with immunosuppressants including steroids.

To test these hypotheses, researchers investigating the pathophysiology of wound chronicity and non-healing should begin with classification of these wounds into two categories: wounds with and without compromised circulation. To explore the pathophysiology of the chronicity or non-healing status of wounds, studies should be performed to quantitatively identify VEGFRs in non-healing wounds as well as during the wound healing processes. Specifically, for wounds with non-compromised circulation, quantitative studies should be performed to identify changes of VEGFR-1; for chronic wounds with compromised circulation, emphasis should be the investigation of VEGFR-2. Complete elucidation of changes of VEGFRs in chronic and non-healing wounds will enhance our understandings in tissue healing and thus better our selection of appropriate treatments for chronic and non-healing wounds.

Conflicts of interest

The authors declare that no competing financial interests exist and this study did not receive any funding.

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