PERSPECTIVE ARTICLE

Growth factors and cytokines in wound healing

Stephan Barrientos[1,2]; Olivera Stojadinovic, MD[2]; Michael S. Golinko, MD[3]; Harold Brem, MD[3]; Marjana Tomic-Canic, PhD[2,4]

1. University of Rochester School of Medicine and Dentistry, Rochester, New York,
2. Tissue Engineering, Repair and Regeneration Program, Hospital for Special Surgery at Weill Medical College of Cornell University, New York, New York,
3. Wound Healing Laboratory, Columbia University College of Physicians and Surgeons, New York, and
4. Department of Dermatology, Weill Medical College of Cornell University, New York

Reprint requests:
Marjana Tomic-Canic, PhD, Hospital for Special Surgery of the Weill Medical College of the Cornell University, Tissue Repair Laboratory, Tissue Engineering, Regeneration and Repair Program, 535 E 70th Street, New York, NY 10021;
Tel: +1 212 774 7160;
Fax: +1 212 249 2373;
Email: tomicm@hss.edu

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ABSTRACT

Wound healing is an evolutionarily conserved, complex, multicellular process that, in skin, aims at barrier restoration. This process involves the coordinated efforts of several cell types including keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets. The migration, infiltration, proliferation, and differentiation of these cells will culminate in an inflammatory response, the formation of new tissue and ultimately wound closure. This complex process is executed and regulated by an equally complex signaling network involving numerous growth factors, cytokines and chemokines. Of particular importance is the epidermal growth factor (EGF) family, transforming growth factor beta (TGF-β) family, fibroblast growth factor (FGF) family, vascular endothelial growth factor (VEGF), granulocyte macrophage colony stimulating factor (GM-CSF), platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), interleukin (IL) family, and tumor nerosis factor-α family. Currently, patients are treated by three growth factors: PDGF-BB, bFGF, and GM-CSF. Only PDGF-BB has successfully completed randomized clinical trials in the Unites States. With gene therapy now in clinical trial and the discovery of biodegradable polymers, fibrin mesh, and human collagen serving as potential delivery systems other growth factors may soon be available to patients. This review will focus on the specific roles of these growth factors and cytokines during the wound healing process.

Wound healing is a complex process involving several overlapping stages that include inflammation, formation of granulation tissue, reepithelialization, matrix formation and remodeling. Upon injury to the skin, the epidermal barrier is disrupted and keratinocytes release prestored interleukin-1 (IL-1). IL-1 is the first signal that alerts surrounding cells to barrier damage.[1–11] In addition, blood components are released into the wound site activating the clotting cascade. The resulting clot induces hemostasis and provides a matrix for the influx of inflammatory cells. Platelets degranulate releasing alpha granules, which secrete growth factors such as: epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β). PDGF, along with proinflammatory cytokines like IL-1, are important in attracting neutrophils to the wound site to remove contaminating bacteria (reviewed in Hantash et al.).[12] With the help of TGF-β, monocytes are converted to macrophages which play an important role in augmenting the inflammatory response and tissue debridement. Macrophages initiate the development of granulation tissue and release a variety of proinflammatory cytokines (IL-1 and IL-6) and growth factors (fibroblast growth factor [FGF], EGF, TGF-β, and PDGF).

With the assistance of platelet released vascular endothelial growth factor (VEGF) and FGF, endothelial cells proliferate and angiogenesis ensues. This process is essential for the synthesis, deposition, and organization of a new extracellular matrix (ECM). FGF, TGF-β, and PDGF then permit fibroblast infiltration. TGF-β and PDGF also initiate phenotypic changes in these cells converting fibroblasts into myofibroblasts which align themselves along the borders of the ECM to generate a constrictive force, facilitating wound closure (reviewed in Hantash et al.).[12]

Within hours of injury, reepithelialization is initiated and the release of EGF, TGF-α, and FGF act to stimulate epithelial cell migration and proliferation. This process begins with the dissolution of cell–cell and cell–substratum contacts followed by polarization and migration of keratinocytes over the provisional ECM. Once wound closure (100% epithelialization) is achieved, keratinocytes undergo stratification and differentiation to restore the barrier (reviewed in[13,14]).

Matrix formation requires the removal of granulation tissue with revascularization. A framework of collagen and elastin fibers replaces the granulation tissue. This framework is then saturated with proteoglycans and glycoproteins. This is followed by tissue remodeling involving the synthesis of new collagen mediated by TGF-β, and the breakdown of old collagen by PDGF. The final product of this process is scar tissue.

The success of the wound healing process depends on growth factors, cytokines, and chemokines involved in a

complex integration of signals that coordinate cellular processes. These agents are biologically active polypeptides that act to alter the growth, differentiation and metabolism of a target cell. They can act by paracrine, autocrine, juxtacrine, or endocrine mechanisms, and effect cell behavior as a consequence of their binding to specific cell surface receptors or ECM proteins. Binding to these receptors triggers a cascade of molecular events. The endpoint of this signaling is the binding of transcription factors to gene promoters that regulate the transcription of proteins controlling the cell cycle, motility, or differentiation patterns.[13] This review will summarize the major growth factors and cytokines involved in wound healing with particular focus on the EGF family, TGF-β family, FGF family, VEGF, granulocyte macrophage colony stimulating factor (GM-CSF), PDGF-BB, CTGF, IL family, and tumor necrosis factor (TNF)-α family (Table 1).

EPIDERMAL GROWTH FACTOR (EGF) FAMILY

Perhaps the best-characterized growth factors in wound healing are those from the EGF family. The ligands

Table 1. Major growth factors and cytokines that participate in wound healing with cell types and their respective roles in both acute and chronic wounds are listed

Growth Factors	Cells	Acute Wound	Function	Chronic Wound
EGF	Platelets Macrophages Fibroblasts[44,45]	Increased levels[46,47]	Reepithelialization[48]	Decreased levels[51]
FGF-2	Keratinocytes Mast Cells Fibroblasts Endothelial cells Smooth muscle cells Chondrocytes[58,75,76]	Increased levels[79,81]	Granulation tissue formation Reepithelialization Matrix formation and remodeling[277]	Decreased levels[52]
TGF-β	Platelets Keratinocytes Macrophages Lymphocytes Fibroblasts[92,93,96]	Increased levels[98]	Inflammation Granulation tissue formation Reepithelialization Matrix formation and remodeling[81,101,107]	Decreased levels[52]
PDGF	Platelets Keratinocytes Macrophages Endothelial cells Fibroblasts[58,140,141]	Increased levels[144]	Inflammation Granulation tissue formation Reepithelialization Matrix formation and remodeling[141,142,146,153]	Decreased levels[52]
VEGF	Platelets Neutrophils Macrophages Endothelial cells Smooth muscle cells Fibroblasts[69,160–164]	Increased levels[185]	Granulation tissue formation[177,180]	Decreased levels[52]
IL-1	Neutrophils Monocytes Macrophages Keratinocytes[13,60]	Increased levels[242]	Inflammation Reepithelialization[244]	Increased levels[51]
IL-6	Neutrophils Macrophages[245]	Increased levels[245]	Inflammation Reepithelialization[77,78]	Increased levels[245]
TNF-α	Neutrophils Macrophages[60,242]	Increased levels[51]	Inflammation Reepithelialization[51]	Increased levels[51]

include: EGF, heparin binding EGF (HB-EGF), transforming growth factor-alpha (TGF-α), epiregulin, amphiregulin, betacellulin, epigen, neuregulin-1 (NRG-1), NRG-2, NRG-3, NRG-4, NRG-5, and NRG-6.[14–26] The main members involved in wound healing include: EGF, TGF-α, and EGF-HB. These ligands bind to the EGF receptor (EGFR), a tyrosine kinase transmembrane protein, resulting in dimerization of the receptor, autophosphorylation, and tyrosine phosphorylation of downstream proteins.[27]

In healthy human epidermis, EGFR can be localized throughout the entire epidermis, although its membranous presence is most prominent in the basal layer.[28,29] There are also ligands for other receptors, such as β-AR agonists (catecholamines), angiotensin II, and antimicrobial hCAP-18, which can transactivate EGFR.[30–32] Ultimately this signaling pathway leads to the activation of a number of converging pathways promoting cell migration and proliferation.

In vitro studies, show that activation of the EGFR plays an important role in reepithelialization by increasing keratinocyte proliferation and cell migration in acute wounds.[33–36] The ligands that bind to EGFR are synthesized as membrane-anchored forms, which are proteolytically processed to bioactive soluble forms. However, EGFR ligand shedding is essential for keratinocyte migration and it has been established that EGF accelerate keratinocyte migration thus promoting reepithelialization.[37,38] It is a potent mitogen for keratinocytes[39,40] and the transmembrane forms are able to stimulate growth of keratinocytes in a juxtacrine manner, suggesting their participation in reepithelialization.[41]

EGF was originally reported by Dr. Stanley Cohen.[42,43] EGF is secreted by platelets, macrophages, and fibroblasts and acts in a paracrine fashion on keratinocytes.[44,45] In vitro studies have shown that EGF is up-regulated after acute injury significantly accelerating reepithelialization[46] and increasing tensile strength in wounds.[47] One mechanism through which EGF functions is by increasing the expression of keratins K6 and K16, involved in the proliferative signaling pathway.[48,49] One in vitro study demonstrated that in the epidermis of nonhealing edges of chronic wounds EGFR was found in the cytoplasm of keratinocytes instead of the membrane.[50] This suggests that the receptor's down-regulation and mis-localization may participate in inhibition of epithelialization in patients with chronic wounds. Other in vitro studies demonstrate substantial degradation of exogenous EGF and the EGFR reversible with the addition of metalloproteinase (MMP) inhibitors in chronic ulcers.[51,52] This suggests that EGF is susceptible to the proteolytic environment found in these wounds. Clinical trials for chronic wound therapeutics show that the addition of topical EGF increased epithelialization and shortened healing time in skin graft donor-healing sites, venous ulcers (VU), and diabetic foot ulcers (DFU).[53–55] Therefore, EGF may still be useful to persons with chronic wounds if delivered by a system, such as gene therapy, polymers, or electrospun nanofibers.[56,57] Such techniques maintain a continuous growth factor concentration, sustaining its presence in the wound and preventing its rapid degradation.

Another member of this family, TGF-α, is secreted by platelets, keratinocytes, macrophages, fibroblasts, and lymphocytes and works in an autocrine fashion on keratinocytes.[22,45,58–61] In vitro studies demonstrate that TGF-α has the ability to increase keratinocyte migration[62] and proliferation[63–65] and induce the expression of K6 and K16.[48] In vivo studies suggest a role in early stimulation and maintenance of wound epithelialization in partial thickness wounds.[66] Despite its seemingly important role in reepithelialization, absence of this growth factor does not hinder wound healing. This can be contributed to a certain degree of compensation by the other growth factors in the EGF-family.[67,68]

HB-EGF is also up-regulated in the acute wound.[69,70] It is secreted by keratinocytes and works in an autocrine fashion[71] by binding to the EGFR subtypes HER1 and HER4[72] promoting reepithelialization.[21] HB-EGF has been implicated in vivo as having a role in wound healing as a major growth factor found in wound fluid[70] and plays a role in promotion of keratinocyte migration suggesting its important role in early stages of reepithelialization.[73] In addition, recent in vitro studies demonstrate a possible role in angiogenesis.[74]

FIBROBLAST GROWTH FACTOR (FGF) FAMILY

The FGF family is composed of 23 members. Of these, the three most important members involved in cutaneous wound healing are FGF-2, FGF-7, and FGF-10. FGFs are produced by keratinocytes, fibroblasts, endothelial cells, smooth muscle cells, chondrocytes, and mast cells.[58,75–78] The high-affinity FGF receptor (FGFR) family, which mediates cellular responses to FGF, comprises four members FGFR1-4. These receptors are tyrosine kinase transmembrane proteins, which work much like EGFR.[79] Essential for activation of the receptor, FGF must bind proteoglycans, such as heparin, that incorporates several ligands together in a web.[80]

FGF-2, or basic FGF, is increased in the acute wound and plays a role in granulation tissue formation, reepithelialization, and tissue remodeling.[79,81] In vitro studies have demonstrated that FGF-2 regulates the synthesis and deposition of various ECM components, increases keratinocyte motility during reepithelialization,[82–84] and promotes the migration of fibroblasts and stimulates them to produce collagenase.[18]

Levels of FGF-2 are decreased in chronic wounds.[52] Clinical trials utilizing FGF-2 in the treatment of DFUs have failed.[85] This is primarily due to FGF-2's inability to maintain its efficacy in these patients. Promising data has been obtained from FGF-2–treated pressure ulcer (PU) patients showing a trend toward faster wound closure.[86]

Other important members of this family include FGF-7, or keratinocyte growth factor-1 (KGF-1), and its homologue FGF-10, or KGF-2, both of which are expressed in acute wounds.[87,88] Both FGF-7 and FGF-10 act in a paracrine fashion through the FGFR2IIIb receptor found only on keratinocytes.[88] FGF-10 is also able to bind to FGFR1IIIb and has been shown to have a mitogenic effect on cells containing this receptor.[88,89] In vitro studies have shown that FGF-7 and FGF-10 stimulate proliferation and migration of keratinocytes playing an important role in reepithelialization. In addition, FGF-7 and FGF-10 increase transcription of factors involved in the detoxification of reactive oxygen species (ROS). This helps to reduce ROS-

induced apoptosis of keratinocytes in the wound bed preserving these cells for reepithelialization (reviewed in Raja et al.[13]). In vitro studies have also shown FGF-7 to be important during the later stages of neovascularization when lumenal spaces and basement membranes are being developed. It is a potent mitogen for vascular endothelial cells and helps in the up-regulation of VEGF. It also stimulates endothelial cells to produce a urokinase type plasminogen activator, a protease required for neovascularization.[90] Because of its potential benefit in reepithelialization, studies have been conducted to evaluate KGF's effect on chronic wounds. One clinical trial using topical application of Repifermin (rh-KGF-2) resulted in accelerated wound healing in VU patients.[91]

TRANSFORMING GROWTH FACTOR-β (TGF-β) FAMILY

The TGF-β family includes the following members: TGF-β1-3, bone morphogenic proteins (BMP), and activins. TGF-β1, TGF-β2, and TGF-β3 are the main forms found in mammals, but TGF-β1 predominates in cutaneous wound healing. They are produced by macrophages, fibroblasts, keratinocytes, and platelets[92–96] and work by binding a heteromeric receptor complex consisting of one type I and one type II receptor, both of which are serine-threonine kinases. In addition, they bind to a nonsignaling type III receptor, which functions in presenting TGF-β to the type II receptor. Once the receptors become autophosphorylated they activate the downstream signaling molecules belonging to the Smad family of transcription factors.[97]

In wound healing, TGF-β1 is important in inflammation, angiogenesis, reepithelialization, and connective tissue regeneration. It is shown to have increased expression with the onset of injury.[98,99] TGF-β1 facilitates the recruitment of additional inflammatory cells and augments macrophage mediated tissue debridement (reviewed in Clark[81]). It is also interesting to note that once the wound field is sterilized, TGF-β1 may be able to deactivate superoxide production from macrophages in vitro.[100] This helps to protect the surrounding healthy tissue and prepares the wound for granulation tissue formation.[101] In vitro studies show that TGF-β1 helps initiate granulation tissue formation by increasing the expression of genes associated with ECM formation including fibronectin, the fibronectin receptor, and collagen and protease inhibitors.[49,102–106] It is also involved in up-regulating the angiogenic growth factor VEGF.[107] In addition, in vitro studies show TGF-β1 playing a role in wound contraction by facilitating fibroblast contraction of the collagen matrix.[108]

During reepithelialization, TGF-β1 shifts keratinocyte integrin expression toward a more migratory phenotype.[62] There are conflicting data as to the role of TGF-β1 in keratinocyte proliferation. Several studies both in vitro and in vivo have demonstrated that TGF-β1 inhibits keratinocyte proliferation.[109–111] Furthermore, animal in vivo studies have shown that Smad3-null (Smad3ex8/ex8) mice have accelerated cutaneous wound healing compared with wild-type mice, characterized by an increased rate of reepithelialization and significantly reduced local infiltration of monocytes.[112] However, other studies show that overexpression of TGF-β1 increases the proliferative phenotype of keratinocytes particularly during the late stages of wound healing.[113,114] This illustrates the complexity of signaling necessary to coordinate cellular processes participating in wound healing, emphasizing the importance of tight spatio-temporal control, in which small changes in levels and timing of any growth factor may have a completely different outcome.

Finally, in the matrix formation and remodeling phase of wound healing, TGF-β1 is involved in collagen production (particularly type I and III). It is also a potent inhibitor of metalloproteinase MMP-1, MMP-3, and MMP-9 and a promoter of tissue inhibitor of metalloproteinase TIMP-1 synthesis, thus inhibiting collagen breakdown.[49,104–106]

TGF-β1's ability to stimulate collagen production is so potent that it can result in pathology. TGF-β1 plays a major role in the pathogenesis of fibrosis by inducing and sustaining activation of keloid fibroblasts.[115] When overexpressed, TGF-β1 has been shown to stimulate connective tissue growth factor (CTGF) also shown to play an important role in the development of hypertrophic and keloid scars.[116] It has been shown that localized increase in the release and activation of TGF-β1 in burn injuries inhibits reepithelialization and enhances fibrosis.[117] Furthermore, in the fetal wound the fetal fibroblast responds to its hypoxic environment by decreasing TGF-β1 transcription that could explain, in part, the scarless healing seen in the fetus.[118–120]

The second isoform, TGF-β2, has also been shown to have a role in wound healing. Like TGF-β1, TGF-β2 is involved in all stages of wound healing. It is involved in recruiting inflammatory cells and fibroblasts to the wound site. In vivo experiments show that TGF-β2 stimulates the formation of granulation tissue by inducing angiogenesis.[121,122] It also has been shown to accelerate reepithelialization in vivo.[121,123] During matrix formation and remodeling, TGF-β2 increases protein, DNA, and collagen production. By stimulating recruitment of fibroblasts to the wound site, the combined result is increased collagen deposition (particularly type I and III) and scar formation in vivo.[121,124]

The third isoform, TGF-β3, has been shown to play a role in wound healing. In vivo studies have shown that TGF-β3 promotes wound healing by recruiting inflammatory cells and fibroblasts to the wound site and by facilitating keratinocyte migration. TGF-β3 has also been shown to be a potent stimulant of neovascularization and vascular rearrangement.[125,126] Furthermore, it has been demonstrated that TGF-β3 is a potent inhibitor of DNA synthesis in human keratinocytes. These findings along with the observation of constitutive TGF-β3 expression in the intact epidermis support the hypothesis that activation of TGF-β3 may be an important stop signal for terminal differentiation in this tissue.[125,127,128] It has also been shown that unlike the other two isoforms which promote, scar formation, TGF-β3 inhibits scarring and promotes better collagen organization in vivo.[124]

In chronic wounds, TGF-βs are significantly decreased[52] possibly due to degradation from proteolytic enzymes, particularly neutrophil elastase.[129] It has also been shown that TGF-βs can be sequestered by molecules like decorin, fibrinogen, albumin and alpha2-macroglobulin, limiting their bioactivity.[130,131] Early work on clinical trials using exogenous TGF-β2 on venous stasis ulcers was promising.[132] Nevertheless, TGF-β has failed multiple clinical trials for treatment of chronic wounds.

ACTIVINS

Activins are members of the TGF-β superfamily produced by fibroblasts and keratinocytes. Their biological functions are mediated by serine/threonine kinase signaling receptors.[133] During wound repair there is up-regulation of activin where it plays a role in reepithelialization. In vitro studies suggest that activin effects keratinocyte proliferation in an indirect fashion by inducing the expression of growth factors in dermal fibroblasts.[134] Activin by itself inhibits keratinocyte proliferation[135] and induces terminal differentiation of keratinocytes[134]. Therefore, a theoretical therapeutic approach for healing chronic wounds could be delivering activin to a wound in the presence of dermal fibroblasts.

BONE MORPHOGENIC PROTEINS (BMPs)

BMPs are also members of the TGF-β superfamily. They also work via a heterodimeric serine/threonine kinase receptor. BMP-2, -4, -6, and -7 are all expressed in the wound tissue.[136] In particular, BMP-6 is highly expressed in regenerated keratinocytes as well as in fibroblasts in the acute wound.[137] After wound closure, BMP-6 accumulates throughout the suprabasal layer of the newly formed epidermis.[137] In vitro studies have shown it to be important in keratinocyte differentiation.[138,139] Furthermore, overexpression of BMP-6 has been shown to severely delay reepithelialization in vivo. There is evidence showing that BMP-6 levels are elevated in chronic wounds perhaps contributing to the pathology of these ulcers.[137]

PLATELET DERIVED GROWTH FACTOR (PDGF)

PDGF comprises a family of homo or heterodimeric growth factors including PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD. PDGFs are produced by platelets, macrophages, vascular endothelium, fibroblasts, and keratinocytes.[58,140,141] These ligands bind to two different transmembrane tyrosine kinase receptors (alpha and beta).[142] Ligand binding causes receptor dimerization, leading to autophosphorylation of the receptors. This creates a docking site for SH2 (Src homology 2) domain-containing signaling molecules, whereby several signaling pathways are then activated.[143]

PDGF plays a role in each stage of wound healing. Upon injury PDGF is released from degranulating platelets and is present in wound fluid.[144,145] This stimulates mitogenicity and chemotaxis of neutrophils, macrophages, fibroblasts, and smooth muscle cells to the wound site.[146] It also stimulates macrophages to produce and secrete growth factors such as TGF-β. Much like TGF-β, PDGF also augments macrophage-mediated tissue debridement and granulation tissue formation.[141] The effects of PDGF on inducing angiogenesis are organ dependent. For example, production of PDGF in cardiac microvascular cells leads to induction of VEGF and VEGF-receptor-2 suggesting an important role in cardiac angiogenesis.[147] With regard to wounding, it has been shown in vitro that PDGF works synergistically with hypoxia to increase the expression of VEGF as seen in ischemic injury.[148] PDGF is particularly important in blood vessel maturation. In vivo experiments demonstrated that PDGF is important in recruiting pericytes to the capillaries and thus increase the structural integrity of these vessels.[149,150] In addition, in vivo studies show that PDGF in combination with VEGF-E not only increases pericyte recruitment but also smooth muscle cells further enhancing the integrity of the capillaries. It should be noted however that PDGF's angiogenic effect is weaker than that of FGF and VEGF and does not appear to be essential for the initial formation of blood vessels.[141] PDGF also plays are role in reepithelialization by up-regulating the production of IGF-1 and thrombospondin-1 in vitro.[151] IGF-1 has been shown to increase keratinocyte motility and thrombospondin-1 delays proteolytic degradation and promotes a proliferative response in the wound in vitro.[38,152] PDGF has also been shown to enhance the proliferation of fibroblasts and thus the production of ECM.[153] In addition, it stimulates fibroblasts to contract collagen matrices and induces the myofibroblast phenotype in these cells.[154] During tissue remodeling, PDGF helps to break down old collagen by up-regulating matrix metalloproteinases.[155]

Levels of PDGF are decreased in chronic wounds.[52] It has been shown that PDGF is susceptible to the proteolytic environment found in the chronic wound and its degradation can be reversed with the addition of MMP inhibitors.[51] It is the increased MMP activity that degrades the ECM inhibiting cell migration and collagen deposition. MMPs also break down growth factors and their target cell receptors.[51]

Recombinant human variants of PDGF-BB (Becaplermin) have been successfully applied in diabetic and PUs and it is the only FDA approved drug for chronic wound treatment. Margolis et al.[156,157] was the first to demonstrate that gene delivery of PDGF can successfully and safely be tested in patients with chronic wounds. Recently, a clinical trial using Adenovirus-PDGF-BB has been initiated for persons with diabetic ulcers.[158] These advances herald in a new era in the treatment of ulcers and growth factor therapy that may enable many of the growth factors that accelerate healing experimentally to be effective in patients, i.e., by safely testing a new delivery system gene therapy.

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

The members of the VEGF family include: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor.[159] VEGF-A is produced by endothelial cells, keratinocytes, fibroblast smooth muscle cells, platelets, neutrophils, and macrophages.[69,160–164] It binds to the tyrosine kinase surface receptors Flt-1 (VEGF receptor-1) and KDR (VEGF receptor–2 [VEGFR-2])[165–167] localized to the endothelial surface of blood vessels.[168–170] These receptors have different functions. KDR is an important mediator of chemotaxis and proliferation of endothelial cells in vitro.[171] It is also responsible for inducing endothelial cell differentiation. In comparison, Flt-1 is required for organization of blood vessels.[172,173] Flt-1 may also be involved in mediating vascular permeability,[174] MMP expression in vascular smooth muscle cells,[175] and the induction of anti-apoptotic proteins.[176]

VEGF-A is important in wound healing because it promotes the early events in angiogenesis, particularly endothelial cell migration[177–179] and proliferation[180–184] as seen

in several in vitro studies. VEGF-A transcription and secretion along with the VEGFR are elevated in the acute wound.[185–187] Upon injury activated platelets release VEGF-A.[161,188] In addition, macrophages release VEGF-A during wound healing[186] as well as releasing TNF-α, which induces VEGF-A expression in keratinocytes and fibroblasts.[185] Other cytokines and growth factors that act as paracrine factors enhancing VEGF-A expression include TGF-β1, EGF, TGF-α, KGF, bFGF, PDGF-BB, and IL-1β.[185,189,190] A major stimulus for the release of VEGF-A in the acute wound setting is hypoxia due to metabolic derangements in the wound environment. The resulting angiogenesis restores tissue perfusion, reestablishes microcirculation, and increases oxygen tension at the wound site.[191] In particular, hypoxia enhances VEGF-A expression in monocytes, fibroblasts, keratinocytes, myocytes, and endothelial cells. It also increases the expression of Flt-1 receptors on endothelial cells.[192] As a result, there is a gradient of VEGF-A expression that parallels the hypoxic gradient.[193] In addition to its angiogenic effects, VEGF-A plays a role in lymphangiogenesis during wound healing. One in vitro study proposed that VEGF-A promotes lymphatic vasculature formation via activation of VEGFR-2.[194]

Chronic wounds such as DFUs,[195–197] venous stasis ulcers,[198,199] and PUs[200–203] have areas of local skin ischemia making VEGF-A a possible therapeutic modality. In animal studies, it has been shown that the administration of VEGF-A restores impaired angiogenesis found in diabetic ischemic limbs.[204–208] Other in vivo experiments show that show that VEGF-A improves reepithelialization of diabetic wounds associated with enhanced vessel formation.[209] Despite these improvements, however, exogenous administration of VEGF induces sustained vascular leakage and promotes the formation of disorganized blood vessels as well as malformed and poorly functional lymphatic vessels.[210,211] In human studies, intramuscular gene transfer of $VEGF_{165}$ to patients with ischemic ulcers and/or rest pain secondary to peripheral arterial disease resulted in limb salvage significantly decreasing rest pain.[212]

VEGF-C is also up-regulated during wound healing. This growth factor is primarily released by macrophages and is important during the inflammatory stage of wound healing.[213] VEGF-C works mostly through the VEGF receptor-3 (VEGFR3), which is expressed in lymphatic endothelium, fenestrated endothelia, and monocytes/macrophages.[213–215] However, the proteolytically processed mature form of VEGF-C can also activate VEGFR-2 in blood vessel endothelium.[216,217] In vitro studies show this growth factor playing a role in facilitating hematopoietic and inflammatory cell recruitment to the wound site both directly and indirectly by binding to VEGFR-2 increasing vascular permeability.[218,219] In vitro studies also show VEGF-C playing a role in lymphoangiogenesis by binding to VEGFR-3[220] and angiogenesis after proteolytic cleavage by binding to VEGFR-2.[216–219] Because DFUs are a result of insufficient blood perfusion coupled with impaired angiogenesis, treatment with VEGF-C has been proposed. In an in vivo animal model VEGF-C was administered via an adenoviral vector to genetically diabetic mice resulting in accelerated healing rate. These results suggest potential therapeutic function in treatment of diabetic wounds.[159]

Placental growth factor (PLGF) is a proangiogenic molecule that is up-regulated during wound healing. In the skin, this growth factor is expressed by keratinocytes and by endothelial cells. This growth factor acts by binding and activating the VEGFR-1. Like VEGF-C, PLGF plays a role during the inflammatory stage of wound healing. It has been shown, in vitro, to promote monocyte chemotaxis and bone marrow-derived precursor cell mobilization.[221–223] It also is involved in promoting granulation tissue formation, maturation, and vascularization. It is thought to work synergistically with VEGF by potentiating its proangiogenic function.[224,225] In addition, PLGF has been shown to directly stimulate cultured fibroblast migration, suggesting a direct role in accelerating granulation tissue maturation. In DFUs, it has been shown that PLGF expression is significantly reduced. The observation that PLGF specifically enhances adult pathophysiological neovascularization[224] does not interfere with lymphatic vessel function, and induces augmented permeability only when administered at high concentration.[210,226] This makes it an ideal candidate for therapeutic modulation for adult angiogenesis. Animal models using genetically diabetic mice have shown that diabetic wound treatment with an adenovirus vector expressing the PLGF gene significantly accelerated the healing process compared with wounds treated with a control vector.[225]

CONNECTIVE TISSUE GROWTH FACTOR (CTGF)

CTGF is an ECM-associated heparin-binding protein that binds directly to integrins. It is synthesized by fibroblasts and stimulates proliferation and chemotaxis of these cells. CTGF expression is increased after injury and is involved in granulation tissue formation, reepithelialization, and matrix formation and remodeling.[227] In vitro experiments have shown that CTGF promotes endothelial proliferation, migration, survival, and adhesions in angiogenesis.[228,229] It has also been demonstrated that CTGF is required for reepithelialization in wound healing by promoting cell migration. It is thought to be induced by TGF-β through the Ras/MEK/ERK MAPK signalling pathway.[230] In addition, CTGF is a strong inducer of ECM proteins, such as collagen type I and fibronectin and their integrin receptors, and acts as a mediator of TGF-β.[231] Much like TGF-β, CTGF also has increased expression in hypertrophic and keloid scars.[116]

GRANULOCYTE MACROPHAGE-COLONY STIMULATING FACTOR (GM-CSF)

GM-CSF has been shown to be increased in the epidermis in wounded skin.[232] It is particularly important during the inflammatory stage of wound healing increasing the number of neutrophils and enhancing their function at the wound site.[233] In vitro studies have shown GM-CSF to increase keratinocyte proliferation and thus enhance reepithelialization. It has been suggested that GM-CSF works directly on the keratinocyte but also indirectly by up-regulating IL-6.[232] In addition, in vitro studies have demonstrated this growth factor to increase migration and proliferation of endothelial cells suggesting a role in

angiogenesis.[234] In patients with DFUs, subcutaneous injections of GM-CSF resulted in quicker resolution of cellulites, a trend toward ulcer healing and lower incidence of amputation.[235] GM-CSF applied locally in the wound is likely to have significant patient benefit for chronic wounds.[236–241] Further study in DFUs and or PUs would be potentially highly useful, and based on the experimental and clinical data this may be another potential therapeutic modality for chronic ulcers.

PROINFLAMMATORY CYTOKINES

Proinflammatory cytokines, particularly IL-1 and interleukin-6, and TNF-α are up-regulated during the inflammatory phase of wound healing.[242] IL-1 is produced by neutrophils, monocytes, macrophages, and keratinocytes. Upon wound healing it is immediately released by keratinocytes. In addition to having a paracrine effect, it also works in an autocrine fashion increasing keratinocyte migration and proliferation (reviewed in Raja et al.[13]). IL-1 has been shown to induce the expression of K6 and K16 in migrating keratinocytes.[1,243] In addition, IL-1 activates fibroblasts and increases the secretion of FGF-7.[244]

IL-6 is produced by neutrophils and monocytes and has been shown to be important in initiating the healing response. Its expression is increased after wounding and tends to persist in older wounds.[83,84,245] It has a mitogenic[77] and proliferative[78,199] effect on keratinocytes and is chemoattractive to neutrophils.

Much like IL-1, TNF-α can induce the production of FGF-7, suggesting that it can indirectly promote reepithelialization.[246,247] Alone, TNF-α has been shown to inhibit wound reepithelialization. The effects of exogenous TNF-α are dependent on concentration and duration of exposure emphasizing the importance of balancing the proinflammatory signals controlling wound healing. TNF-α, at low levels, can promote wound healing by indirectly stimulating inflammation and increasing macrophage produced growth factors. However, at higher levels, especially for prolonged periods of time, TNF-α has a detrimental effect on healing. TNF-α suppresses synthesis of ECM proteins and TIMPs while increasing synthesis of MMPs (MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, and MT1-MMP).[248–251] In addition, elevated levels of IL-1β have a similar response to that of TNF-α. Both TNF-α and IL-1β have been shown to perpetuate each others expression and therefore amplify this signal.[51]

Levels of TNF-α and IL-1β are elevated in chronic wounds.[252,253] In addition, infection that is common in chronic wounds further contributes to prolonged inflammation. Furthermore, nonhealing wounds also exhibit elevated levels of interstitial collagenases, gelatinases, and stromelysins that have been shown to be induced by TNF-α and IL-1β.[252] It has, therefore, been hypothesized that in chronic wounds, chronic inflammation causes inflammatory cells to secrete TNF-α and IL-1β that synergistically increase production of MMPs while reducing synthesis of TIMPs. It is increased MMP activity that degrades the ECM inhibiting cell migration and collagen deposition. MMPs also break down growth factors and their target cell receptors.[51]

CHEMOKINES

Chemokines are also active participants in the wound healing process because they stimulate the migration of multiple cell types in the wound site particularly inflammatory cells. In addition, the presence of chemokine receptors on resident cells suggests that they also contribute to the regulation of reepithelialization, tissue remodeling, and angiogenesis (reviewed in Raja et al.[13]). The CXC, CC, and C families of ligands act by binding to G protein-coupled surface receptors, CXC-receptors and the CC-receptor.

Macrophage chemo-attractant protein (MCP-1 or CCL2) is a CC family chemokine. MCP-1 is induced in keratinocytes upon wounding. It is a chemoattractant for monocytes/macrophages, T-cells, and mast cells.[254] Sustained expression of this chemokine permits a prolonged presence of neutrophils and macrophages in the chronic wound contributing to a prolonged inflammatory response.[255] However, lack of MCP-1 in vivo significantly delays wound healing particularly with reepithelialization, angiogenesis, and collagen synthesis as seen in mouse models.[256] This suggests that in the mouse MCP-1 may be influencing gene expression/protein synthesis of growth factors in murine macrophages. However, in humans MCP-1 does not seem to regulate growth factor production by these cells.[257] Addition of exogenous MCP-1 to wounds in animals yielded only moderate improvements in wound healing.[258]

Interferon inducible protein 10 (IP-10 or CXCL10) is another cytokine part of the CXC family. In acute wounds and chronic inflammatory states, there is increased expression by keratinocytes. IP-10 has been demonstrated to negatively impact wound healing. Overexpression of IP-10 results in a more intense inflammatory response by recruiting lymphocytes to the wound site.[257,259] In vitro studies show that IP-10 delays reepithelialization and prolongs the granulation phase. This cytokine inhibits the migration of dermal fibroblasts by blocking their release from the substratum regulated by IP-10 inhibition of EGF and heparin-binding EGF-like growth factor receptor-mediated calpain activity.[44] In addition, it has been shown that IP-10 inhibits angiogenesis (reviewed in Belperio et al.[260]). A suggested mechanism can be seen in the related cytokine, PF4. PF4 inhibits endothelial cell migration, proliferation, and angiogenesis in response to bFGF. PF4 inhibits bFGF binding its receptor by forming heterodimeric complexes via heparin binding. It has been suggested that IP-10 might work in a similar fashion.[261]

Interleukin-8 (IL-8 or CXCL8) is a member of the CXC family.[13] Its expression is increased in acute wounds[257] and it has been shown to play a role in reepithelialization by increasing keratinocyte migration and proliferation.[262,263] It also induces the expression of MMPs in leukocytes, stimulating tissue remodeling.[257] It is, however, a strong chemoattractant for neutrophils, thus participating in the inflammatory response.[264] High levels of this chemokine accumulate in nonhealing wounds. Furthermore, addition of IL-8 in high levels decreases keratinocyte proliferation and collagen lattice contraction by fibroblasts.[265] It has been shown that there are relatively low levels of IL-8 in the fetus. This finding may be responsible for the lack of inflammation during the fetal wound healing and contribute to scarless wounds.[266]

The GRO-α (CXCL1) chemokine is also a member of the CXC family. This cytokine is a potent regulator of neu-

trophil chemotaxis and is up-regulated in the acute wound. In vitro studies suggest a role in reepithelialization by promoting keratinocyte migration.[257,259]

The SDF-1 (CXCL12) chemokine is a member of the CXC family and works via the CXCR4 receptor. It plays a role in the inflammatory response by recruiting lymphocytes to the wound and promoting angiogenesis. Endothelial cells, myofibroblasts, and keratinocytes express SDF-1. When homeostasis is disturbed in an acute wound SDF-1 is seen at increased levels at the wound margin.[267] An in vivo study has demonstrated that SDF-1 promotes proliferation and migration of endothelial cells.[268] In addition, it recruits proangiogenic subpopulations of hematopoietic cells (bone marrow progenitors) from circulation to peripheral tissues.[269] SDF-1 may also enhance keratinocyte proliferation thus contributing to reepithelialization.[270] It has been suggested that due to the chemokines tightly controlled expression, both site and time point of interference indicates the outcome of intervention.[267] Recently, it has been shown in diabetic mouse (db/db) wound model that decreased level of SDF1α prevents circulating bone marrow progenitor cell migration into the wound site.[271,272]

SUMMARY

Growth factors, cytokines and chemokines are crucial for coordinating multiple cell types during the healing process, making cutaneous wound healing possible. Proper wound healing is guided by stringent regulation of these agents as well as a wound environment that favors their activity. In the acute wound, the healing process is controlled by spatio-temporal action of these growth factors, cytokines and chemokines leading through progression of healing and resulting in the reestablishment of the skin's barrier function. This is contrasted by the chronic wound, which is arrested in a state of chronic inflammation. As a consequence, the generation of a proteolytic environment by inflammatory cells infiltrating the wound site as well as prolonged up-regulation of pro-inflammatory cytokines and chemokines inhibits normal progression of wound healing. This environment subjects various growth factors and cytokines to degradation and sequestration in the wound site inhibiting their function.

Topical delivery of growth factors to chronic wounds must be resistant to rapid degradation form the wounds proteolytic environment and have sustained release. This is readily being accomplished using gene therapy. Currently, multiple novel delivery systems, including adenovirus and slow-releasing polymers are being investigated as growth factor delivery systems. The most promising growth factors that require clinical testing are VEGF, bFGF, and GM-CSF. PDGF-BB has already been approved by the FDA and is currently used in the treatment of chronic ulcers. Living cell therapy, which is also FDA approved, may be considered as sustained, simultaneous multiple growth factor therapy. Both healthy keratinocytes and fibroblasts produce at least 17 different growth factors[273] and secrete these factors stimulating patients' cells to participate in healing process.[274,275] Despite these novel approaches, wound debridement should remain an integral component in treating chronic wounds. Debridement facilitates growth factor delivery by restoring the expression of growth factor receptors that are not properly expressed at the nonhealing edge of chronic ulcers, making cells unresponsive to exogenous growth factor therapy.[50,273]

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