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REVIEW

A potential role of SDF-1/CXCR4 chemotactic pathway in wound healing and hypertrophic scar formation

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Fibroproliferative disorders are an ongoing clinical issue that is prevalent within society today. These disorders generally manifest themselves by an overproduction of fibrotic tissue with unknown provocation resulting in numerous detrimental defects. Cellular migration of blood-borne cells via the chemotactic pathway, consisting of stromal cell-derived factor 1 and its receptor, CXCR4, has been strongly implicated in post-burn hypertrophic scar formation. Evidence has shown this pathway has potential as a therapeutic target in the formation of hypertrophic scar and likely in other fibroproliferative disorders.

Keywords: Hypertrophic scar; fibroproliferative disorders; SDF-1/CXCR4 pathway; CXCR4 antagonist

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Introduction

Wound Healing Process

The mechanism of wound healing involves numerous cellular components, their migration and subsequent production of cytokines, which stimulate effector responses. Generally, this process involves three overlapping phases consisting of inflammation, proliferation and maturation and remodeling, with the antecedent of these being hemostasis [1].

Hemostasis

In the event of an injury, hemostasis or the cessation of bleeding is immediately initiated and occurs for a brief period prior to inflammation [2]. As blood comes into contact with the open wound, platelets are stimulated to release clotting factors and growth factors. This release is due to contact with exposed collagen, extracellular matrix (ECM) and other tissue elements [3]. Blood vessels constrict and complement-clotting cascades are activated, initiating the formation of a fibrin clot [1]. This clot consists primarily of platelets embedded in a cross-linked mesh of fibrin fibers that serves many functions. It acts as a temporary protective shield for the wound and as a medium through which various inflammatory cells can migrate [4]. It also serves as a potent cytokine and growth factor reservoir during platelet degranulation [5]. Granules such as α-granules are found in platelets and release a number of potent cytokines. These include epidermal growth factor, platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), vascular growth factor, fibroblast growth factor 2 and insulin-like growth factor, many of which are involved in chemotactic homing of inflammatory cells, fibroblast migration and cellular proliferation [6].

Inflammation

Inflammation plays a significant role in stimulating proliferation for wound closure. During inflammation vasodilation occurs, increasing vascular permeability for invading inflammatory cells [4]. The proliferation and differentiation of these cells is a necessity for the phagocytosis of bacteria, damaged tissue and any other foreign material [7]. Neutrophils are generally the first cells to arrive after injury and debride the wound of denatured tissue via protease production [8]. Peripheral blood monocytes then infiltrate the tissue and differentiate into macrophages, which continue to debride the wound area.

Macrophages secrete a vast array of cytokines that stimulate further attraction of fibroblasts and smooth muscle cells. They also stimulate collagen expression and promote re-epithelialization and angiogenesis. Consequently, macrophages play an important part in the transition between the inflammatory phase and proliferative phase, given the latter is heavily dependent on their cytokine secretion profile [2,3,9]. Although it is not a definitive end, the presence of macrophages can act as a tentative marker to denote the end of the inflammatory phase and the start of the proliferative phase [3].

Proliferation

The proliferative phase involves a number of reparative processes for the epidermal and dermal layers of the skin that include ECM deposition, reepithelialization, continued cellular migration and angiogenesis [2,10]. Many of the cytokines and growth factors that are released by platelets and macrophages are held within the fibrin clot and act to stimulate cells as they enter the wound area [11]. Fibroblasts and endothelial cells are generally regarded as the most significant proliferative cells within this phase [4]. Migratory and resident fibroblasts in conjunction with macrophages, fibrocytes and endothelial cells form granulation tissue, which bridges the wound gap and leads to vascular ingrowth. Activated fibroblasts synthesize type III collagen, ECM and other constituents to form this tissue, which eventually replaces the fibrin clot [1,12-14].

During injury, damaged blood vessels within tissue are repaired by angiogenesis, a process stimulated by changes in the tissue environment and a host of cytokines and growth factors [15]. Matrix metalloproteinases degrade and dissect the basement membrane and ECM, thus permitting endothelial cells to migrate, form tubules and eventually new capillaries [2].

Re-epithelialization also occurs at the site of injury. Epithelial cells are stimulated to proliferate and migrate to prevent further fluid loss and bacterial invasion [4]. Their migration is mediated via cytokine and growth factor secretions by platelets, macrophages and fibroblasts. Proliferating keratinocytes eventually progress across the granulation tissue until wound closure is achieved, thus marking the end of the proliferative phase [1,12].

Maturation and Remodeling

The maturation/remodeling phase is the longest phase in wound healing. Its main feature constitutes ECM modification and collagen deposition. Type III collagen is degraded and replaced by thicker type I collagen fibers [3,4,14]. These new collagen fibers are then broken down and arranged in a cross-linked, organized manner that differs from the morphology observed in uninjured tissue [3,4]. In addition to collagen deposition, wound contraction also occurs in the final stage of scar maturation by fibroblasts that have differentiated into myofibroblasts expressing contractile myofilaments, including α-smooth muscle actin [2,16].

The final stage in scar formation can persist for long periods during which contraction and ECM remodeling continue to occur until cellular activity ceases and apoptosis occurs [1].

SDF-1/CXCR4 Chemotactic Pathway

Regulation of cellular migration is generally the result of chemokine stimulation [17]. Chemokines are a subset of pro-inflammatory cytokines whose functions are specifically correlated to cellular migration. They act as chemoattractants, stimulating the migration of various cell types [4]. The SDF-1/CXCR4 pathway consists of stromal cell-derived factor 1 (SDF-1) also known as CXCL12, a chemokine that belongs to the CXC family, where its structure consists of N-terminal cysteines (C) that are separated by single or multiple amino acids (X), and its receptor CXCR4[18]. Initially it was believed that SDF-1 was exclusive to one receptor (CXCR4); however, recent evidence has shown it may also bind with CXCR7 as well [19,20].

SDF-1 is similarly expressed in human, swine and rat skin and is produced by fibroblasts, endothelial cells, myofibroblasts and keratinocytes [21,22]. Expression of CXCR4 is present on bone marrow-derived stem cells and other circulatory cell types such as $CD14^+$ monocytes and fibrocytes [18].

The chemotactic pathway that SDF-1 and its receptor CXCR4 constitute has involvement in the migration of bone

marrow-derived stem cells, or more specifically CD14 and CXCR4 expressing cells to damaged tissue [18,23,24]. SDF-1 gradients attract circulating cells expressing CXCR4 into peripheral tissue where adherence is facilitated by CXCR4 signaling [25].

SDF-1/CXCR4 Pathway in Normal Wound Healing

In normal wound healing, the SDF-1/CXCR4 pathway facilitates cellular migration of bone marrow-derived stem cells that migrate from the blood to injured tissue in the wound healing process [26]. The SDF-1/CXCR4 pathway is a crucial chemotactic mechanism that has been implicated in this migration, primarily in the inflammatory and proliferative phase [27]. Bone marrow-derived peripheral blood mononuclear cells (PBMCs) differentiate into epithelial-like cells, endothelial cells, macrophages, as well as myofibroblasts via an intermediary form called the fibrocyte. Differentiated epithelial-like cells and endothelial cells take part in re-epithelialization and angiogenesis, while macrophage, myofibroblasts and fibrocytes contribute to connective tissue production and contracture, which enhance overall wound healing [24,28-30]. Thus, the SDF-1/CXCR4 chemotactic pathway is pertinent to wound healing as it facilitates the migration of cells that play prominent roles in the reparative process.

Fibroproliferative Disorders

Fibroproliferative disorders (FPD) may be benign or malignant in nature and include idiopathic pulmonary fibrosis, systemic sclerosis, hepatic cirrhosis, myelofibrosis, atherosclerosis, hypertrophic scars (HTS), keloids and numerous other conditions. Although their exact pathophysiology is unknown, their pathological definition is characteristic of excessive fibrosis, often as a consequence of inflammation to injury/tissue damage [31].

Fibrosis due to prolonged deposition of ECM due to an imbalance between the synthesis of new connective tissue (ECM and local cells) and its degradation, results in a net increase of newly synthesized fibrous connective tissue [32-35]. This change in the amount of fibrous tissue and its composition ultimately result in the deterioration of structure and function.

Fibroblasts and myofibroblasts are the primary cells in fibrosis and are responsible for secretion of ECM [36], where their stimulation and effector responses are controlled by the release and subsequent activation of various growth factors and cytokines from a variety of cellular sources [4]. One of the most prevalent cytokines is TGF-β, a primary functional mediator that stimulates the production of collagen and a number of other components such as elastin, fibronectin and glycosaminoglycans that constitute the ECM in scar tissue [34,35]. Activated fibroblasts also have the potential to differentiate into myofibroblasts, which express α-smooth muscle actin, myofilaments that allow them to exhibit contractile properties essential to wound closure [8,16] but are also capable of producing components of fibrotic tissue in FPD[37]. Ultimately fibroblasts and myofibroblasts predominate in fibrotic tissue production and remodeling such that regulation of their functional properties is an important target for developing therapeutic strategies in a variety of fibroproliferative conditions.

The Role of Blood-Borne Cell Migration in Fibrosis

Recently PBMCs have been implicated as significant contributors to fibrosis. Mildner *et al* concluded that the secretome of PBMCs has positive effects on the wound healing process and enhanced angiogenic effects [38] while Zhang and Huang [29] outlined the differentiation potential of PBMCs into various other cells. Peripheral blood $CD14^+$ monocytes constitute part of the PBMC fraction, which can differentiate into fibrocytes and macrophages in fibroproliferative conditions [4].

Fibrocytes can perform a number of functions similar to fibroblasts including collagen synthesis, although to a lesser degree. In addition, fibrocytes also secrete various pro-inflammatory cytokines and growth factors including interleukin 6, 10, TGF-β, PDGF and tumor necrosis factor alpha, with TGF-β being the most prominent[9,10]. Both human and murine fibrocytes express CXCR4 and have been found to migrate in response to SDF-1 expression *in vitro* and *in vivo*, indicating the significance of the SDF-1/CXCR4 pathway in fibrosis [39]. Furthermore, a study conducted by Mori *et al* [40] has confirmed that fibrocytes are capable of differentiating into myofibroblasts and thus, contribute to wound contraction.

Unlike fibrocytes, macrophages have a more indirect contribution to fibrosis through their roles in chronic inflammation by producing a wide array of cytokines that stimulate fibroblast proliferation and production of ECM proteins [15]. The most significant effector cytokines secreted by macrophages include TGF-β, PDGF, fibroblast growth factor 2 and insulin-like growth factor 1, all of which have profibrotic effects on fibroblasts [41]. Macrophages play pivotal, yet divergent roles in the development of hepatic fibrosis via ECM accumulation during injury and matrix degradation during the recovery period [42].

TGF-β is one of the most highly regarded cytokines involved in the wound healing process. It is secreted by

macrophages and fibrocytes in addition to a variety of other cells [43]. Three homologous mammalian forms exist (TGF-β 1, 2 and 3), with TGF-β1 being the most prevalent and most investigated [44]. TGF-β promotes ECM protein synthesis for granulation tissue. It acts as chemoattractant for inflammatory cellular migration and is a primary cytokine involved in modulating keratinocyte and fibroblast interactions, aiding contraction by stimulating fibroblast differentiation into myofibroblasts and promoting angiogenesis and re-epithelialization [44]. Unusually high amounts of TGF-β1 have been found in HTS tissue, supporting its profibrotic properties in fibroproliferative disorders [45,46].

Although tissue-derived fibroblasts are the most pertinent cells in fibrosis, recognition of the implicit role that blood-borne cells play cannot be diminished. Up-regulation of fibroblast activity is facilitated by a number of migratory cells including $CD14^+$ $CXCR4^+$ monocytes that differentiate into fibrocytes and macrophages, which produce a number of profibrotic cytokines including TGF-β and migrate via the SDF-1/CXCR4 chemotactic pathway, substantiating the role of PBMCs in normal wound healing.

PBMC Chemotaxis in HTS Formation

Recent studies have demonstrated an up-regulation of the SDF-1/CXCR4 pathway in the development of FPD [18,23]. According to studies conducted by Xu *et al* [23], SDF-1 and CXCR4 expressing cells were clearly identified in human patients with idiopathic pulmonary fibrosis, similar to earlier results in their murine models. More recently, Ding *et al* [18] showed signaling of the SDF-1/CXCR4 in post-burn HTS patients, was up-regulated by an increase in the expression of SDF-1 within tissue and serum and an increased expression of its receptor CXCR4. Chronic expression of SDF-1 facilitates the attraction of CXCR4 expressive cells, a mechanism that is up-regulated in other fibroproliferative disorders [18,23,47]. Consequently, increased $CD14^+$ CXCR4 positive cells, most of which appeared to be monocytes [18] differentiate into profibrotic macrophages and fibrocytes [8,9] via the SDF-1/CXCR4 chemotactic pathway perpetuating fibrosis.

Recently, the down regulation of the SDF-1/CXCR4 pathway in HTS formation was assessed by Ding *et al*[47] using a human HTS-like nude mouse model[46]. Morphological changes due to blood-borne cellular migration included tissue exhibiting a typical HTS aesthetic appearance where the scars are thickened, raised, firm and contracted. Epidermal and dermal layers were thicker and characteristic of a lack of rete ridges. Collagen fibers and fiber bundlers were more compact and disoriented as in HTS and the gene expression of type 1 collagen was gradually up-regulated [47].

Inhibition of Blood-Borne Cell Chemotaxis via the CXCR4 Antagonist, CTCE-9908 Attenuates Scar Formation

Inhibition of the SDF-1/CXCR4 pathway has distinct implications in fibrotic development. Zgraggen *et a l*[48] emphasized the role of the SDF-1/CXCR4 pathway in skin inflammation and indicated its inhibition as a potential therapeutic strategy. Interferon alpha 2b, an anti-proliferative cytokine that has been used to treat fibrotic conditions [49,50] improved scar remodeling by decreasing SDF-1/CXCR4 signaling and expression as well as down regulating TGF-β1 and promoting myofibroblast apoptosis [18].

In a more recent study by Ding *et al* [47] the use of CTCE-9908, a CXCR4 antagonist, was tested therapeutically on the SDF-1/CXCR4 pathway. It was shown to improve a number of HTS scar features in a human HTS-like nude mouse model including reduced scar thickness, cellularity, vascularity, contraction and thinner and softer engrafted tissue. Macrophage and myofibroblast populations were also observed to decrease, implicating a reduction in chemotaxis of peripheral blood cells and substantiating the potential of SDF-1/CXCR4 signaling as a therapeutic target for HTS development. Other antagonists of the SDF-1/CXCR4 have also been shown to have attenuating effects on fibrosis in other FPD. These include plerixafor (AMD3100), 4F-benzoyl-TN14003 (T140) and tetramethylpyrazine (TMP) [23,51,52].

Clinical Significance

HTS is a FPD with an unclear pathophysiology that may follow trauma, various surgical proceedings or thermal injury [46]. Typically manifested by pruritus, pain and discomfort, contractures and restriction of mobility, in addition to cosmetic impairment for the affected individual, rendering new novel therapeutic techniques very desirable [53]. The blood-borne cell migration caused by the SDF-1/CXCR4 chemotactic pathway is one such favorable target in the development of an anti-fibrotic therapy not only for HTS, but other fibroproliferative conditions as well. However, more rigorous studies are needed to further quantify the specific spectrum of effects that various blood-borne cells have on FPD including HTS.

Summary

Fibroproliferative conditions as HTS are characteristically unfavorable aesthetically, physiologically and psychologically. The exact etiology of excessive fibrosis in

these disorders such as HTS is still unknown but is associated with an imbalance in collagen synthesis and degradation resulting in a more fibrous scar. Blood-borne cell involvement via the SDF-1/CXCR4 chemotactic pathway has been implicated as having a significant role in the development of excessively fibrous scars with respect to cytokine secretory profiles of migratory cells and their ability to differentiate into other effector cells. When this pathway is up-regulated in HTS development, there is an increase in migratory $CD14^+$ $CXCR4^+$ cells, most of which appeared to be monocytes. Increased macrophages and fibrocytes in fibrotic tissues derived from circulating monocytes make significant contributions to HTS tissue, substantiating the importance of the SDF-1/CXCR4 pathway within FPD.

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Conflicts of Interest

The authors declared no conflicts of interest.

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