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Professor Special Lecture: 149th Regular Meeting of the Medical Society of Toho University

Review Article

Tissue Repair Mediated by Basic Fibroblast Growth Factor in Wounds

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ABSTRACT: Basic fibroblast growth factor stimulates granulation tissue formation and promotes scarless repair, a process that is partly mediated by decreased alpha smooth muscle actin (α-SMA) expression. Basic fibroblast growth factor also specifically induces angiogenesis by inducing an angiogenic phenotype of CD34+/pro-collagen I+ fibrocytes. This leads to a possible mechanism of disappearance of the newly formed vessels through conversion of angiogenic phenotype of fibrocytes into fibroblasts.

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KEYWORDS: bFGF, fibroblasts, endothelial cells, fibrocytes

Wound healing

Wound healing following injury is a complex and multicellular process that relies on numerous growth factors. These factors regulate a complex network that alters growth and differentiation of target cells.[1] With the assistance of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), endothelial cell proliferation results in angiogenesis. Within hours of injury, re-epithelialization begins, and release of epidermal growth factor (EGF), transforming growth factor (TGF)-α, and FGF stimulates epithelial cell migration and proliferation. Matrix formation requires removal of granulation tissues with revascularization, which is followed by tissue remodeling involving synthesis of new collagen mediated by TGF-β. Wound healing thus depends on growth factors, cytokines, and chemokines involved in a complex, integrated signaling system that coordinates cellular processes.[1]

Scar formation inhibited by basic fibroblast growth factor

Basic fibroblast growth factor (bFGF) is present in higher concentrations in acute wounds and is important in granulation tissue formation, re-epithelialization, and tissue remodeling. Previous studies reported that bFGF regulates synthesis and deposition of extracellular matrix (ECM) components, increases keratinocyte motility during re-epithelialization, and promotes migration of fibroblasts, thus inducing them to produce collagenase. Administration of bFGF into skin wounds accelerates dermal wound healing.[2]

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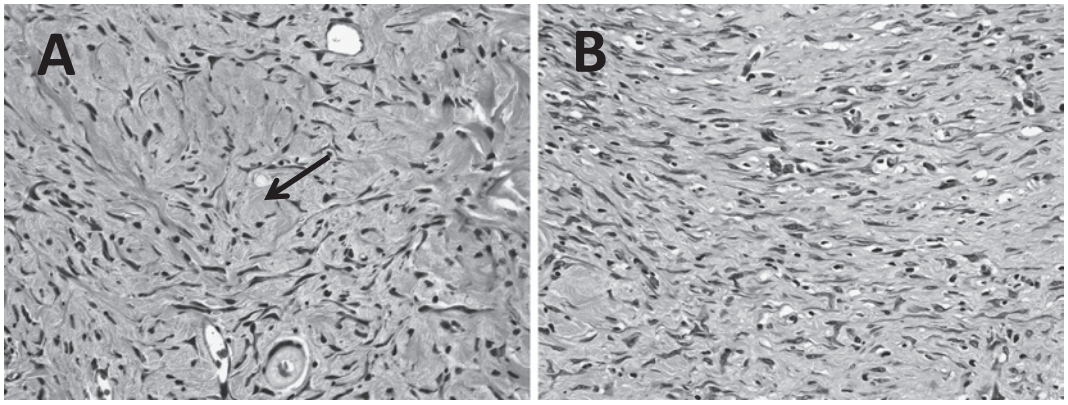

Fig. 1 Histological changes in bFGF-treated wounds
Comparative histological analysis of wounds showing that collagen bundles in control wounds (A) are thicker than those in bFGF-treated wounds (B). Bundles (arrow) in control wounds (A) were more randomly distributed and thicker than in bFGF-treated wounds (B), indicating the effectiveness of bFGF for inhibiting mature collagen formation in wounds. Well-arranged fibroblasts are present in bFGF-treated wounds. HE staining (magnification: 40×).
bFGF: basic fibroblast growth factor, HE staining: hematoxylin eosin staining

We previously assessed tissue response in wounds after bFGF treatment and found that the width of incisional wounds treated with bFGF exceeded that of non-treated wounds after treatment for 7 days.[3] Therefore, tissue response in bFGF-treated wounds was greater than that in the control group. However, after day 7, the width of bFGF-treated wounds diminished and was significantly less than that of the control group.[3] Therefore, granulation tissue formation after bFGF treatment from day 7 markedly decreased by day 28.

The decrease in tissue cellularity during healing was reported to be involved in apoptosis.[4,5] Thus, we previously assessed the relationship between the degree of wound healing and the level of apoptosis in bFGF-treated wounds. The level of apoptosis in bFGF-treated wounds was significantly greater than in the control.[3] Therefore, inhibition of scar formation in bFGF-treated wounds is likely mainly due to increased apoptosis in granulation tissue cells. Interestingly, vimentin-positive fibroblasts in bFGF-treated wounds were well arranged within the dermis and tended to run in parallel, whereas fibroblasts in control wounds showed little uniformity of arrangement.[3] Our subsequent study showed that collagen bundles in control wounds had become thicker and more numerous.[6] The orientation of bundles was more random and thicker in a control group than in a bFGF group (Fig. 1). Morphometric analysis revealed significantly fewer mature collagen bundles in the bFGF group than in the control group, which suggests that bFGF in wounds markedly inhibited formation of mature collagen. Morphometric analysis showed noticeably less contraction in bFGF-treated wounds, and inhibition of wound contraction was significantly greater in bFGF-treated wounds than in the control.[6,7]

Differentiation of fibroblasts into myofibroblasts is associated with alpha-smooth muscle actin (α-SMA) expression. We found that α-SMA expression was diminished in bFGF-treated wounds. This indicates that bFGF treatment of wounds reduces α-SMA expression in treated wounds. In addition, we examined the level of apoptosis in treated wounds.[6] bFGF-treated wounds exhibited less α-SMA expression and significantly increased apoptosis as compared with control wounds.[6] Further study revealed that apoptosis in α-SMA－positive fibroblastic cells was significantly increased in bFGF-treated wounds. This showed that bFGF is a potent inducer of myofibroblastic apoptosis, which partly contributes to reduced wound contraction. Recently, we revealed strong evidence of effective inhibition of collagen expression by bFGF in wounds. Collagen type I messenger ribonucleic acid (mRNA) expression was specifically inhibited in bFGF-treated wounds, without inhibition of collagen type III or IV mRNA, as cleared by FGF receptor 1 (FGFR1) small interfering RNA (siRNA)－injected wounds with the capability of knocking down the bFGF/FGFR1 signaling system[8] (Fig. 2).

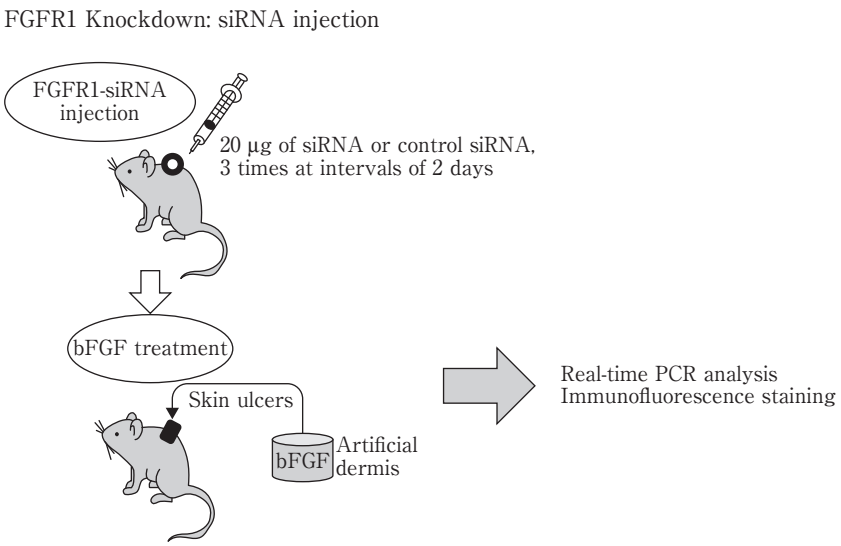

Fig. 2 Knock down of FGFR1 by FGFR1 siRNA injection and assessment of the specificity of bFGF tissue response
Full-thickness wounds were created and injected with siRNA or control siRNA. The wounds were then treated with bFGF-treated artificial dermis, and skin tissue samples were excised from wounds for immunofluorescence staining and PCR analysis.
FGFR1: fibroblast growth factor, siRNA: small interfering ribonucleic acid, bFGF: basic fibroblast growth factor, PCR: polymerase chain reaction

bFGF promotes angiogenesis by inducing fibrocytes

bFGF positively regulates angiogenesis by recruiting inflammatory cells such as monocytes, macrophages, and neutrophils. By promoting the early events of angiogenesis, VEGF-A also positively regulates angiogenesis, particularly promotion of endothelial cell migration.[1] A previous study reported that endothelial cell precursors appear to be induced by bFGF, which suggests that bFGF has a role in inducing endothelial cell precursors.[9] In contrast, VEGFR-2 appears to be expressed on endothelial cell precursors, as the precursors adopt a mesenchymal morphology. Because VEGFR-2 is the first VEGF receptor to be expressed, VEGF might be not involved in the induction of endothelial cell precursors. However, VEGF appears to be important in the growth and morphogenesis of endothelial cell precursors into vascular patterns.[9]

Among the types of repaired tissue (granulation tissue) cells, endothelial cells are believed to originate in bone marrow cells. Numerous studies have investigated the pathways by which bone marrow cells undergo differentiation into endothelial cells, thereby resulting in vascularization in granulation tissues.[10] In an *in vitro* experiment, Bucala et al discovered another type of bone marrow－derived cell with fibroblast-like properties, which they termed "fibrocytes".[11] During tissue repair, fibrocytes might infiltrate extravascular tissues and differentiate into myofibroblasts, leading to collagen production and subsequent scar formation.[11, 12] However, the mechanisms underlying fibrocyte-mediated granulation tissue formation, especially the relationship between endothelial cells and fibroblasts, are unclear.

Recent findings suggest that fibrocytes are important in angiogenesis: *in vitro* evidence showed that fibrocytes express high levels of angiogenic growth factors such as VEGF, bFGF, and platelet derived growth factor (PDGF).[13] We therefore examined the role of fibrocytes in angiogenesis and investigated the mechanisms by which the angiogenic properties of fibrocytes might contribute to angiogenesis after bFGF stimulation in wounds. We first assessed the numbers of fibrocytes during healing after bFGF treatment of wounds.[8] Double immunofluorescence (IF) staining focusing on fibrocyte detection revealed a loose cellular network mainly composed of CD34+/pro-collagen I+ fibrocytes in wounds after bFGF treatment for 4 days. However, on day 6, the CD34+/pro-collagen I+ fibrocytes in bFGF-treated wounds appeared

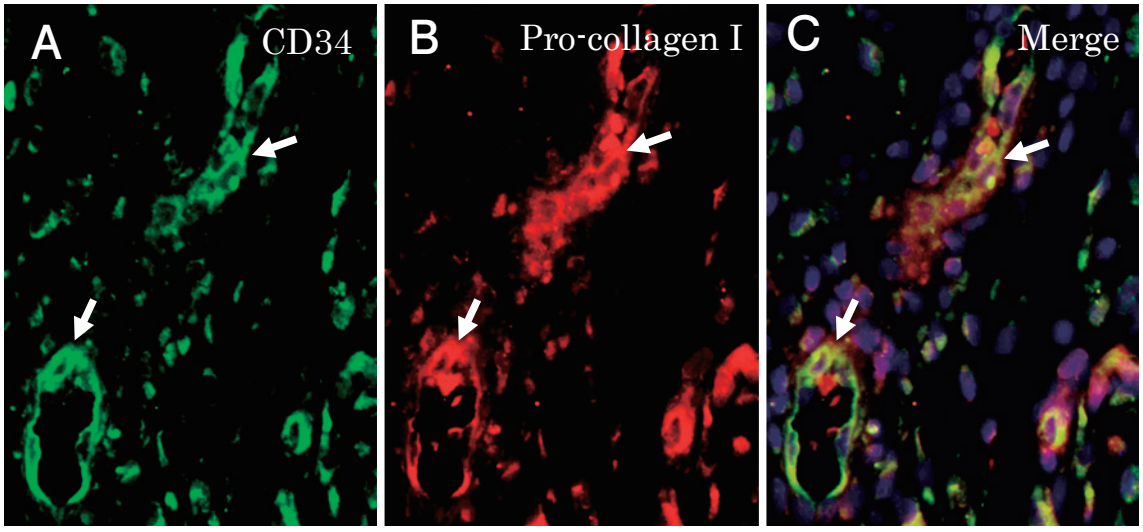

Fig. 3 bFGF induces CD34＋/pro-collagen I＋fibrocytes in wounds
Double immunofluorescence staining of CD34 (green: A) and pro-collagen I (red: B) in wounds. The merged image in C shows CD34＋/pro-collagen I＋double positive fibrocytes as yellow fluorescence (arrow). The tubular structure composed of double-positive fibrocytes was evident in bFGF-treated wounds (magnification: 100×).
bFGF: basic fibroblast growth factor

to form thin-walled, capillary-like structures (Fig. 3), which were absent in non－bFGF-treated wounds. Statistical analysis showed that the number of CD34+/pro-collagen I+ fibrocytes in bFGF-treated wounds increased rapidly after wound creation, peaked by day 6, and then declined until day 14. The average number of CD34+/pro-collagen I+ fibrocytes was significantly higher than in control wounds on days 4, 6, and 7. Regarding the accumulation patterns for other types of fibrocytes, there were increased numbers pf CD45+/pro-collagen I+ and CD11b+/pro-collagen I+ fibrocytes after wound creation, although the numbers of these two types of fibrocytes did not differ between bFGF-treated and non－bFGF-treated wounds. The bFGF-treated wounds also lacked capillary-like structures composed of either type of fibrocyte, *i.e.*, double positivity for CD45/pro-collagen I and CD11b/pro-collagen I, at all time points. In sum, these results show that bFGF treatment specifically induces capillary-like structures composed of CD34+/pro-collagen I+ fibrocytes in wounds.[8)]

To confirm the specificity of bFGF-induced capillary-like structures, we knocked down FGFR1 by FGFR1 siRNA injection. After real-time PCR analysis confirmed the effectiveness of FGFR1 knockdown by FGFR1 siRNA injection, double IF staining clearly showed fewer capillary-like structures with CD34+/pro-collagen I+ fibrocytes in the siFGFR1 group than in the respective small interfering CON group. We consistently noted significantly fewer CD34+/pro-collagen I+ fibrocytes in the small interfering FGFR1 group than in the respective small interfering CON group. These results indicate that the bFGF/FGFR1 system is required for induction of the capillary-like structures composed of CD34+/pro-collagen I+ fibrocytes in wounds.[8)]

Regression of vessel growth associated with scar formation

Regarding the transformation of endothelial cells into fibroblasts, a previous study reported that bFGF was important in endothelial mesenchymal transformation (EMT) in corneal endothelial cells *in vitro*. During this process, bFGF promoted secretion of collagen type I from cells, presumably leading to retrocorneal fibrous membrane formation.[14–16)] In the present study, bFGF treatment induced the angiogenic phenotype of fibrocytes positive for CD34 and pro-collagen type I in wounds. Thus, we speculate that during further differentiation of CD34+/pro-collagen I+ fibrocytes this type of fibrocyte

increases production and secretion of collagen type I and ultimately transforms into fibroblasts/myofibroblasts through EMT, which is specifically induced by bFGF.[8] Fibrocytes ultimately differentiate into fibroblasts/myofibroblasts *in vitro*. Therefore, during fibrocyte differentiation associated with phenotypic changes, EMT might be part of the process by which the angiogenic phenotype of fibrocytes (*e.g.* CD34+/pro-collagen I+ fibrocytes) can further differentiate into fibroblasts/myofibroblasts.

During resolution of healing, most new vessels disappear, and the vessel bed is pruned back to normal vascular density. Although proangiogenic factors within healing wounds are well understood, regulation of vascular pruning remains unclear. Onset of vascular regression coincides with a decrease in proangiogenic stimuli, and a previously accepted mechanism was that the simple decrease in the proangiogenic signal led to loss of vessels through apoptosis.[17] However, subsequent experiments revealed that high levels of wound vascularity could not be maintained only by continued presence of high levels of proangiogenic factors.[18] On the basis of these findings, we propose a possible mechanism of regression of vessels, namely, that newly formed vessels composed of angiogenic phenotype of fibrocytes might disappear through differentiation of this type of fibrocyte into fibroblasts/myofibroblasts.

In conclusion, bFGF treatment leads to granulation tissue formation and promotes scarless repair, which is partly mediated by induction of apoptosis in α-SMA–positive myofibroblasts, leading to inhibition of α-SMA expression. bFGF also specifically induces an angiogenic phenotype of CD34+/pro-collagen I+ fibrocytes in granulation tissues, which are main contributor for bFGF-induced angiogenesis in wounds. These findings indicate that these newly formed vessels can disappear caused by conversion of angiogenic phenotype of fibrocytes into fibroblasts in wounds, along with further differentiation.

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March	1984	Graduated from Teikyo University School of Medicine
April	1984	Entered Keio University Graduate School of Medicine
April	1988	Assistant, Department of Pathology, Keio University School of Medicine
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April	2005	Associate Professor, Department of Pathology, School of Medicine, Faculty of Medicine, Toho University
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Awards

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