The Effects of Basic Fibroblast Growth Factor(bFGF) on Type I and VII Collagen Gene Expression in Cultured Dermal Fibroblast

Young Wook Ryoo, M.D., Dong Won Choi, M.D*., Kyu Suk Lee, M.D.

*Departmemt of Dermatology, Department of Plastic Surgery**
Keimyung Unuversity School of Medicine, Taegu, Korea

Background **: bFGF, a member of the fibroblast growth factor family, potently induces vascular smooth muscle cell proliferation and decreased synthesis of the collagens.**

Objective **: For further investigation of the effect of bFGF on extracellular matrix homeostasis in the skin, we evaluated the expression of type I and type VII collagen gene at the transcriptional levels.**

Method **: We examined that recombinant human bFGF affects the expression of genes involved in ECM synthesis and remodeling in human dermal fibroblasts cultures as judged by Northern blot analysis.**

Results **: The steady state levels of type I and VII collagen gene mRNA were decreased with age dependent pattern up to 0.13 and 0.44 folds respectively. The transcriptional levels of type I collagen mRNA were increased by TGF-ß, treatment but markedly decreased by bFGF as well as TNF-α. But there were no synergistic effects bFGF and TNF-α on type I collagen gene expression. The levels of type VII collagen gene expression were increased by both bFGF and TGF-ß,. The TNF-α showed slightly antagnostic effects on type VII collagen gene expression.**

Conclusion **: The type I and VII collagen gene expression in dermal fibroblasts is clearly subjected to modulation by the cytokines including bFGF with uncoordinate regulatory pathway. In addition to its function of vascular proliferation, bFGF also may play a major role in physiologic skin condition and in repair process such as formation of a stable dermoepidermal junction during skin wound healing. (Ann Dermatol 11(3) 147~152, 1999).**

Key Words : bFGF, Type I and VII collagen

Basic fibroblast growth factor(bFGF) belongs to the family of multifunctional fibroblast growth factor(FGF) that exhibit mitogenic, chemotatic, neurotropic and angiogenic activities in vivo and in vitro[1]. The bFGF is expressed by many tissues and organs and has been localized in the basement membrane of blood vessels, muscles, nerves cells, and sweat glands[2] and is believed to stimulate angiogenesis in many normal and pathological processes[3,4]. Consistent with its role as an angiogenic factor, bFGF stimulates migration and proliferation of vascular endothelial cells in vitro[5]. Microvessel endothelial cells are highly dependent on matrix interaction for normal function and differentiation. The extracellular matrix is believed to regulate microvessel endothelial cell phenotypes by providing mechanical and chemical signals[6]. Recently, it has been reported that bFGF may play a role in metabolism of the extracellular matrix(ECM) of skin[7,8]. One of the prominent components of the ECM is collagen, the main fibrillar components of the connective tissues, which provides the tensile

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Reprint request to : Young Wook Ryoo, M.D., Department of Dermatology, School of Medicine Keimyung University 194 Dong San-Dong Joong-Gu Taegu, Korea 700-712
Tel) 053-250-7621-4 Fax) 053-250-7626

Table 1. Steady-state levels of type I and Ⅶ collagen mRNA in cultured normal skin fibroblasts of different age

Age(year old)	type I/GAPDH	type Ⅶ/GAPDH
2	882.2±39.24	102.2±9.22
2	7 152.4±15.82	80.7±5.81
4	7 112.6±5.90	45.2±1.92

The values are means± SD(n=3) and expressed as densitometric absorbance unit which are percentage of the values of GAPDH ($p< 0.05$)

Table 3. Steady-state levels of type Ⅶ collagen mRNA in cultured dermal fibroblast cell lines from 2 year of age

	type Ⅶ collagen/GAPDH
Control	38±3.2
bFGF	65±6.1
TGF-ß	79±6.8
TGF-ß + TNF-α	60±5.3
bFGF + TNF-α	23±2.4

The values are means± SD(n=3) and expressed as densitometric absorbance unit which are percentage of the values of GAPDH($p< 0.05$)

properties that allows skin to serve as a protective organ against external trauma. In human skin dermis, type I collagen make about 80% of the collagenous materials and type III collagen makes up to 15%. The remaining 5% consists of type IV, V, VI and type VII collagen[9]. The type VII collagen is found exclusively in the basement membrane zone of stratified squamous epithelia, such as in the skin, various mucous membrane and the cornea of the eye[10]. Specifically, type VII collagen is the predominant, if not the exclusive, component of anchoring fibrils, attachment structures that play a critical role in ensuring stability to the association by basement membrane zone with the underlying papillary dermis[11]. In spite of the current knowledge concerning bFGF activity, little is known about the mechanism by which bFGF regulate ECM homeostasis in human dermal skin fibroblasts. So we hypothesized that bFGF is involved in type I collagen synthesis as well as type VII collagen and investigated the potential role of bFGF, TGF-ß, and TNF-α on the transcriptional regulation of type I and VII collagen synthesis using northern blot analysis in cultured skin fibroblasts.

Table 2. Steady-state levels of type I collagen mRNA in cultured dermal fibroblast cell lines from 2 year of age

	type I collagen/GAPDH
Control	40±3.3
TGF-ß	92±7.6
TGF-ß + TNF-α	51±4.0
bFGF	24±1.8
bFGF + TNF-α	25±2.4

The values are means± SD(n=3) and expressed as densitometric absorbance unit which are percentage of the values of GAPDH($p< 0.05$)

MATERIALS AND METHODS

Cell culture

Human skin fibroblasts cultures were established from tissue specimens obtained from 2, 27 and 42 years of age without any known systemic condition. Cells were maintained in Dulbeco's modified Eagle's medium (DMEM) with 10% fetal bovine serum, penicillin(100 U/ml), streptomycin (100 μg/ml), and amphotericine B(1μg/ml) at 37℃ in a humidified atmosphere of 5% CO_2. Cells in passages 5 to 8 were used in the experiments described below.

Treatment of bFGF, TGF-ß, and TNF-α.

Recombinant human bFGF(1ng/ml), TGF-ß(10ng/ml) and TNF-α (1ng/ml) obtained from Sigma Co (MO, USA) in lyophilized form was dissolved in water and further dissolved in DMEM for 48 hours in the absence of fetal bovine serum.

cDNA probe preparation

The following human-sequence-specific cDNAs were utilized in this study: a 1.8kb αcDNA[12] for α type 1(I) collagen ; a 1.9kb cDNA[13] for type VII collagen ; a 1.3kb cDNA[14] for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The cDNAs were labeled with [^{32}P]-dCTP(NEG 036H, New England Nuclear, U.S.A.) by nick translation[15] to a specific activity of approximately 1×10^8cpm/μg.

Quantitation of messenger RNA levels by northern blot analysis

Total RNA was isolated by the methods of Chomzynski and Sacchi from cultured normal skin fibroblasts[16]. Total RNA (5 - 15 μg) was frac-

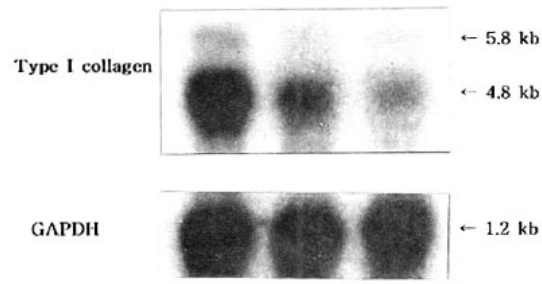

Fig. 1. Northen blot analysis of type I collagen mRNA transcripts from cultured normal skin fibroblasts. of different ages.
Lane 1 2yr old
Lane 2 27yr old
Lane 2 42yr old

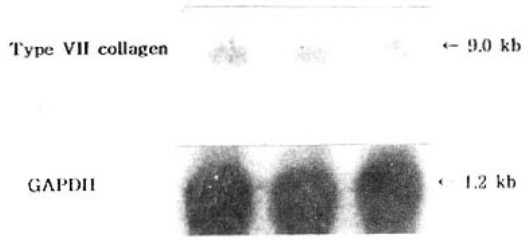

Fig. 2. Northen blot analysis of type VII collagen mRNA transcripts from cultured normal skin fibroblasts. of different ages.
Lane 1 2yr old
Lane 2 27yr old
Lane 2 42yr old

tionated by 1% agarose gel electrophoresis(85 volt, 5 hours) after denaturating the samples with formaldehyde and formamide[17]. RNA transcripts obtained were transferred to the charged nylon filter(Zeta-probe, BioRad, CA, USA) in 20× SSC overnight at 4℃ [18]. The filters was prehybridized for 12-18 hours at 42℃ with prehybridization mixture(50% formamide, 0.1% SDS, 3×SSC, 1× Denhart's solution, 50 g/ml ss-DNA) and hybridized with ^{32}P-labeled cDNA by nick translation at 42℃ for 24 to 36 hours. Following hybridization, the filters were washed and autoradiography was performed.

RESULTS

Steady-state levels of type I and VII collagen mRNA transcripts from cultured cells with different age.

In northern blot analysis of cultured normal skin fibroblasts, [^{32}P] labeled pro 1 type I and type VII collagen and GAPDH cDNA probes specifically hybridized with each mRNA. Pro- 1(I) collagen revealed two mRNA transcripts, 5.8-kb and 4.8-kb, while type VII and GAPDH revealed one transcript, 7-kb and 1.4-kb respectively(Fig. 1, 2). There were no changes in size, indicating no alteration in quality. Type I and type VII collagen mRNA levels were measured as densitometric absorbance units(D.A.U.) and the values are the means from triplicate wells±SD. Steady-state levels of pro- 1(I) collagens mRNAs were 882.2±39.24 D.A.U. at 2 years of age, 152.4±15.82 at 27 years of age, 112.6±5.90 at 42 years of age and showed the age dependent decrement pattern up to 0.13 folds. Steady-state levels of pro- 1(VII) collagens mRNAs were 102.1±9.22 at 2 years of age, 80.2±5.81 at 27 years of age, 45.2±1.92 at 42 years of age, and showed the age dependent decrement pattern up to 0.44 folds(Fig. 1, 2, Table 1).

Effects of the bFGF, TGF-α, and TNF-α on steady-state levels of type I collagen mRNA

The expressions of type I collagen gene were examined in dermal fibroblast cell lines from only 2 year of age by northern transfer analysis. The mean±SD of 3 replicate wells for type I collagen were quantified as D.A.U. The levels of type I collagen mRNAs were 40±3.3 in control, 92±7.6 in TGF-ß, 51±4.0 in both TGF-ß, and TNF-α, 24±1.8 in bFGF, 25±2.4 in both bFGF and TNF-α treated group. TGF-ß, up-regulated type I collagen gene expression, but the up-regulated expression by TGF-ß, was inhibited in the presence of TNF-α. The expression of type I collagen gene was inhibited by bFGF(0.6 folds compared to control group) but the down-regulated expression by bFGF was not interrupted by TNF-α(Fig.3, Table 2). The steady-state level of GAPDH mRNA, as indicated northern transfer analysis, was not affected by the various experimental conditions, reflecting equal RNA loading.

Effects of the bFGF, TGF-ß, and TNF-α on steady-state levels of type VII collagen mRNA

The expressions of type VII collagen gene were examined in dermal fibroblast cell lines from only 2

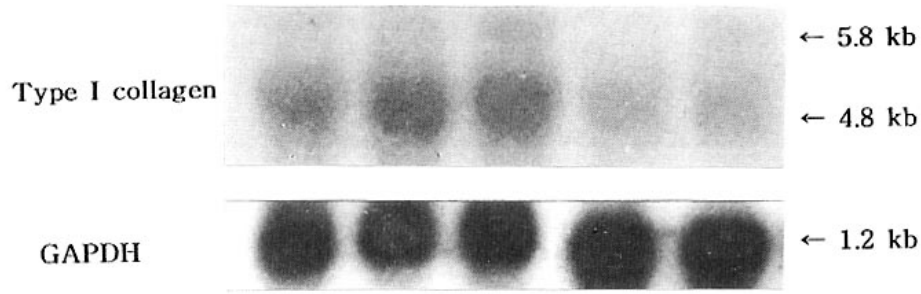

Fig. 3. Northen blot analysis of type I collagen mRNA transcripts from cultured normal skin fibroblasts.
Lane 1 Control
Lane 2 Incubated With TGF-ß + TNF-*α*
Lane 3 Incubated With TGF-ß
Lane 4 Incubated With bFGF
Lane 5 Incubated With bFGF + TNF-*α*

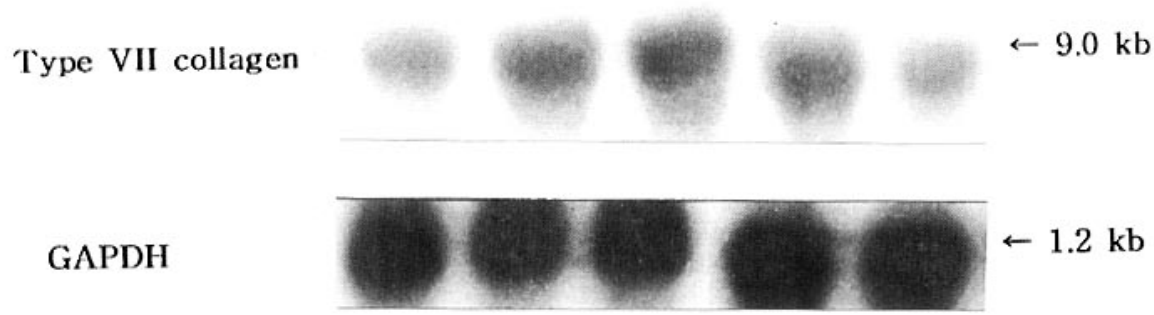

Fig. 4. Northen blot analysis of type Ⅶ collagen mRNA transcripts from cultured normal skin fibroblasts.
Lane 1 Control
Lane 2 Incubated With bFGF
Lane 3 Incubated With TGF-ß
Lane 4 Incubated With TGF-ß + TNF-*α*
Lane 5 Incubated With bFGF + TNF-*α*

year of age by northern transfer analysis. The mean±SD of 3 replicate wells for type VII collagen were quantified as D.A.U. The levels of type VII collagen mRNAs were 38±3.2 in control, 65±6.1 in bFGF, 79± 6.8 in TGF-ß,, 60± 5.3 in both TGF-ß, and TNF-*α*, 23±2.4 in both bFGF and TNF-T*α* treated group. The expression of type VII collagen gene were increased by bFGF and TGF-ß,(1.7 and 2.1 folds compare to control group). The up-regulated expression by bFGF and TGF-ß, was inhibited in the presence of TNF-*α* (Fig. 4, Table 3).

DISCUSSION

Collagens comprise a family of closely related, yet genetically distinct proteins, and currently at least 18 different collagens have been identified[9]. These genetically distinct collagens exhibit characteristic tissue distribution. For example, type I collagen is the most abundant protein of the body and is widely distributed, while certain minor collagens, such as type VII collagen, have restricted anatomical location[10]. Type VII collagen molecules aggregate to form anchoring fibrils, which play an essential role in stabilizing the association of the cutaneous membrane zone to the underlying papillary dermis[10,11,19]. Some age associated manifestations in the skin, such as fragility and impaired epidermal wound healing, might be attributable to diminished collagenous material synthesis and reduced anchoring fibril in the elderly individuals[20,21]. It is noteworthy to evaluate the age dependent basal expression of the type I and VII col-

lagen expression from dermal fibroblasts cultures. From our results, the steady-state levels of type I and VII collagens mRNA showed the age dependent decrement pattern up to 12.7 % and 44.3% respectively at the transcriptional level. During development and inflammatory processes such as wound repair, resident and newly recruited cells are flooded by a host of signals, including the polypeptide cytokines[4,22]. The precise sequence and proportion in which these and other cytokines appear in active, bioavailable form during development or tissue repair is still not certain, although it is likely that all of these stimuli are required for appropriate biological response. The fibroblast growth factor(FGF) polypeptides influence proliferation, angiogenesis, differentiation, and other cellular activities[23]. In vitro, the FGF are capable of inducing various functional responses in a broad spectrum of cell types. For instance, bFGF has plasminogen activator inducing capacity in bovine aortic endothelial cells[24]. Functional interactions between endothelial cells and fibroblasts may be important in wound healing, atherogenesis, and postangioplasty restenosis[25]. There is considerable experimental evidence to support this, and many candidate mediators have been proposed, including bFGF, TGF-ß,, platelet derived growth factor (PDGF) and connective tissue growth factor[26]. The effect of the bFGF on the ECM have not been examined extensively, particularly at the level of gene for the type I and VII collagens. Here, we provide evidence that bFGF oppositely regulates the type I and VII collagen gene expression in dermal fibroblasts cultures. The expression of type I collagen gene was down-regulated upto 60% but the type VII collagen expression was up-regulated upto 1.4 times by bFGF, whereas the TGF-ß,, a potent mitognic cytokine, showed consistently up-regulated expression of both collagens. The study is supported by Kim et al[27], in which TNF-α also showed opposite effect on the expression of type I and type VII collgen genes. These results suggest that there is a different response pathway between type I and VII collagen expression to the exogenous cytokine, bFGF. Each collagen takes part in the different stages of wound healing process or pathogenesis of other connective tissue diseases. Cytokine mediated homeostasis of collagenous material in response to environmental stimuli is the major histopathological feature of maintenance of ECM. Focusing on the pathophysiological relationship between many cytokines, especially bFGF, and ECM components will be an interesting experimental field. In conclusion, type VII collagen gene expression is clearly subject to modulation by bFGF in physiologic condition, such as anchoring fibril assembly during fetal development, or in repair processes, such as formation of a stable dermoepidermal junction during epidermal wound healing.

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