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Basic FGF Regulates Interstitial Collagenase Gene Expression in Human Smooth Muscle Cells

Susan H. Kennedy,[1] Susan Rouda,[1] Huiping Qin,[2] Sirpa Aho,[2] Jesse Selber,[2] and Elaine M.L. Tan[1,3]*

[1]Departments of Pathology, Anatomy, and Cell Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania
[2]Department of Dermatology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania
[3]Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania

Abstract Basic fibroblast growth factor (bFGF) is a mitogenic factor that is implicated in smooth muscle cell growth in atherosclerosis and vascular restenosis. In this study, we examined the effect of bFGF on the expression of the interstitial collagenase gene in human vascular smooth muscle cells. Results from Northern transfer analysis showed that bFGF increased collagenase mRNA levels greater than threefold as early as 24 h. Collagenase pre-mRNA levels were elevated approximately threefold by bFGF, according to RT-PCR analysis. Transient transfections of the smooth muscle cells with a 4.4-kb human collagenase promoter-CAT reporter gene, however, failed to show upregulation of the promoter activity by bFGF. Interestingly, transfections with deleted fragments containing promoter sequences from −1047 to −2271 resulted in modest stimulation of the collagenase-CAT promoter activity by bFGF. bFGF did not alter the stability of the collagenase mRNA, as demonstrated by degradation studies. The enhanced collagenase mRNA levels elicited by bFGF were reflected in increased amounts of collagenase protein that were detected by Western blot analysis. In summary, bFGF upregulates the interstitial collagenase expression, resulting in turnover of the extracellular matrix, an event that could facilitate smooth muscle cell migration and proliferation during the early stages of atherosclerosis and restenosis. J. Cell. Biochem. 65:32–41. © 1997 Wiley-Liss, Inc.

Key words: bFGF; interstitial collagenase; mRNA; extracellular matrix; atherosclerosis and restenosis

Vascular smooth muscle cell proliferation is recognized as a hallmark feature of atherosclerosis and restenosis following balloon angioplasty or endarterectomy [Ross, 1986; Hanke et al., 1990; Casscells, 1992]. The stimulation of smooth muscle cell growth is an early pathologic event that ensues from some form of insult or injury to the vessel wall [Ross, 1986]. Cytokines have been documented to play a role in the activation of smooth muscle cell migration and proliferation [Ferns et al., 1991; Majesky et al., 1991; Jackson and Reidy, 1993].

A major cytokine that has been shown to be a potent smooth muscle cell mitogen is basic fibroblast growth factor (bFGF), a pluripotent member of the fibroblast growth factor family. Polypeptides of this family are well known for their mitogenic effects in a variety of cells. bFGF and a related member, acidic FGF (aFGF), are also involved in the induction of angiogenesis and differentiation of mesodermal, endodermal, and ectodermal cells [Gospodarowicz et al., 1987; Klagsbrun and Edelman, 1989; Burgess and Maciag, 1989; Rifkin and Moscatelli, 1989].

bFGF, in particular, is known to be expressed in early vascular lesions by endothelial, smooth muscle cells, and macrophages. However, the lack of a signal peptide sequence precludes conventional secretion of the cytokine into the extracellular milieu. Its extracellular localization is thought to occur primarily via mechanical release from damaged cells following injury to the vessel wall. The FGFs bind to sulfated proteoglycans, particularly to the heparan sulfate proteoglycans in the extracellular matrix, which serves as a storage depot for the growth factors. The biologic effects of the FGFs are mediated by binding to high-affinity cell sur-

*Correspondence to: Elaine M.L. Tan, Ph.D., Department of Pathology, Anatomy, and Cell Biology, 422 Bluemle Life Sciences Building, 233 S. 10th Street, Philadelphia, PA 19107.

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face receptors associated with tyrosine kinase activity [Neufeld and Gospodarowicz, 1985]. In addition to the high-affinity receptor-mediated pathway, bFGF is capable of interacting directly with cell surface heparan sulfate proteoglycans and is internalized by the cell [Roghani and Moscatelli, 1992].

We and other investigators have shown previously that bFGF or aFGF in the presence of heparin is capable of regulating the expression of extracellular matrix macromolecules. Specifically, the FGFs have been shown to alter collagen [Tan et al., 1993] and interstitial collagenase gene expression by human fibroblasts in a divergent manner [Chua et al., 1985, 1991]. We have also documented the suppression of collagen expression by bFGF in vascular smooth muscle cells [Kennedy et al., 1995]. The changes in the abundance and composition of the extracellular matrix macromolecules have been shown to influence cellular migration and proliferation. These events are integral in wound healing [Saarialho-Kere et al., 1993; Juhasz et al., 1993].

In vivo, collagen is present as an abundant protein in the blood vessel wall. Several types of collagens are expressed in the vasculature; a major collagen is type I collagen [Tan and Uitto, 1994]. The smooth muscle cells express the pro-α1(I) and pro-α2(I) type I collagen chains both in vitro and in vivo. The turnover of type I collagen is mediated by interstitial collagenase, a major metalloproteinase that is secreted by both smooth muscle and endothelial cells [Tan and Uitto, 1994].

The objective of the present study is to elucidate the regulatory control of the interstitial collagenase gene expression by bFGF in human vascular smooth muscle cells. Our results suggest that bFGF is a potent cytokine that upregulates the interstitial collagenase gene expression in human smooth muscle cells. The control of the gene expression by bFGF appears to be regulated primarily at the transcriptional level.

METHODS

Cell Culture

Human vascular smooth muscle cells were derived from adult iliac arteries of brain-dead, heart-beating cadaver renal donors by an explant method [Hoover et al., 1980]. The vascular smooth muscle cells were grown in Medium 199 and 10% fetal bovine serum (FBS) (Intergen Corp., Purchase, NY), 2 mM glutamine, 200 U/ml penicillin, and 200 µg/ml of streptomycin (GIBCO, Grand Island, NY) and were positively identified as such by their characteristic "hill and valley" growth morphology, positive staining for α- and γ-actin, and negative staining for factor VIII-related antigen expression. Eight donor smooth muscle cell strains were employed for the experiments in this study.

Quantitation of mRNA Steady-State Levels by Northern Blot

Early confluent smooth muscle cell cultures were incubated without or with 50 ng/ml of bFGF for varying periods. To determine whether de novo protein synthesis was required for induction of gene expression by bFGF, the cells were pretreated with 10 µg/ml of cycloheximide for 1 h and then incubated without or with bFGF for 24 or 48 h. Total RNA samples were prepared for Northern blot analysis. Briefly, total RNA was isolated by extraction of the cells with 4 M guanidinium isothiocyanate, pH 7.0, containing 5 mM sodium citrate, 0.5% sarkosyl, and 0.1 M 2-mercaptoethanol. The RNA samples were isolated by the chloroform–phenol extraction method [Chomcynski and Sacchi, 1987], and the amount was quantitated by spectrophotometric absorbance. The RNAs were electrophoresed in 1% agarose gels under denaturing conditions and were processed for Northern blot as described previously [Thomas, 1980]. A 2-kb full-length human collagenase [Goldberg et al., 1986] and 7S ribosomal [Balmain et al., 1982] cDNA probes were used for the Northern analysis. The latter cDNA was used as an internal control to standardize the collagenase mRNA levels in the untreated control versus bFGF-treated samples. The autoradiograms were scanned with a BioRad Gel Doc 1000 with Molecular Analyst/PC Software (BioRad, Hercules, CA).

Reverse Transcription and Polymerase Chain Reaction

Reverse transcription–polymerase chain reaction (RT-PCR) was employed to quantitate collagenase pre-mRNA levels using primers specific for the sequence of the first intron of the human interstitial collagenase gene. Total RNA in an amount of 5 µg was reverse transcribed using 200 units of Superscript II RNase H^- reverse transcriptase (Superscript Preamplification System, GIBCO BRL). Primers for the

first intron of the human interstitial collagenase, 5′-ATGCATACGCTCTCTTTTCGAG-3′ (forward primer) and 5′-TGCATGGGAAATCTTTCTCATTA-3′ (reverse primer) were used. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplimer sets (Clontech, Palo Alto, CA) were used with each of the RT reactions. One-tenth of the RT reaction was used in the PCR reaction in a 50-µl volume containing 100 nM of the forward and 100 nM of reverse primers; 2 units of Taq polymerase (Boehringer Mannheim); 200 µM of each dATP, dCTP, dTTP, dGTP (Perkin-Elmer Cetus, Norwich, CT); and 10 mM Tris–HCl, pH 8.3, with 1.5 mM $MgCl_2$, and 50 mM KCl. The mixture was overlayed with 2 drops of mineral oil and amplified in a thermal cycler for 25 cycles. An initial denaturation step was employed at 95°C for 5 min, and then 25 repeat cycles of 95°C for 1 min (denaturation), 55°C for 45 s (annealing) and 72°C for 1 min (elongation). The samples were incubated at 72°C for 5 min and stored at 4°C; 15 µl of the PCR samples was electrophoresed on a 2.0% agarose gel and stained with ethidium bromide. The size of the PCR products was determined by accompanying standard DNA markers obtained from GenSura Laboratories (Del Mar, CA). The ethidium bromide bands were quantitated using the Gel Doc 1000 with Molecular Analyst/PC Software (BioRad).

Transient Transfections

Human vascular smooth muscle cells at early confluence were transfected with 40 µg of pCLCAT3, which contains ~4.4 kb of the 5′ flanking DNA of the human interstitial collagenase gene linked to a chloramphenicol acetyltransferase (CAT) reporter gene [Frisch et al., 1990]. Transient transfections of smooth muscle cells were also performed with deletion constructs of the collagenase promoter. The constructs were prepared using restriction enzymes with single recognition sites within the pCLCAT3. Specifically, double digests with *Hin*dIII and *Sac*I, *Eco*RV, *Bgl*II, *Stu*I, or *Kpn*I were conducted to generate expression plasmids: p345, which contains 522 bp, p346 with 893 bp, p347 with 1,047 bp, p348 with 1,194 bp, p349 with 1,548 bp, and finally p352 containing 2,271 bp of the upstream sequences. The plasmids were ligated with a *Hin*dIII–*Sac*I linker or *Hin*dIII–*Kpn*I linker isolated from the multiple cloning site of Bluescript KS (Stratagene, La Jolla, CA). The DNA sequences were established by automated DNA sequencing (Applied Biosystems models 373A and 377 DNA Sequencing Systems, Foster City, CA), using the Prism Ready Reaction DyeDeoxy Terminator Cycle Sequencing kit. The cells were incubated in the absence and presence of 50 ng/ml of bFGF for 24 h prior to transfection with Lipofectin (GIBCO-BRL, Grand Island, NY) or Profectin (Promega, Madison, WI), after which the respective cultures were restored in medium without and with bFGF for 48 h.

CAT Assay

Smooth muscle cell cultures were harvested and lysed in reporter lysis buffer (Promega); the protein concentration of each sample was determined by a dye-binding assay (BioRad). Identical amounts of the samples were incubated with [^{14}C]chloramphenicol as detailed previously for the CAT reporter assay [Gorman et al., 1982]. The acetylated and nonacetylated forms of radioactive chloramphenicol were separated by thin-layer chromatography (TLC) and visualized by autoradiography. Parallel transfections were performed with pBSOCAT construct lacking the promoter and with pSV2CAT construct containing the SV40 early region promoter linked to the reporter gene [Gorman et al., 1982]. Alternatively, a FLASH^R CAT nonradioactive assay (Stratagene) was used to separate the acetylated from the nonacetylated products by TLC, after extraction with ethyl acetate. This assay employs a fluorescent chloramphenicol substrate labeled with a borondipyrromethane (BODIPY) difluoride fluorophore [Lefevre et al., 1995]. The products were visualized under long-wavelength ultraviolet (UV) in the Gel Doc 1000 system.

Determination of mRNA Stability

To determine whether bFGF exerted a post-transcriptional influence on the stability of the interstitial collagenase mRNA transcripts, smooth muscle cells were treated with 60 µM of 5,6-dichloro-1-β-D-ribofuranosylbenzimidizole (DRB). Following DRB treatment to inhibit transcription, the cells were incubated without and with 50 ng/ml of bFGF for various periods. Total RNA from untreated and bFGF-treated samples at each time point were prepared for Northern transfer analysis. Independent experiments using 5 µg/ml of actinomycin D to inhibit transcription were also conducted, and the de-

cay rate of the transcripts was determined over several time points.

Western Blot Analysis

Cell cultures were incubated without and with 50 ng/ml of bFGF for 24 or 48 h. The media and cell layers were harvested and prepared for Western blot analysis. Samples representing similar amounts of total protein were subjected to electrophoresis using a NOVEX Mini-cell System (San Diego, CA). After transferring the proteins to nitrocellulose (MSI, Westboro, MA), 5% nonfat milk in Tris-buffered saline (TBS) was added for 2 h to block nonspecific reaction. The antiserum against the human collagenase (kindly provided by Dr. Gregory Goldberg) was added and incubated overnight. After several washes, a secondary antibody conjugated with alkaline phosphatase was added in a 1:7,500 dilution and incubated for 45 min. The bands were visualized with Western Blue substrate (Promega).

RESULTS

bFGF Regulates mRNA Steady-State Levels of Interstitial Collagenase

Northern transfer analysis demonstrated that bFGF enhanced collagenase mRNA levels at least twofold and more after 24 and 48 h of incubation. Stimulation of collagenase gene expression by bFGF was apparent at concentrations ranging from 5 to 50 ng/ml of bFGF, as shown in the representative experiment (Fig. 1A). The elevated collagenase mRNA levels were normalized with constitutively expressed levels of 7S RNAs (Fig. 1B). Several Northern blots were performed during the course of study, and enhancement of the collagenase mRNA abundance was noted consistently in all the human donor smooth muscle cell strains. These findings are also in agreement with those of an earlier study [Kennedy et al., 1995].

De Novo Protein Synthesis Is Required for bFGF-Induced Stimulation of Interstitial Collagenase Gene Expression

To ascertain whether de novo protein synthesis is necessary for the induction of collagenase expression by bFGF, smooth muscle cell cultures were pretreated with 10 µg/ml of cycloheximide for 1 h and then incubated both in the absence and in the presence of 50 ng/ml bFGF for 24 or 48 h (Fig. 2). Enhanced collagenase gene expression was noted in the cultures treated with bFGF, as early as 24 h and substantially after 48 h. Cycloheximide-treated cells in the absence of bFGF exhibited limited reduction in collagenase mRNA levels. However, the diminution of collagenase mRNAs by cycloheximide was clearly apparent in the bFGF-treated cells, particularly after 48 h, at which time the level was reduced to that of the control nontreated cultures. The results suggest that ongoing protein synthesis is required for upregulation of collagenase gene expression by bFGF.

bFGF Enhances Interstitial Collagenase Pre-mRNA Levels

RT-PCR analysis, using primers specific for the sequence of the first intron of the collagenase gene, was performed to quantitate the abundance of collagenase pre-mRNA. The results revealed that bFGF, at concentrations of 5 and 50 ng/ml, elicited an increase in collagenase pre-mRNA levels after 24 and 48 h (Fig. 3). The stimulation of transcription by bFGF was evident during the course of 96 h (data not shown). The induction of the collagenase pre-mRNA by bFGF was specific relative to the respective GAPDH pre-mRNA levels determined under identical experimental conditions.

Effect of bFGF on Collagenase Promoter Activity

Collagenase deletion fragments with the CAT reporter gene were prepared as depicted in Figure 4A. The regulation of the collagenase promoter activity was studied in the smooth muscle cells by transient transfection/CAT reporter assays, using deletion and full-length promoter constructs. No dose- or time-dependent alterations of promoter activity by bFGF were noted with the full-length, 4.4 kb, collagenase promoter (pCLCAT3)/CAT construct (Fig. 4B). Furthermore, the results from the deletion constructs (Fig. 4A) demonstrated that the activity of the 0.5-kb or that of the 0.8-kb promoter fragment (p345 and p346, respectively) was not regulated by bFGF. The p345 contains a single AP-1 site and the p346 has two AP-1 motifs. The activities of the larger fragments, p347, p348, p349, and p352 containing 1.0 kb, 1.2 kb, 1.5 kb, and 2.3 kb, respectively, of the 5′ flanking sequences, showed minor response, about 1.7- to 2-fold to bFGF (Fig. 4B). The modest promoter activity elicited by bFGF was consistent particularly with the p348 and p349 promoter fragments transfected into various

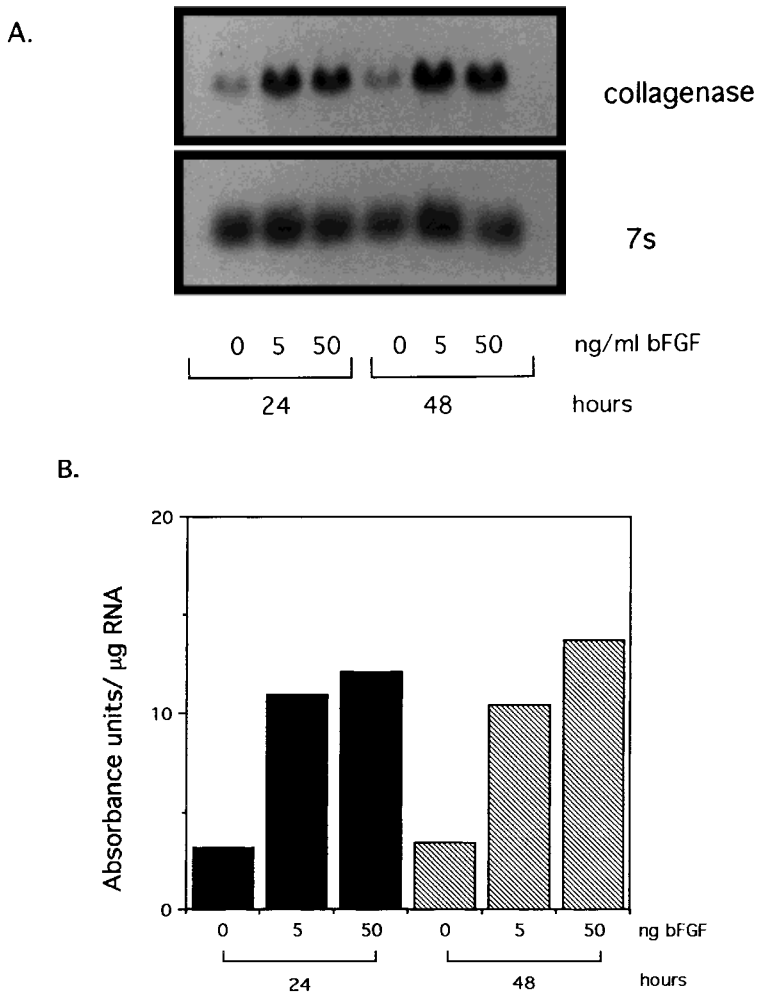


Fig. 1. Time- and concentration-dependent effects of bFGF on interstitial collagenase mRNA steady-state levels in human smooth muscle cells. Early confluent cultures of human smooth muscle cells were incubated with 0, 5, and 50 ng/ml of bFGF for 24 and 48 h. **A:** Total RNA samples were extracted from the cell cultures and Northern blot hybridizations were performed with a human interstitial collagenase cDNA and 7S probes. **B:** Collagenase mRNA levels were quantitated and normalized to corresponding 7S levels.

human donor smooth muscle cell strains. It is possible that partial or minor regulatory elements for bFGF-induction of the promoter activity may be located in the intermediate region of the collagenase promoter. Numerous transient transfections with the full-length collagenase promoter and CAT assays of several human donor smooth muscle cell strains incubated with bFGF at concentrations known to elicit major increase in collagenase mRNA levels failed consistently to reveal any alteration of the promoter activity. That the findings of bFGF-induced collagenase pre-mRNA and mRNA by bFGF are not readily explained by corresponding alterations of the promoter activity strongly suggest that other pretranslational/transcriptional processing are likely to be involved in the upregulation of the collagenase gene expression by bFGF in smooth muscle cells.

mRNA Stability in bFGF-Treated Smooth Muscle Cells

To determine whether the apparent increase of the collagenase mRNA levels in bFGF-treated smooth muscle cell cultures was due to altered stability of the transcript, early confluent cultures were pretreated with bFGF and incubated in the presence of DRB and bFGF (Fig. 5). Examination of the mRNA levels at 0, 12, 24, and 36 h showed that the decay of the collagenase transcript was evident over the course of time in both bFGF-treated and parallel control nontreated smooth muscle cultures, with one-half of the collagenase mRNA decayed by about

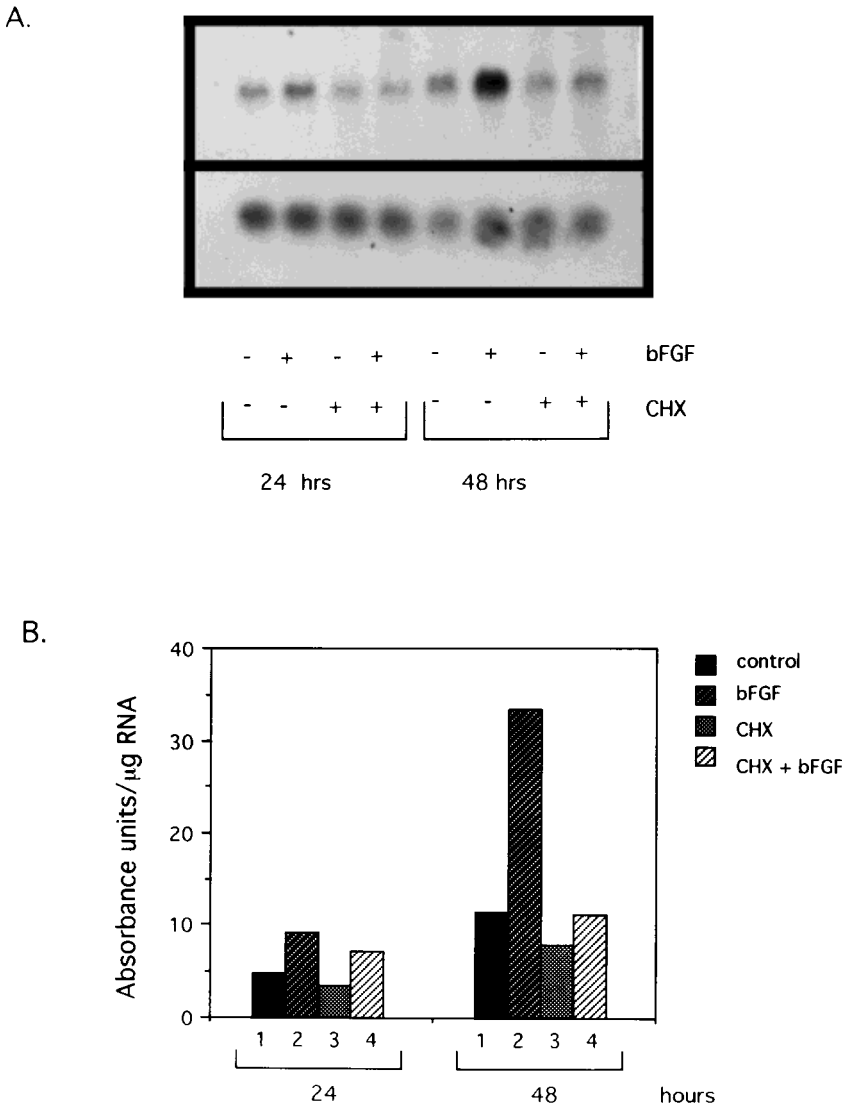


Fig. 2. Effect of cycloheximide on bFGF-induced changes in interstitial collagenase gene expression. Human smooth muscle cells were incubated without (−) and with (+) 50 ng/ml of bFGF, provided 1 h after the addition of 10 μg/ml of cycloheximide (CHX), for a duration of 24 or 48 h. **A:** Total RNA was analyzed by Northern blot hybridization with a cDNA probe for human interstitial collagenase. **B:** mRNA values were normalized with the respective 7S.

16 h. There was no major alteration in the rate of mRNA degradation in cultures exposed to bFGF when compared to the corresponding control nontreated cells (Fig. 5). Therefore, the enhanced expression of collagenase gene expression by bFGF is not attributed to increased post-transcriptional stability of the mRNA.

bFGF Alters Protein Expression of Interstitial Collagenase

To determine whether corresponding change of the collagenase protein levels was elicited by bFGF treatment, Western blot analysis was performed on cell cultures, using the same amount of total cellular protein incubated both in the absence and in the presence of 50 ng/ml of bFGF for 24 and 48 h. The total cellular protein content is not altered by bFGF when administered to confluent cell cultures for the duration of 48 h. The amount of collagenase is not substantial relative to overall protein content; thus, using identical amounts of total cellular protein for each sample serves to normalize the specific changes of the collagenase content by bFGF. The results demonstrate that the basal level of collagenase protein was apparent after 48 h and the synthesis of collagenase was upregulated as early as 24 h and enhanced

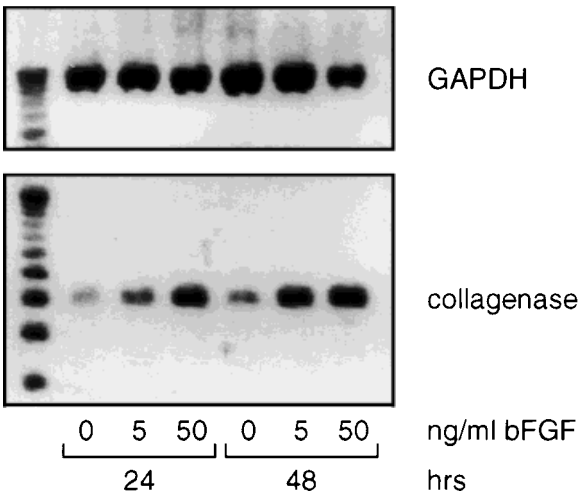


Fig. 3. Effect of bFGF on collagenase pre-mRNA levels as determined by RT-PCR. RT-PCR analysis of the pre-mRNA abundance of collagenase was conducted on total RNA extracted from confluent cultures of human smooth muscle cells incubated with 0, 5, and 50 ng/ml of bFGF for 24 and 48 h.

after 48 h of treatment with bFGF (Fig. 6). Thus, the elevated mRNA steady-state level of collagenase by bFGF is reflected in corresponding increase at the protein level.

DISCUSSION

bFGF is a well-characterized pluripotent member of the fibroblast growth factor family, known for potent mitogenic effects for various cell types. The cytokine has been associated with smooth muscle cell proliferation in vascular diseases, such as atherosclerosis and restenosis [Winkles et al., 1987; Lindner et al., 1991]. Previous studies have also demonstrated that the proliferative potential of cells in vitro is influenced by the surrounding extracellular matrix [Saarialho-Kere et al., 1993; Juhasz et al., 1993]. bFGF has been shown to inhibit collagen synthesis significantly by human fibroblasts [Tan et al., 1993], immortalized osteoblast-like cells [Hurley et al., 1993], and vascular smooth muscle cells [Majors and Ehrhart, 1993; Kennedy et al., 1995]. In addition, enhanced synthesis of interstitial collagenase by bFGF has been documented in fibroblast cultures [Chua et al., 1985, 1991]. The present study aims to elucidate the mechanism for the alteration of interstitial collagenase gene expression by bFGF in human vascular smooth muscle cells.

Northern analysis in this study demonstrated major enhancement of collagenase mRNA levels expressed by human smooth muscle cells incubated with bFGF. Upregulation of the collagenase gene expression was noted in all of the human donor smooth muscle cell strains. Our previous study showed that the increase was time and dose dependent and was accompanied by concomitant reduction in the pro-α1(I) collagen levels in bFGF-treated smooth muscle cells [Kennedy et al., 1995]. Thus, bFGF elicits divergent regulation of interstitial collagenase and type I collagen gene expression. It is, however, unclear whether the regulation of the expression of the genes is controlled at the transcriptional and/or post-transcriptional level. Nonetheless, the alterations of the extracellular matrix components are known to facilitate migration and subsequent proliferation of smooth muscle cells.

The results from this study suggest that upregulation of the interstitial collagenase gene expression by bFGF is likely related to transcriptional modification. Results from the RT-PCR analysis showed elevated amounts of collagenase pre-mRNA suggesting that transcriptional activity of the collagenase gene is enhanced by bFGF. Transient transfection experiments, however, revealed that the promoter activity of the full-length promoter was not altered by bFGF in the smooth muscle cells. The activity of the shortest promoter fragment of 0.5 kb that contained an AP-1 motif was detectable in the smooth muscle cell cultures but was not responsive to bFGF. The presence of the first and second AP-1 sites was apparently not required for bFGF induction of the collagenase promoter activity in the human smooth muscle cell cultures. Even though a modest stimulation of the promoter activity was noted with the regulatory elements present upstream of -1047 bp but was absent with longer sequences, the overall results suggest that other poorly defined transcriptional mechanism(s) are likely to be involved in the induction of the collagenase pre-mRNA abundance by bFGF.

mRNA stability studies demonstrated that the half-life of the collagenase mRNA was approximately 16 h for human smooth muscle cells and that bFGF did not alter the rate of degradation of the collagenase transcript. The rate of degradation of collagenase mRNA in bFGF-treated smooth muscle cells was comparable to that of parallel nontreated control cultures, indicating that bFGF does not increase

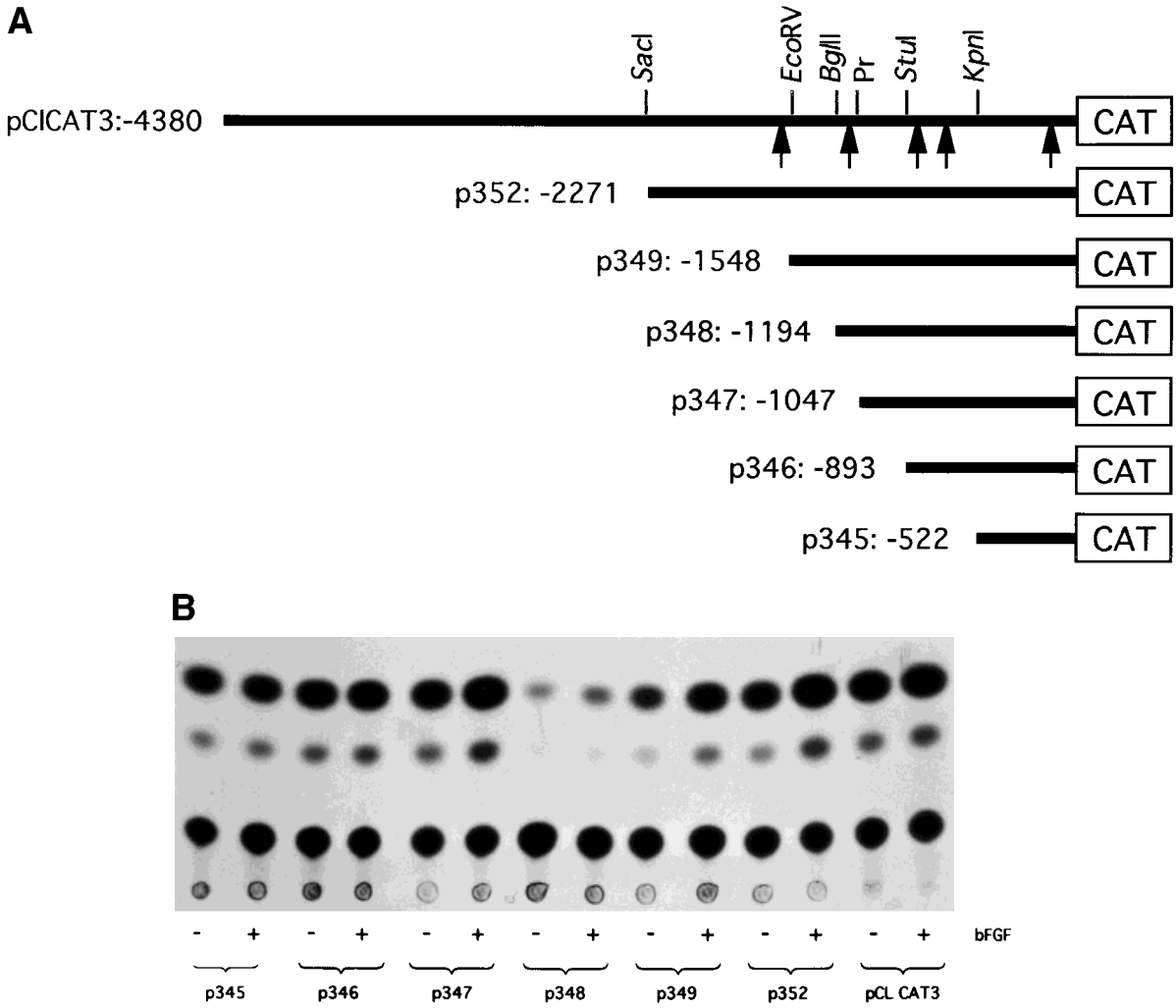
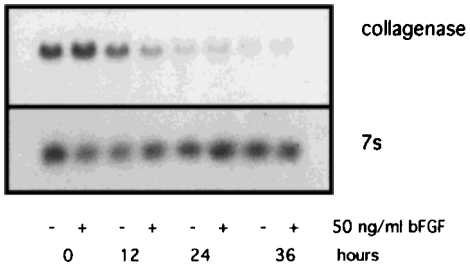


Fig. 4. Collagenase promoter activity of full-length and deleted promoter fragments in control and bFGF-treated smooth muscle cells. **A:** Size and spatial location of the deletion fragments. Arrows point to AP-1 consensus sequences. **B:** Early confluent smooth muscle cells were grown for 24 h and the promoter constructs, p345, p346, p347, p348, p349, p352 as well as the full-length 4.4-kb pCL CAT3 promoter, were transfected into the cells with Lipofectin. The cultures were treated without and with 50 ng/ml of bFGF for 48 h and analyzed for CAT promoter activity as described in Materials and Methods.

the stability of the collagenase mRNA transcripts. Thus, bFGF does not appear to regulate gene expression post-transcriptionally.

Induction of the collagenase gene expression by bFGF resulted in increased collagenase protein as detected by Western blot analysis, particularly after 48 h. Previously, the suppression of the pro-α1(I) collagen mRNA levels by bFGF in human smooth muscle cells was also found to correlate with a major reduction of collagen protein synthesis [Kennedy et al., 1995]. Thus, the alterations caused by bFGF at the mRNA level are reflected in corresponding changes detected at the protein level.

Enhanced transcription of collagenase gene expression by other cytokines has been documented previously. These cytokines include interleukin-1β (IL-1β) [Vincenti et al., 1994] and tumor necrosis factor-α (TNF-α) [Dayer et al., 1985]. IL-1β stimulates collagenase gene expression through increased transcription of the collagenase gene [Vincenti et al., 1994]. Induction of transcription has been reported to involve the AP-1 binding site, which is apparently necessary but not always adequate for mediating the promoter activity. The results from our study demonstrate that the full-length collagenase promoter, which has several AP-1 sites (unpublished results), is not regulated by bFGF, and suggest that the in vitro regulation of the collagenase gene expression in smooth muscle cells is not dependent on the AP-1 site.



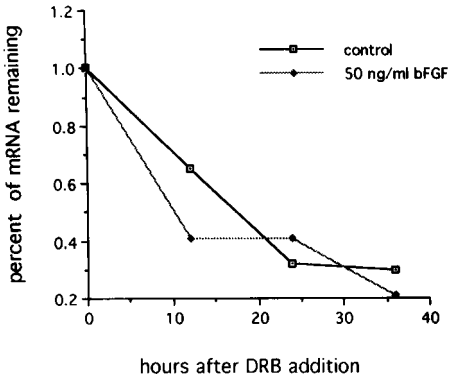


Fig. 5. Degradation of interstitial collagenase mRNA. Smooth muscle cells were treated with 60 μM of DRB both in the absence (−) and in the presence (+) of 50 ng/ml of bFGF for varying time periods. Northern transfer analysis was performed and the abundance of collagenase and 7S mRNAs were quantitated by densitometric scanning. The mRNA level of collagenase at each time point was normalized to the corresponding 7S value. At time 0 h, the percentage of mRNA was depicted as 100% and the percentage of mRNAs remaining at subsequent time points relative to time 0 hour is shown.

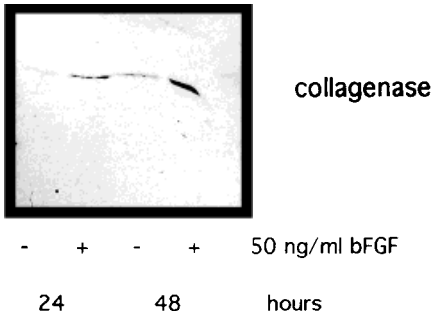


Fig. 6. Western blot analysis of the effect of bFGF on interstitial collagenase protein level. Smooth muscle cells were incubated without (−) and with (+) 50 ng/ml of bFGF for 24 and 48 h. The media at each time point were dialyzed, concentrated, and equal amounts of protein were applied to each lane before incubation with an antibody against human collagenase.

Cytokines, such as bFGF, influence cellular migration and proliferation of vascular smooth muscle cells. These biologic events are elicited directly by bFGF or may also be influenced by bFGF-induced alterations of the extracellular matrix macromolecules. Indeed, it is well known that cellular migration is increased when the extracellular matrix of the cells is degraded by proteinases, as in the case of metastatic cells [Xie et al., 1994; Southgate et al., 1992]. Moreover, it has been shown that smooth muscle cell migration and proliferation are suppressed when metalloproteinases are inhibited by synthetic inhibitors [Starkey et al., 1987]. Thus, the increased turnover of collagen in the extracellular matrix could well enhance in vivo cellular movement and growth, hallmark features of early atherogenesis and restenosis. Finally, the findings from this study highlight the complexity of the pretranslational effects of bFGF on the expression of the interstitial collagenase gene, independent of its mitogenic action in human vascular smooth muscle cells.

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REFERENCES

Balmain A, Krumlauf R, Vass JK, Birnie GD (1982): Cloning and characterization of the abundant cytoplasmic 7S RNA from mouse cells. Nucleic Acids Res 10:4259–4277.

Burgess WH, Maciag T (1989): The heparin-binding (fibroblast) growth factor family of proteins. Annu Rev Biochem 58:575–606.

Casscells W (1992): Migration of smooth muscle and endothelial cells—Critical events in restenosis. Circulation 86:723–729.

Chomcynski P, Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 162:156–159.

Chua CC, Geiman DE, Keller GH, Ladda RL (1985): Induction of collagenase secretion in human fibroblast cultures by growth promoting factors. J Biol Chem 260:5213–5216.

Chua CC, Chua BHL, Zhao ZY, Krebs C, Diglio C, Perrin E (1991): Effect of growth factors on collagen metabolism in cultured human heart fibroblasts. Connective Tissue Res 26:271–281.

Dayer JM, Beutler B, Cerami A (1985): Cachetin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. J Exp Med 162:2163–2168.

Ferns GAA, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross R (1991): Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. Science 253:1129–1132.

Frisch SM, Reich R, Collier IE, Genrich LT, Martin G, Goldberg GI (1990): Adenovirus E1A represses protease gene expression and inhibits metastasis of human tumor cells. Oncogene 5:75–83.

Goldberg GI, Wilhelm SM, Kronberger A, Bauer EA, Grant GA, Eisen AZ (1986): Human fibroblast collagenase. Complete primary structure and homology to an oncogene transformation-induced rat protein. J Biol Chem 261: 6600–6605.

Gorman CM, Moffat LF, Howard BH (1982): Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol Cell Biol 2:358–363.

Gospodarowicz D, Ferrara N, Schweigerer L, Neufeld G (1987): Structural characterization and biological functions of fibroblast growth factor. Endocrine Rev 8:95–114.

Hanke H, Strohschneider T, Oberhoff M, Betz E, Karsch KR (1990): Time course of smooth muscle cell proliferation in the intima and media of arteries following experimental angioplasty. Circ Res 67:651–659.

Hoover RL, Rosenberg R, Haering W, Karnovsky MJ (1980): Inhibition of rat arterial smooth muscle cell proliferation by heparin. II. In vitro studies. Circ Res 47:578–583.

Hurley MM, Abreu C, Harrison JR, Lichtler AC, Raisz LG, Kream BE (1993): Basic fibroblast growth factor inhibits type I collagen gene expression in osteoblastic MC3T3-E1 cells. J Biol Chem 268:5588–5593.

Jackson CL, Reidy MA (1993): Basic fibroblast growth factor: Its role in the control of smooth muscle cell migration. Am J Pathol 143:1024–1031.

Juhasz I, Murphy GF, Yan H-C, Herlyn M, Albelda SM (1993): Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous human wound healing in vivo. Am J Pathol 143:1458–1469.

Kennedy SH, Qin H, Lin L, Tan EML (1995): Basic fibroblast growth factor regulates type I collagen and collagenase gene expression in human smooth muscle cells. Am J Pathol 146:764–771.

Klagsbrun M, Edelman ER (1989): Biological and biochemical properties of fibroblast growth factors—implications for the pathogenesis of atherosclerosis. Arteriosclerosis 9:269–278.

Lefevre CK, Singer VL, Kang HC, Haugland RP (1995): Quantitative nonradioactive CAT assays using fluorescent BODIPY 1-deoxychloramphenicol substrates. Biotechniques 19:488–493.

Lindner V, Lappi DA, Baird A, Majack RA, Reidy MA (1991): Role of basic fibroblast growth factor in vascular lesion formation. Circ Res 68:106–113.

Majesky MW, Lindner V, Twardzik DR, Schwartz SM, Reidy MA (1991): Production of transforming growth factor β during repair of arterial injury. J Clin Invest 88:904–910.

Majors A, Ehrhart LA (1993): Basic fibroblast growth factor in the extracellular matrix suppresses collagen synthesis and type III procollagen mRNA levels in arterial smooth muscle cell cultures. Arterioscler Thromb 13:681–686.

Neufeld G, Gospodarowicz D (1985): The identification and partial characterization of the fibroblast growth factor receptor of baby hamster kidney cells. J Biol Chem 260: 13860–13868.

Rifkin DB, Moscatelli D (1989): Recent developments in the cell biology of basic fibroblast growth factor. J Cell Biol 109:1–6.

Roghani M, Moscatelli D (1992): Basic fibroblast growth factor is internalized through both receptor-mediated and heparan sulfate-mediated mechanisms. J Biol Chem 267:22156–22162.

Ross R (1986): The pathogenesis of atherosclerosis. N Engl J Med 314:488–500.

Saarialho-Kere UK, Kovacs SO, Pentland AP, Olerud JE, Welgus JE, Parks WC (1993): Cell-matrix interactions modulate interstitial collagenase expression by human keratinocytes actively involved in wound healing. J Clin Invest 92:2858–2866.

Southgate KM, Davies M, Booth RFG, Newby AC (1992): Involvement of extracellular-matrix-degrading metalloproteinases in rabbit aortic smooth muscle cells. Biochem J 288:93–99.

Starkey JR, Stanford DR, Magnuson JA, Hamner S, Robertson NP, Gasic GJ (1987): Comparison of basement membrane matrix degradation by purified proteases and by metastatic tumor cells. J Cell Biochem 35:31–49.

Tan EML, Uitto J (1994): Extracellular matrix. In White RA, Hollier LH (eds): "Vascular Surgery: Basic Science and Clinical Correlations." Philadelphia: JB Lippincott, pp 31–45.

Tan EML, Rouda S, Greenbaum SS, Moore JH Jr, Fox IV JW, Solberg S (1993): Acidic and basic fibroblast growth factors down-regulate collagen gene expression in keloid fibroblasts. Am J Pathol 142:463–470.

Thomas PS (1980): Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77:5201–5205.

Vincenti MP, Coon CI, Lee O, Brinckerhoff CE (1994): Regulation of collagenase gene expression by IL-1β requires transcriptional and post-transcriptional mechanisms. Nucleic Acids Res 22:4818–4827.

Winkles JA, Friesel R, Burgess WH, Howk R, Mehlman T, Weinstein R, Maciag T (1987): Human vascular smooth muscle cells both express and respond to heparin-binding growth factor I (endothelial cell growth factor). Proc Natl Acad Sci USA 84:7124–7128.

Xie B, Bucana CD, Fidler IJ (1994): Density-dependent induction of 92-kd type IV collagenase activity in cultures of A431 human epidermoid carcinoma cells. Am J Pathol 144:1058–1067.