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Basic Fibroblast Growth Factor Decreases Type V/XI Collagen Expression in Cultured Bovine Aortic Smooth Muscle Cells

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Abstract Vascular smooth muscle cells (SMCs), the major cellular constituent of an artery, synthesize the bulk of fibrillar collagens, including type V/XI, which regulates heterotypic collagen fibril assembly. Basic fibroblast growth factor (bFGF) is a heparin-binding polypeptide growth factor that has been implicated in important events during the development of atherosclerosis, such as early intimal SMC proliferation. Here we have investigated the effects of bFGF on aortic SMC expression of type V/XI collagen. Treatment of exponentially growing or serum-deprived subconfluent cultures of bovine aortic SMCs with bFGF decreased the steady-state levels of the mRNAs for collagen type V/XI, including α1(V), α2(V), and α1(XI). The effect of bFGF was time dependent with a two- and a fourfold decrease in α2(V) mRNA observed after treatment for 24 and 48 h, respectively. This decrease resulted from a drop in the rate of α2(V) gene transcription; no change was observed in the stability of the α2(V) mRNA. Furthermore, accumulation of collagen protein decreased upon bFGF treatment. As expected, treatment with bFGF increased the rate of proliferation of serum-deprived SMCs, as judged by DNA content in the cultures, thymidine incorporation, and steady-state mRNA levels of the S-phase-expressed histone H3.2. These results suggest that bFGF plays an important role in the regulation of collagen fibril structure, with potential implications for the development and organization of an atherosclerotic lesion. J. Cell. Biochem. 68:247–258, 1998.

Key words: SMCs; bFGF; collagen fibril structure; mRNA; atherosclerotic lesion

Smooth muscle cells (SMCs) are the major constituents of the medial layer of an artery. During the development of the artery, SMCs go through an initial highly proliferative phase, followed by the synthesis of extracellular matrix components, including collagens, elastin, and proteoglycans, as well as the enzymes involved in matrix protein deposition [reviewed in Ross, 1993]. Once the artery has been fully formed, SMCs differentiate into a contractile phenotype in which they normally remain [Chamley-Campbell et al., 1979]. SMCs also play a crucial role in the development of atherosclerosis. During lesion formation, SMCs migrate from the medial layer into the intima, as a normal response to injury [Ross, 1993]. In this new environment, some initial rounds of cell proliferation occur, which are followed by the synthesis and deposition of the components of the fibrotic plaque, including the major fibrillar collagen types I and III and the minor fibrillar collagen V/XI [Poole et al., 1971; Ross, 1993; Schwartz et al., 1985; Vuorio and deCrombrugghe, 1990; Gordon et al., 1990; Strauss et al., 1994].

Type V collagen belongs to the group I fibrillar collagens and was originally discovered in skin [Chung et al., 1976] and in human placenta [Sage and Bornstein, 1979]. It is also expressed in a variety of other tissues, including smooth muscle tissue [Liau and Chan, 1989]. Type XI collagen, another group I fibrillar collagen, was known to be closely related structurally to type V collagen and share similar properties [Eyre and Wu, 1987; Morris et al., 1990]; however, these two species were originally denoted as different collagen types based on their

Abbreviations: SMCs, smooth muscle cells; bFGF, basic fibroblast growth factor; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; DRB, 5,6-dichlorobenzimidazole riboside.

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apparent separate sites of synthesis. In particular, type XI collagen was thought to be expressed exclusively in cartilage; however, more recently type XI chains were detected in bone in trimeric structures with type V [Niyibizi and Eyre, 1989]. In addition, mRNA for various type XI chains were found in the placenta [Bernard et al., 1988], and in rhabdomyosarcoma cells [Kleman et al., 1992]. We demonstrated that bovine vascular SMCs synthesize the α1 chain, but not the α2 or α3 chain of type XI collagen [Brown et al., 1991]. As a result of these findings, types V and XI are now considered a single family. Immunolocalization and chemical cross-linking studies have shown that type V/XI collagen can participate along with other fibrillar collagens in heterotypic fibrils [Fitch et al., 1984, 1988; Birk et al., 1988; Mendler et al., 1989]. Evidence obtained originally in vitro [Birk et al., 1990] and more recently in vivo [Andrikopoulos et al., 1995; Marchant et al., 1997] indicates that type V collagen plays a very important role in the regulation of the diameter and architecture of heterotypic fibrils. In atherosclerotic lesions in humans and in animal models, the ratio of type V collagen to types I and III collagen was found to be increased [Ooshima, 1981; Morton and Barnes, 1982; Murata et al., 1986] suggesting that this collagen family may play a significant role in the pathology of atherosclerosis.

The cytokine basic fibroblast growth factor (bFGF) was originally identified as an activity in pituitary extracts that stimulated growth of Swiss hamster 3T3 fibroblasts [Gospodarowicz et al., 1984]. bFGF is known for a multiplicity of biological activities both in vivo and in vitro [Klagsbrun and Edelman, 1989; Burgess and Maciag, 1989; Rifkin and Moscatelli, 1989]. For example, it has been shown to act as a potent mitogen for a variety of cells in culture, including SMCs [Winkles et al., 1987]. bFGF is a single polypeptide with microheterogenity at the N-terminal region that yields proteins of molecular weights ranging from 16 to 24 kDa [reviewed in Newby and George, 1993]. bFGF lacks the classical signal peptide that targets various proteins to the secretory pathway and the mechanisms of its release remain obscure. Previous studies have shown that viable cells deposit bFGF into the extracellular matrix [Gospodarowicz et al., 1983; Vlodavsky et al., 1987b], while more recent studies have shown that bFGF is secreted upon cell injury or lysis [Abraham et al., 1986; McNeil et al., 1989; Brooks et al., 1991; Muthukrishnan et al., 1991; Villaschi and Nicosia, 1993]. Following injury to the intima, SMCs are exposed to a variety of cytokines and factors, such as bFGF, secreted by the endothelial cells, smooth muscle cells, and macrophages [Baird et al., 1985; Vlodavsky et al., 1987a,b; Schweigerer et al., 1987; Sarzani et al., 1989; Mansson et al., 1990; Speir et al., 1991; Lindner et al., 1991]. Expression of bFGF correlates with the migration and rapid proliferation of vascular SMCs during the very early stages of restenosis after balloon injury [Lindner and Reidy, 1991]. In culture, treatment of vascular SMCs with bFGF decreased expression of collagen type I and type III [Majors and Ehrhart, 1993; Kennedy et al., 1995]. Here, we examined the effect of bFGF treatment on the expression of type V/XI collagen. Treatment of bovine aortic SMCs with bFGF caused a significant drop in the levels of type V/XI collagen mRNA and protein, due in part to a decreased rate of gene transcription.

MATERIALS AND METHODS

Cell Culture and Treatment Conditions

Tissue culture reagents were purchased from Life Technologies, Inc., except for Dulbecco's modified Eagle's medium (DMEM), which was purchased from JRH Biosciences. SMC explants were derived from the aortic arches of female calves, as we have described previously [Beldekas et al., 1982]. Cultures were fed every 2–3 days. Second and third passage cells were used for experiments.

For treatment with bFGF (unless otherwise indicated), cells were plated at an initial density of 5.0×10^5 cells/P150 dish (0.33×10^4 cells/cm^2) and grown in medium supplemented with 10% fetal bovine serum (FBS) (10% FBS-DMEM). Subconfluent cultures were serum starved for 1–3 days in medium containing 0.5% FBS (0.5% FBS–DMEM). Following addition of bFGF (0.5–5 ng/ml dissolved in carrier solution: 50 mM Tris–HCl, pH 7.5, 0.3 M NaCl, 1 mM dithiothreitol, 0.05% gelatin), or the equivalent volume of carrier solution, cultures were incubated for the times indicated.

DNA Synthesis

SMCs were seeded at 60,000 cells/P35 tissue culture dish in triplicate and grown for 1 day. Cultures were then switched to 0.5% FBS–

DMEM for 24 h, and treated with bFGF or carrier solution for an additional 24 h. During the final hour of treatment, the cells were pulsed with 2 μCi/ml of [^{3}H]thymidine (Dupont NEN; 80 Ci/mmol). Cells were washed twice in ice-cold phosphate-buffered saline (PBS) and lysed with addition of 1 ml of 10% trichloroacetic acid. After 30 min, the resulting precipitate was washed once with 10% trichloroacetic acid, solubilized by incubation with 0.5 ml of 0.4 N NaOH for 1 h, diluted to 5 ml with Formula-963 aqueous counting mixture (DuPont NEN) and quantitated in a LB 1217 Rack Beta liquid scintillation counter. The results are reported as the mean ±SD.

RNA Isolation and Hybridization Analysis

Total cellular RNA was isolated by guanidinium isothiocyanate extraction followed by purification on CsCl density gradients, as described [Sambrook et al., 1989]. For Northern blot analysis, RNA samples (10 or 15 μg) were denatured and separated by electrophoresis on 1.0% formaldehyde-agarose gels [Dean et al., 1983]. RNA was stained with ethidium bromide to verify integrity and equal loading and then transferred to GeneScreen Plus (DuPont NEN). RNA was cross-linked to the membrane by ultraviolet (UV) irradiation (Stratalinker, Stratagene) at 0.12 joules/cm^2 for 30 s. Probes, prepared by random priming, were used as described previously [Kindy and Sonenshein, 1986], except that 2.0×10^6 cpm/ml [^{32}P]-labeled DNA was employed. Quantitation by scanning densitometry was performed using a Molecular Dynamics 300A computing densitometer.

Pepsin Digestion of Extracellular Matrix Proteins

Cultures, treated with bFGF or carrier solution alone for 24 h, were labeled with 25 μCi/ml [^{3}H]proline, as described previously [Lawrence et al., 1994]. The medium was removed, and the cell layers were washed three times in ice-cold PBS. The cell layer was scraped in pepsin digestion buffer, transferred to a 50-ml Falcon conical tube, and digested at 4°C for 16 h. The pepsin-digested cell-layer-associated matrix was dialyzed exhaustively against 0.5 M HAc, lyophilized, and reconstituted to 1 ml distilled H_2O supplemented with protease inhibitors (2 mM phenylmethylsulfonyl fluoride and 2 mM p-hydroxymercuribenzoate). Samples were normalized for DNA content according to Burton (1956) and resolved by electrophoresis in a 5–15% polyacrylamide gradient sodium dodecyl sulfate (SDS) gel. The radioactive signal was enhanced using fluorography with 2,5-diphenyloxazole/dimethylsulfoxide (DMSO), as described [Laskey and Mills, 1975]. Molecular mass markers used included species at 200, 97.4, 69, 46, 30, 21.5, and 14.3 kDa (Rainbow Molecular Mass Markers, Amersham, Arlington Heights, IL). The molecular mass of the species was estimated based on their migration relative to the markers.

Cloned DNA Species

The cDNAs used included α1(III) collagen: pCg6, 1.05-kb bovine cDNA [Stepp et al., 1985]; α1(V) collagen: pTV302, 3.2-kb human cDNA [Takahara et al., 1991]; α2(V) collagen: Hf511, 2.5-kb human cDNA [Weil et al., 1987]; α1(XI) collagen: pMU5G3, 2.7-kb bovine cDNA [Brown et al., 1991]. The histone H3.2: pRAH3.2, a genomic fragment encoding amino acids 57–125 of histone H3.2 [Alterman et al., 1984] was also employed.

Runoff Transcription Assays

Nuclear runoff analysis was performed by a modification of the method of Greenberg and Ziff (1984). Briefly, approximately 1×10^7 nuclei were incubated in the presence of 250 μCi of [^{32}P]-UTP (Dupont NEN; 3,200 Ci/mmole) for 30 min at 30°C. Labeled RNA was isolated, and equal amounts of radiolabeled RNA (4.5×10^6 cpm/ml of hybridization buffer) were hybridized to plasmid DNA (10 μg/sample) immobilized onto GeneScreen Plus by slot blotting followed by UV irradiation; after hybridization blots were washed as described previously [Marhamati and Sonenshein, 1996].

RESULTS

Treatment With bFGF Decreases the Steady-State Levels of Type V/XI Collagen mRNA in Cultures of Aortic SMCs

We first addressed the question of whether treatment of SMCs with bFGF affects type V/XI collagen mRNA expression. Subconfluent cultures of bovine aortic SMCs were incubated in DMEM medium containing 10% FBS (10% FBS–DMEM) or under serum deprivation conditions (0.5% FBS–DMEM) for 24 h and then treated with 2 ng/ml bFGF, or the equivalent volume of carrier solution as control, for an additional 24 h. Total RNA was extracted from

the cultures and samples were subjected to Northern blot analysis for collagen chain expression. Treatment with bFGF caused a decrease in the steady-state mRNA levels of the α1(V), α2(V), and α1(XI) collagen chains at either serum concentration (Fig. 1A,1B). A drop was also detected in the α1(III) mRNA levels (Fig. 1A), consistent with previously published results [Kennedy et al., 1995]. As expected, based on previous work in our laboratory [Brown et al., 1991], serum deprivation of subconfluent cultures resulted in increased mRNA levels for all fibrillar collagens. Collagen type VI is a nonfibrillar collagen, which is expressed in a variety of cells [Timpl and Engel, 1987]. In contrast to the effects of serum deprivation on the expression of fibrillar collagen mRNAs, the levels of collagen α1(VI) mRNA were not affected (Fig. 1B and data not shown). Furthermore, treatment with bFGF had no effect on the levels of α1(VI) collagen mRNA. Thus, bFGF selectively mediates a significant reduction in the steady-state mRNA levels for the chains of type V/XI collagen in vascular smooth muscle cells.

Dose- and Time-Dependent Effect of bFGF on the Levels of α2(V) and α1(XI) Collagen mRNA

To test whether the effects of bFGF are dose dependent, the levels of procollagen α2(V) and α1(XI) mRNA were measured as a function of bFGF concentration. Cultures of aortic SMCs were seeded at 1×10^6 cells per P-150 tissue culture plate in 10% FBS-DMEM and treated with 0.5, 1, 2, and 5 ng/ml bFGF for 24 h and RNA isolated and analyzed by Northern blotting (Fig. 2). A detectable decrease in the levels of α2(V) and α1(XI) mRNA was observed with a concentration of bFGF as low as 0.5 ng/ml. Treatment with increasing concentrations of bFGF resulted in further decreases in the mRNA levels of these chains, with the lowest levels observed at 2–5 ng/ml bFGF. Higher concentrations of bFGF did not result in a further decrease (data not shown). Thus, a concentration of 2 ng/ml bFGF was selected for the remaining studies.

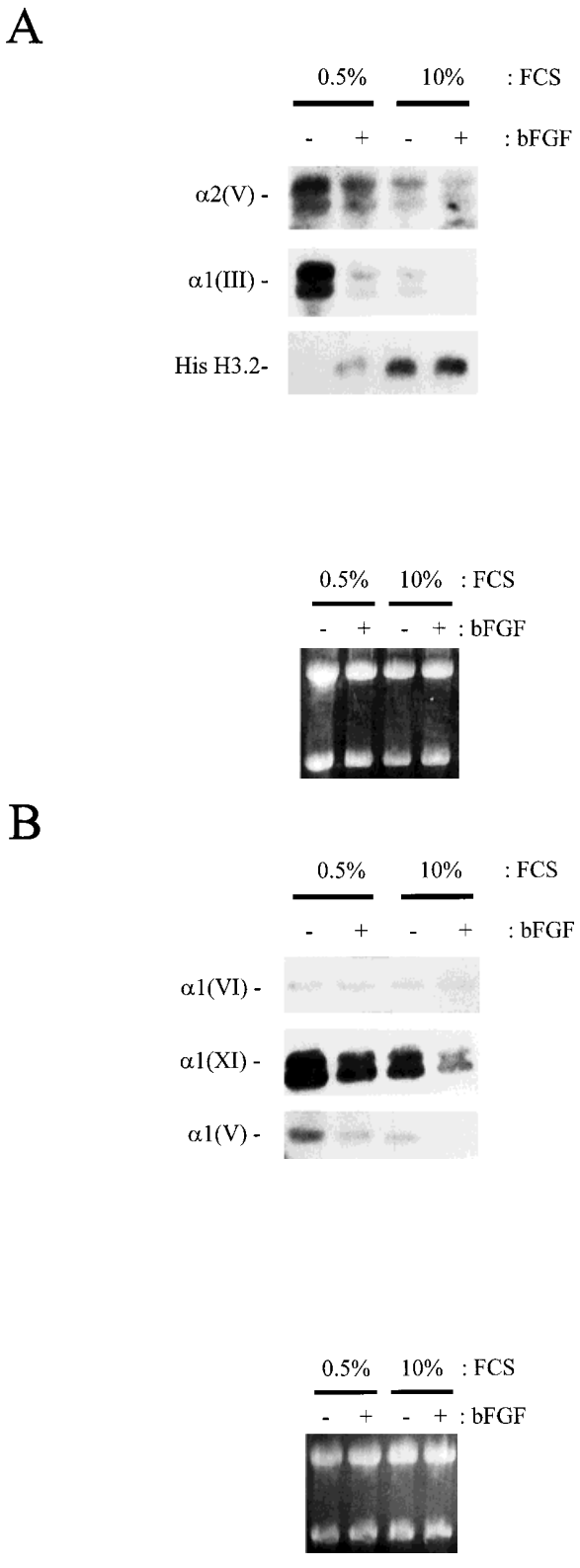
To determine the kinetics of the response to bFGF, subconfluent SMC cultures, rendered quiescent by serum deprivation for 72 h, were treated with 2 ng/ml bFGF for 24 or 48 h, or carrier solution for 48 h. RNA was extracted from these cultures and analyzed by Northern blotting for α2(V) collagen expression (Fig. 3). High levels of α2(V) mRNA were detected in quiescent cultures. Addition of bFGF for 24 or 48 h resulted in a two- and fourfold drop, respectively in the levels of this message, in comparison to the levels detected in quiescent, or control cultures treated with carrier solution for 48 h. Thus, the reduction in the steady-state levels of α2(V) mRNA caused by bFGF occurs in a time-dependent fashion.

bFGF Decreases Collagen Protein Expression in Aortic SMCs

To measure the effects of bFGF on collagen protein expression, a radiolabeling experiment was performed. Subconfluent cultures of aortic SMCs, maintained in 10% FBS–DMEM or 0.5% FBS–DMEM for 24 h, were treated with either 2 ng/ml bFGF or equivalent volume of carrier solution. Cultures were then labeled with [^{3}H]proline for 8 h, washed, and the cell layers digested with 0.1 mg/ml pepsin at 4°C for 24 h. Samples were normalized for DNA content and analyzed by electrophoresis in 5–15% SDS–PAGE (Fig. 4). Cells treated with carrier solution exhibited major bands that correspond to α1(V)/α1(XI), α2(V), α1(I)/α1(III), and α2(I), as identified previously [Lawrence et al., 1994]. Treatment with bFGF, in either serum concentration, resulted in a reduction in the levels of type V/XI collagen proteins, as well as types I and III. These findings are consistent with the observed drop in mRNA levels of these fibrillar collagens.

bFGF Does Not Decrease the Stability of Procollagen mRNA

To investigate whether bFGF affects the stability of collagen mRNA, subconfluent cultures of SMCs were treated with 2 ng/ml bFGF or carrier solution for 24 h under serum deprivation conditions. The selective inhibitor of RNA polymerase II 5,6-dichlorobenzimidazole riboside (DRB) was then added to each culture and total RNA isolated at the indicated time points. As shown in Figure 5, treatment with bFGF for 24 h (0 h DRB) led to the expected drop in the α2(V), α1(XI), and α1(III) mRNA levels. No significant change in the rate of collagen mRNA decay was seen in bFGF treated versus control cultures. DRB treatment had the expected effect on the mRNA levels of the labile c-*jun* message in control and bFGF treated cultures, verifying the efficacy of the inhibitor. Thus, bFGF did not decrease the stability of the mRNA species for these collagen chains, suggesting



that other mechanisms are involved in the drop in steady-state levels of these mRNAs.

Down-Regulation of Collagen Gene Transcription by bFGF

To determine whether transcriptional control mechanisms play a role in the decrease in the steady-state collagen mRNA levels, nuclear run-off analysis was performed. Nuclei were isolated from subconfluent cultures treated with 2 ng/ml bFGF or equivalent volume of carrier solution for 24 h under serum deprivation conditions. Hybridization of run-off transcripts to α2(V), α1(XI), and α1(III) probes decreased significantly upon bFGF treatment (Fig. 6). In contrast, bFGF treatment slightly increased the rate of transcription of the cell-cycle regulated gene B-*myb*, whose steady-state mRNA levels increased in the presence of bFGF (data not shown). Thus, bFGF treatment results in a drop in the rate of transcription of α2(V), α1(XI), and α1(III) procollagen mRNA and this drop accounts, at least in part, for the change in the steady state mRNA levels of these collagens.

bFGF Increases the Rate of Bovine Aortic SMC Proliferation

bFGF was found to stimulate growth in a variety of cells, including SMCs [Winkles et al., 1987; Goldring and Goldring, 1991; Newby and George, 1993; Nugent et al., 1993]. To determine the extent of induction of cell growth upon bFGF treatment with the bovine aortic SMCs, the mRNA levels of the S-phase expressed gene histone H3.2 were measured. The decrease in the collagen mRNA levels was accompanied by an increase in histone H3.2 mRNA levels. Treatment with bFGF caused a very dramatic increase in H3.2 mRNA levels under serum deprivation conditions (0.5% FBS–DMEM) but had only a minor effect in exponentially growing cells, incubated in 10% FBS–DMEM (Fig. 1A).

Fig. 1. Treatment with bFGF decreases matrix gene and increases growth-related mRNA expression in aortic SMCs. Subconfluent SMC cultures were incubated for 24 h in complete medium (10% FBS–DMEM), or under serum deprivation conditions (0.5% FBS–DMEM), and then treated either with 2 ng/ml bFGF (in carrier solution) (+), or the equivalent volume of carrier solution (−) for an additional 24 h. Total RNA was isolated and samples (15 μg) subjected to Northern blot analysis for the indicated genes analyzed in two separate gels **(A,B).** Bottom panels, ethidium bromide-stained gel confirming RNA quality and equal loading.

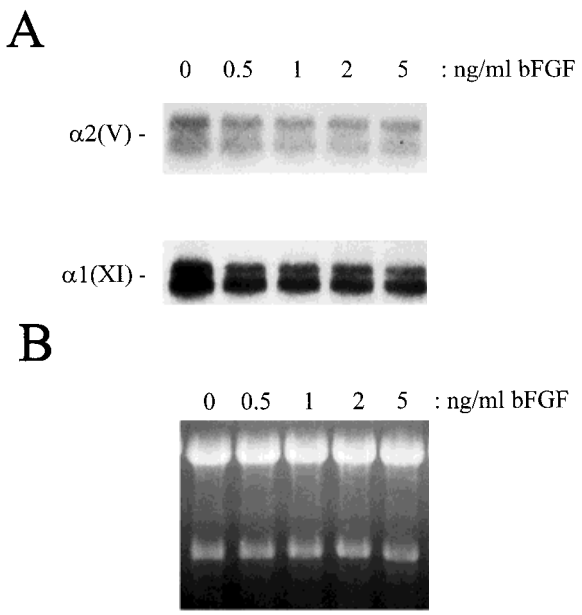


Fig. 2. Dose-dependent effects of bFGF on gene expression. Subconfluent cultures of SMCs were maintained under serum deprivation conditions for 24 h and then treated with carrier solution (0 ng/ml) or the indicated concentration of bFGF for an additional 24-h period. **A:** Total RNA was isolated and samples (15 μg) subjected to Northern blot analysis for the indicated genes. **B:** Ethidium bromide-stained gel, confirming RNA quality and equal loading, is shown.

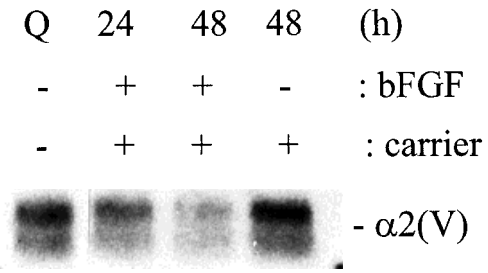


Fig. 3. Time-dependent effects of bFGF on α2(V) collagen mRNA expression. Subconfluent cultures of SMCs were maintained under serum deprivation conditions for 72 h, at which time they were in quiescence (Q). The indicated cultures were then treated either with 2 ng/ml bFGF for an additional 24 or 48 h or with carrier solution for 48 h. Total RNA was isolated and samples (10 μg) subjected to Northern blot analysis for α2(V) collagen gene expression.

To test directly for the mitogenic effect of bFGF under serum deprivation conditions, SMCs were plated at very low density and grown for 1 day in 10% FBS–DMEM (subconfluence), switched to 0.5% FBS–DMEM for 24 h, and treated with increasing amounts of bFGF or carrier solution as control, for an additional 24 h. Following pulse labeling with 2 μCi/ml [^{3}H]thymidine for 1 h, cells were harvested. A 3-fold increase in

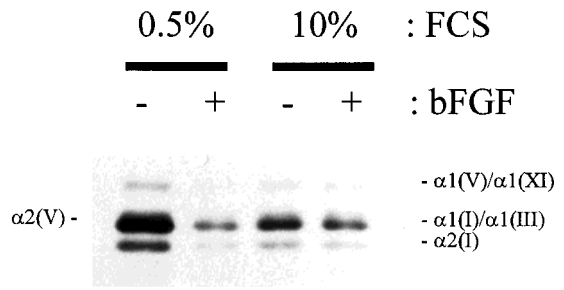


Fig. 4. Treatment with bFGF decreases the levels of cell layer-associated collagen protein. Subconfluent cultures were incubated for 24 h in 10% FBS–DMEM or 0.5% FBS–DMEM, and then treated with 2 ng/ml bFGF (+) or carrier solution (−) for an additional 24 h period. Cultures were then incubated in the presence of 25 μCi/ml [^{3}H]proline for 8 h and the cell layer-associated, pepsin-resistant material extracted. Samples, normalized for DNA content, were subjected to 5–15% SDS–PAGE and proteins visualized by autoradiography. Positions of the types I, III, and V/XI collagen protein chains were made on the basis of comparison to molecular weight and purified collagen protein standards [Lawrence et al., 1994] (data not shown).

thymidine incorporation was observed at every concentration of bFGF used, in comparison to the control serum deprived cultures (Fig. 7). A distinct change in SMC morphology, was also noted upon bFGF treatment. Control cultures (treated with carrier solution) displayed a flattened, spread phenotype, typical of quiescence. In contrast, cells treated with bFGF were more spindle shaped and thinner; furthermore cells undergoing cytokinesis were plainly visible (Fig. 8). Thus, treatment of bovine aortic SMCs with bFGF results in increased cell proliferation, with a more pronounced effect under serum deprivation conditions.

DISCUSSION

Treatment with bFGF decreased type V/XI collagen expression by bovine aortic SMCs. A drop in type V/XI collagen protein expression was paralleled by a drop in the steady-state levels of the collagen α1(V), α2(V), and α1(XI) mRNAs. Consistent with the work of others [Kennedy et al., 1995], we observed that bFGF decreased the steady-state levels of α1(III) mRNAs. The decrease in the collagen mRNAs occurred in a dose- and time-dependent fashion. Treatment with bFGF resulted in a decreased rate of transcription of genes for the α2(V), α1(XI), and α1(III) collagen chains, but did not apparently decrease the stability of their mRNAs. In contrast to these effects on fibrillar collagens, no change in the steady-state level of α1(VI) mRNA was observed. Treat-

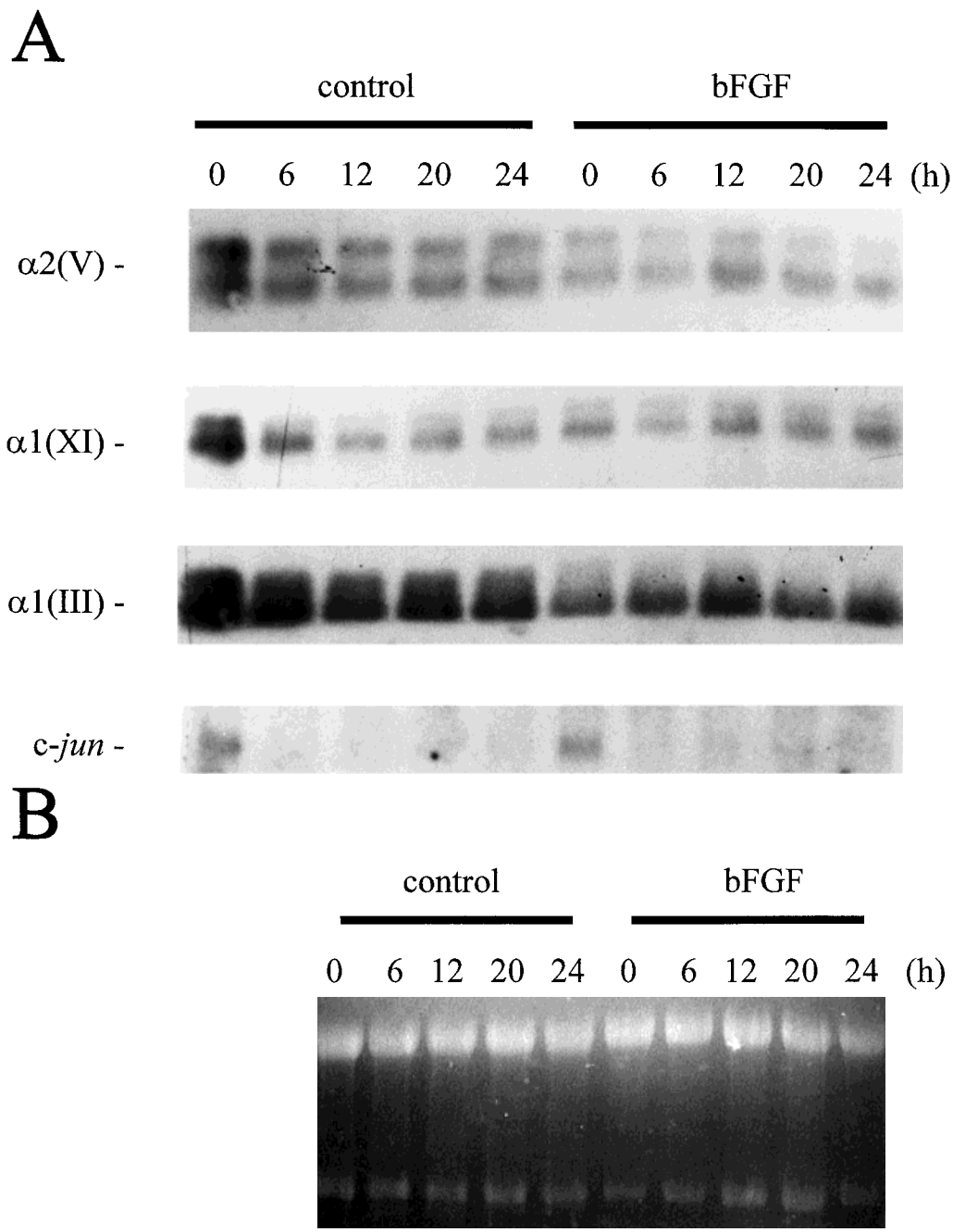


Fig. 5. Treatment with bFGF does not decrease the half-life of decay of collagen mRNAs. Subconfluent cultures were maintained under serum deprivation conditions for 24 h and then treated with carrier solution or 2 ng/ml bFGF for an additional 24-h period. Following replacement with fresh 0.5% FBS-DMEM containing bFGF or carrier solution, DRB was added to 30 μg/ml and total RNA isolated after 0, 6, 12, 20, or 24 h. **A:** RNA samples (15 μg) were subjected to Northern blot analysis for the indicated genes. **B:** Ethidium bromide-stained gel is shown.

ment with bFGF increased the rate of proliferation of SMCs when incubated under serum deprivation conditions, while only a modest effect was seen in complete medium. Since the decrease in type V/XI collagen expression was seen in SMCs incubated with bFGF under either growth conditions, its effects on this collagen type appear to occur independent of the growth state of the cells. Thus, our results extend the findings of the effects of bFGF to type V/XI collagen which regulate heterotypic collagen fibril assembly, suggesting bFGF plays

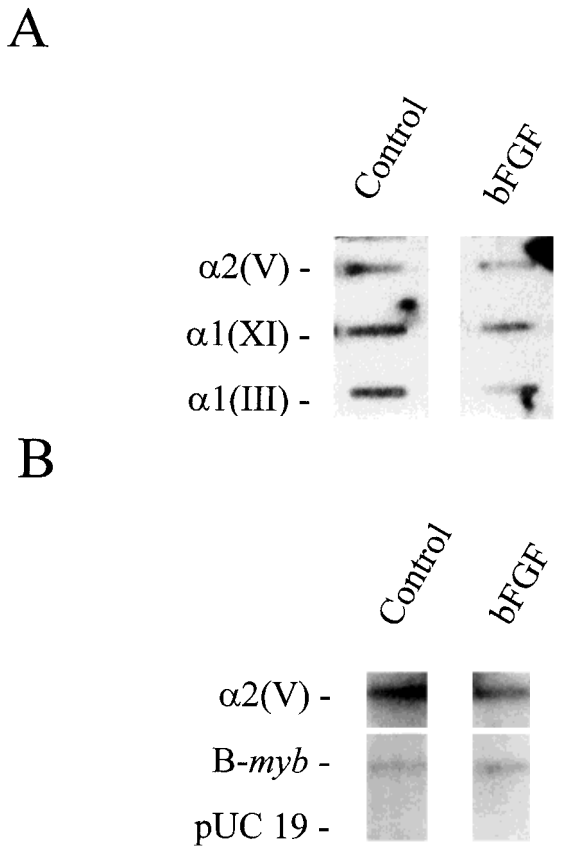


Fig. 6. The rate of transcription of collagen genes is decreased and B-*myb* increased by bFGF treatment. Subconfluent cultures were maintained under serum deprivation conditions for 24 h and then treated with carrier solution or 2 ng/ml bFGF for an additional 24-h period. Nuclei from two independent experiments **(A,B)** were isolated; the resulting radiolabeled RNAs (runoff transcripts) were hybridized to the 10-µg/slot of the indicated cDNA probe or pUC 19 vector DNA immobilized on nylon membrane.

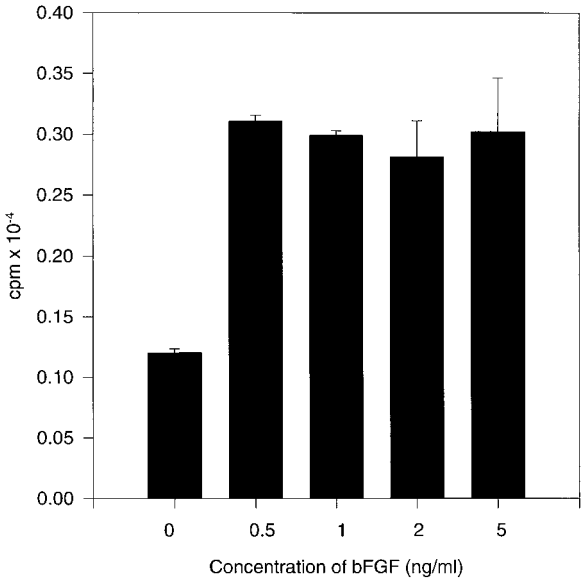


Fig. 7. Treatment with bFGF increases [^{3}H]thymidine incorporation. Subconfluent cultures were maintained under serum deprivation conditions for 24 h and then treated with carrier solution (0 ng/ml) or the indicated concentration of bFGF for an additional 24-h period. During the last 1 h, cultures were incubated in the presence of 2 µCi/ml [^{3}H]thymidine, and the acid-insoluble radioactive material determined.

an extremely important role in the regulation of the architecture of the collagen fibrils.

Work from other groups has shown that type V collagen plays a very important role in the structural assembly of heterotypic collagen fibrils in cornea where it assembles along with type I collagen [Fitch et al., 1984, 1988; Birk et al., 1988; Mendler et al., 1989]. Collagen type V is assembled first inside these fibrils, and appears to regulate overall fibril diameter [Birk et al., 1988]. Recently, data from our laboratory indicate that type V/XI collagen plays a similar role in the formation and regulation of fibril architecture by vascular SMCs. In particular, type V/XI collagen participated with type I in heterotypic fibrils, and the ratio of type V/XI to type I collagen correlated with fibril diameter [K.E. Kypreos, D.E. Birk, V. Trinkaus-Randall, and G.E. Sonenshein, manuscript in preparation].

Although the initiating events in the development of the atherosclerotic plaque remain obscure, the effect of bFGF in promoting proliferation of SMCs in the medial layer of the artery appears to be a key element [Klagsbrun and Edelman, 1989]. Expression of bFGF by endothelial cells and SMCs has been observed in human atherosclerotic lesions [Hughes et al., 1993]. In animal models, antibody to bFGF prevented restenosis after balloon injury [Lindner and Reidy, 1991; Edelman et al., 1992]. In the extracellular matrix, bFGF is associated with heparan sulfate proteoglycans which serves as a storage site for a more localized and regulated response to injury. Heparin bound bFGF appears to be biologically more active and very stable against denaturation and proteolytic degradation by proteinases such as plasmin [Saksela et al., 1988]. Other studies, have shown that elastase is able to release heparin bound bFGF from the extracellular matrix, an observation that may explain, at least partially, the medial hypertrophy and neointimal proliferation caused by ECM-degrading proteinases

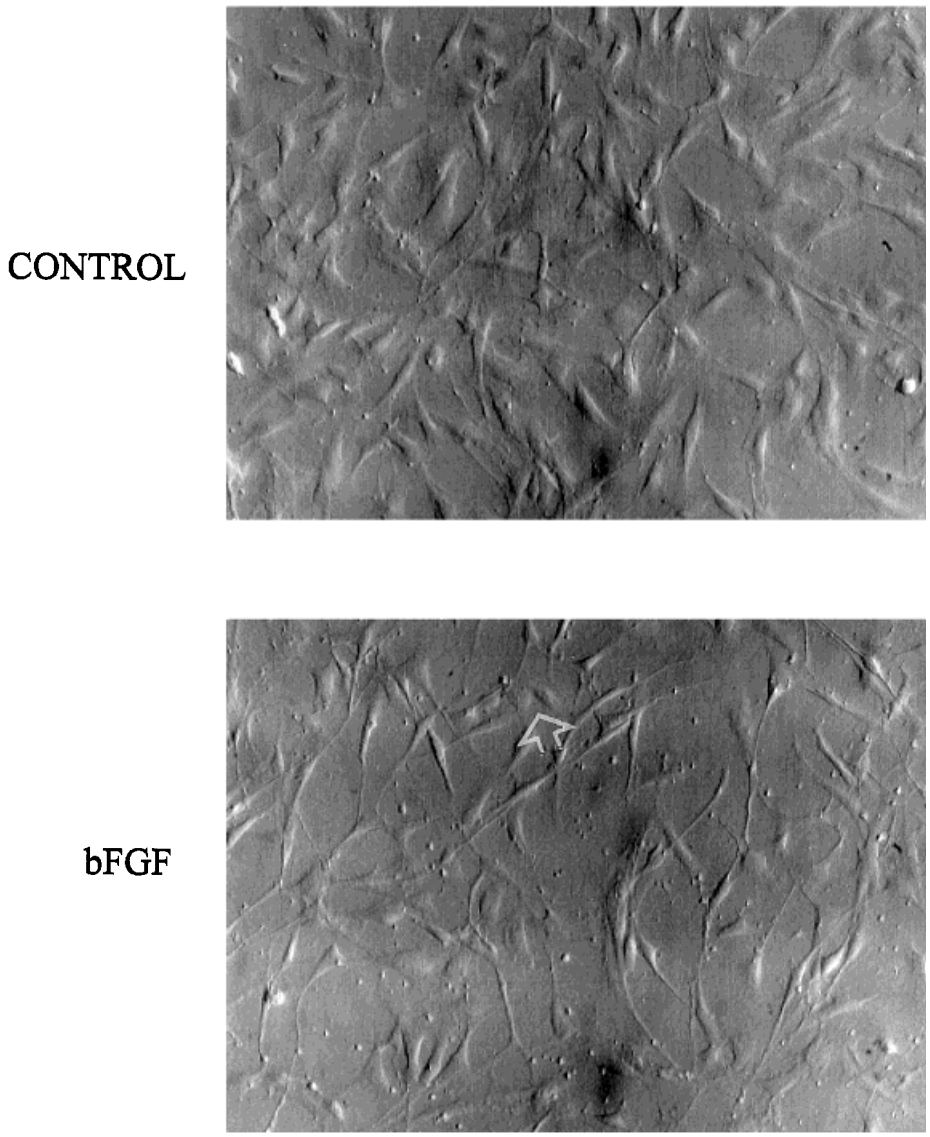


Fig. 8. Treatment of serum-deprived SMCs with bFGF, displaying an altered morphology. Subconfluent cultures, maintained under serum deprivation conditions for 24 h, were treated either with carrier solution (control) or with 2 ng/ml bFGF (bFGF) for an additional 24-h period. Cells undergoing cytokinesis were plainly visible (arrow).

in vascular disease [Thompson and Rabinovitch, 1996]. Nugent and Newman [1989] and Koyama et al. [1996] have shown that polymeric type I collagen inhibits proliferation of vascular SMCs in culture, suggesting that the capacity of SMCs to respond to mitogenic stimuli may be highly regulated by changes in the extracellular matrix. Our results suggest that bFGF may prevent collagen deposition early, during the initial stages of plaque formation; decreased collagen deposition may result in a less dense extracellular matrix that allows SMCs to migrate from the medial layer into the intima where they are able to proliferate. However the effects of bFGF appear to attenuate later, during the most advance stages of the lesion, where collagen secretion and accumulation become major events.

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