Basic Fibroblast Growth Factor Induces The Expression of Matrix Metalloproteinase-3 in Human Periodontal Ligament Cells Through the MEK2 Mitogen-Activated Protein Kinase Pathway

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Basic fibroblast growth factor (bFGF, FGF-2) is one of the potent mitogens for periodontal ligament (PDL) cells. However, the role of bFGF on the matrix metalloproteinase (MMP) -3 expression in PDL cells is unknown. In this study, the effect of bFGF on MMP-3 expression in PDL cells and the mechanism of this process were examined. Human PDL cells were exposed to bFGF at various concentrations (0.01 - 10 ng/ml) in monolayer cultures. bFGF increased [^{3}H]-thymidine incorporation and suppressed proteoglycan synthesis concentration-dependently. However, similar concentration ranges of bFGF increased the release of the cell-associated proteoglycans into the medium. Furthermore, bFGF increased MMP-3 mRNA levels concentration-dependently as examined by reverse transcription-polymerase chain reaction. Induction of MMP-3 after the stimulation with bFGF was observed as early as 12 h with maximal at 24 h. Thereafter, the MMP-3 mRNA level gradually decreased until 72 h. Cycloheximide blocked the induction of MMP-3 by bFGF, indicating the requirement of *de novo* protein synthesis for this stimulation. Furthermore, MMP-3 expression induced by bFGF was abrogated by U0126, a specific inhibitor of MEK1/2 and ERK1/2 in mitogen-activated protein (MAP) kinase pathway, not by PD98059, a specific inhibitor of MEK1. In addition, bFGF up-regulated the phosphorylated ERK1/2 in 5 min with the maximal at 20 min as examined by Western blotting, and U0126 inhibited the ERK1/2 phosphorylation induced by bFGF. These findings suggest that bFGF induces MMP-3 expression in PDL cells through the activation of the MEK2 in MAP kinase pathway. bFGF stimulation on MMP-3 synthesis may be involved in the control of the cell-associated proteoglycans in PDL cells during periodontal regeneration and degradation.

Keywords; periodontal ligament (PDL), basic fibroblast growth factor (bFGF, FGF-2), matrix metalloproteinase (MMP) -3, mitogen-activated protein (MAP) kinase.

Introduction

During the periodontal regeneration process, periodontal ligament (PDL) cells play an important role in forming new attachments between these tissues as well as cementogenesis and osteogenesis. PDL cells initially extend and migrate on the root surface, and then the cells proliferate and secret various extracellular matrix (ECM) components (1, 2). In this process, various hormones and growth factors modulate the proliferation and differentiation of PDL cells. Among them, basic fibroblast growth factor (bFGF, FGF-2) is a member of the heparin-binding growth factor family (3) and the most potent mitogen for PDL cells (4-6). bFGF increases the cell number and inhibits alkaline phosphatase activity in these cells. When bFGF is applied to alveolar bone defects, bFGF induces new PDL and new bone formation without ankylosis or root resorption (7). In addition, PDL cells express both bFGF and FGF receptor mRNAs and proteins. bFGF increases the number of PDL cells without affecting their potency for calcification (7). bFGF may function as both autocrine and paracrine factors in the early wound-healing process of PDL. bFGF may be one of the key factors in periodontal regeneration.

Recently, we reported that PDL cells express FGF receptor mRNA as well as syndecans, a family of heparan sulfate proteoglycans (HSPGs) core proteins, and that the expression levels of these molecules decrease during the differentiation process of PDL cells (6). Differentiated PDL cells show a lower response to bFGF. PDL cells control their growth and differentiation by changing the levels of FGF receptors and syndecans. Furthermore, removing HSPGs from PDL cells results in a decrease in the stimulatory effect of bFGF (6). HSPGs form complexes with bFGF on the FGF receptors to activate cells (8). HSPGs function as a low affinity receptor for bFGF and store bFGF in the ECM for protection against proteolytic degradation (9, 10).

Matrix metalloproteinases (MMPs) are a family of proteolytic zinc enzymes responsible for the degradation of various ECM components (11, 12). Among this family, MMP-3 (stromelysin-1) targets and digests proteoglycan, types IV and IX collagen, laminin, fibronectin, and gelatin (11, 12). MMP-3 plays a role in connective tissue breakdown associated with several inflammatory diseases (12). Interleukin-1ß (IL-1ß), which is an inflammatory cytokine produced by monocytes and macrophages, induces the release of MMP-3 in PDL cells *in vitro* (13, 14). IL-1ß and MMP-3 are found in inflamed periodontal tissues and the gingival crevicular fluid of periodontitis (13).

Previous studies suggested that bFGF and HSPGs may play a role in the control of growth and differentiation of PDL cells. However, it is unknown whether and how bFGF regulates HSPGs turnover in periodontal regeneration. Therefore, this study was designed to investigate the effect of bFGF on the MMP-3 expression and the mechanism of this process in human PDL cells.

Material and methods

PDL cell cultures and treatment with bFGF

Human PDL cells were isolated from healthy periodontal ligaments of the first premolars of individuals undergoing tooth extraction for orthodontic treatment. Informed consent was obtained from all patients before surgical procedures. Healthy periodontal tissue was removed from the middle third of the root surface, and then transferred to 100 mm plastic culture dishes. The explants were cultured in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO) supplemented with 10% fetal calf serum (FCS; GIBCO, Grand Island, NY), 100 units/ml of penicillin and 100 μg/ml of streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. When the cells growing from the explants became confluent, they were harvested with 0.125% trypsin in phosphate buffered saline and transferred to plastic culture dishes at a 1:3 split ratio. For experiments, the cells were trypsinized and cultured in 16 mm wells of a 24-well plate or 100 mm culture dishes at a density of 5 x 10^4 cells/ml in alpha modified minimum essential medium (□-MEM; Sigma) supplemented with 10% FCS and antibiotics until confluence. After confluence, the cells were washed twice with serum-free □-MEM and further cultured with serum free □-MEM for another 24 h. Then the cells were exposed to various concentrations (0.01 - 10 ng/ml) of bFGF (PeproTech EC, London, UK). Experiments were carried out with cells from the fourth to eighth passaged cultures and all cell lines provided similar results in each experiment.

Effect of bFGF on DNA synthesis

PDL cells were labeled with [^{3}H]-thymidine (Japan Atomic Energy Institute, Tokyo, Japan; final concentration, 10 μCi/ml) for the last 4 h of the 24 h treatment as described previously (6). At the end of labeling, the cell layers were washed three times with PBS, twice with 10% trichloroacetic acid and twice with ethanol/diethyl ether (3:1, vol/vol) on ice. The residues in the wells were solubilized with 0.1 N NaOH, the solution was neutralized with 6 N HCl, and radioactivity was measured in a liquid scintillation spectrometer (Aloka, Tokyo, Japan).

Effect of bFGF on proteoglycan synthesis

PDL cells were labeled with [^{35}S]-sulfate (Japan Atomic Energy Institute; final concentration, 20 μCi/ml) for 24 h as described previously (15). Proteoglycan synthesis was determined by measurement of [^{35}S]-sulfate incorporation into material precipitated with cetylpridinium chloride after pronase E treatment.

Effect of bFGF on the release of proteoglycans from the cell matrix layer into the medium

PDL cells were labeled with [^{35}S]-sulfate (Japan Atomic Energy Institute; final

concentration, 20 μCi/ml) for 48 h. The cell matrix layers were washed 5 times with medium as described previously (15), and the cells were then incubated for another 48 h in the presence or absence of various concentrations of bFGF. At the end of treatment, the medium containing the released proteoglycans was collected and the amount of the released proteoglycans was determined by measurement of [^{35}S]-sulfate incorporation into material precipitated with cetylpridinium chloride.

RNA isolation and MMP-3 detection by reverse transcription-polymerase chain reaction (RT-PCR)

PDL cells treated with bFGF were incubated for various lengths of time prior to isolating total RNAs. Total RNAs were isolated from these PDL cells by the guanidinium isothiocyanate method (16) and treated with DNAase I (Promega, Madison, WI) to avoid contamination of genomic DNA. The primers used for RT-PCR were designed based on the published sequence data for corresponding human MMP-3 (sense primer; 5'-TAA AGA CAG GCA CTT TTG GCG C, antisense primer; 5'-CTG CAT CGA TTT TCC TCA CGG T, expected size; 245 bp) (12), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (sense primer; 5'-CCA TGG AGA AGG CTG GGG, antisense primer; 5'-CAA AGT TGT CAT GGA TGA CC, expected size; 210 bp) (17). Aliquots of 1 μg of total RNA were reverse transcribed using avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL) with these 3'-specific primers in 25 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM DTT, 5 mM $MgCl_2$, and 1 mM of each dGTP, dTTP, dCTP and dATP at 64°C for 30 min, 99°C for 5 min and 4°C for 5 min. Amplification was performed for 1 cycle at 95°C for 12 min, 28 cycles at 94°C for 30 sec and 64°C for 1 min, 1 cycle at 64°C for 10 min and 4°C for 5 min using Ampri*Taq* Gold polymerase (Perkin-Elmer, Foster City, CA) with the 3' and 5'- specific primers in 15 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.4 mM DTT, 2.5 mM $MgCl_2$, and 200 μM of each dATP, dCTP, dGTP and dTTP. The PCR products were separated on 2% agarose gels containing ethidium bromide, and then observed on an ultraviolet transilluminator. PCR products in the gels were scanned and the images were quantified using image processing and analysis software (NIH Image version 1.62, National Institutes of Health, Bethesda, MD).

Sequencing of PCR products

All PCR products were separated in 2% low melting-point agarose gels, purified with a DNA fragment extraction kit (QIAGEN, Hilden, Germany) and cloned into the pGEM-5Zf(+) vector (Promega). Sequencing was carried out by the single primer extension method with an Applied Biosystems 377 Sequencer (Perkin-Elmer). The identities of the cloned DNAs were confirmed by comparison with sequences in the GenBank/EMBL/DDBJ database.

Immunoblotting analysis for phosphorylation of ERK1/2

PDL cells were treated with 10 ng/ml of bFGF for the times indicated. The cells were washed with ice-cold 150 mM NaCl and lysed with 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 100 mM Na_3VO_4, 20 μg/ml aprotinin, 25 μg/ml leupeptin, and 1% Triton-X 100. The proteins were concentrated by centrifugal filtration (10 kDa cut-off, Millipore, Bedford, MA), and the protein concentration was measured using a BCA protein assay kit (Pierce, Rockford, IL). Then 40 μg samples were subjected to 10% SDS-PAGE using the procedure of Laemmli (18) under nonreducing conditions, and proteins separated in the gel were subsequently electrotransferred onto PVDF membranes (Immobilon, Millipore). The membranes were incubated with polyclonal rabbit antibody against phosphorylated ERK1/2 (pTEpY, 1 : 4,000, Promega) in 1% casein and TBS (0.15 M NaCl, 20 mM Tris-HCl, pH 8.0) for 1 h at room temperature. After washing with TBS, the membranes were incubated with biotinylated goat anti-rabbit IgG (1 : 2,000, Vector Laboratories, Burlingame, CA) for 30 min, followed by washing with TBS, and then coupled to a peroxidase biotin-avidin complex (Vector). The membranes were incubated in the color reagent (50 mM Tris-HCl, pH 8.0, 0.1% 3,3-diaminobenzidine tetrahydrochloride, and H_2O_2), until brown reaction products were obtained. Reactions were terminated by flushing with water and drying.

Statistical analysis

Values were assessed by analysis of variance (ANOVA) to evaluate the differences and were considered significant at * $p<0.05$ and ** $p<0.01$. Values were expressed as means ± SEM (standard error of the mean).

Results

Human PDL cells were exposed to various concentrations (0.01 - 10 ng/ml) of bFGF for 24 h. bFGF increased [^{3}H]-thymidine incorporation into DNA in a concentration-dependent manner (Fig. 1A). This stimulatory effect was detected at 0.01 ng/ml ($p<0.05$) and maximal at 10 ng/ml ($p<0.01$). On the other hand, bFGF decreased proteoglycan synthesis in PDL cells in a concentration-dependent manner (Fig. 1B). The maximum inhibitory effect was detected at 1 ng/ml ($p<0.01$).

To determine whether bFGF influences the degradation of the cell-associated proteoglycans in PDL cells, PDL cells were labeled with [^{35}S]-sulfate for 48 h. Then various concentrations of bFGF were added to the medium, and the released proteoglycans into the medium were determined. bFGF increased the release of proteoglycans into the medium in a concentration-dependent manner (Fig. 2). This effect was detected at a 0.1 ng/ml ($p<0.01$) and maximal at 10 ng/ml ($p<0.01$).

Next, we examined whether MMP-3 was induced by bFGF in PDL cells, because MMP-3 is known to degrade proteoglycans. The effect on the expression of MMP-3 was assessed by RT-PCR. MMP-3 mRNA was barely detected in control PDL cells (Fig. 3A, B). However, the expression of MMP-3 was observed as an expected 245 bp band in bFGF-exposed cells and the maximal expression was observed at 10 ng/ml (Fig. 3A, B). The DNA sequence of the cloned PCR product was identical to the human MMP-3 sequence (data not shown). In addition, the induction of MMP-3 after the stimulation with bFGF in PDL cells was observed as early as 12 h and became maximal at 24 h (Fig. 4A, B). Thereafter, the expression gradually decreased until 72 h.

To elucidate whether the bFGF stimulation in MMP-3 expression requires newly synthesized protein, the induction of MMP-3 by bFGF was assessed in the presence or absence of a protein synthesis inhibitor, cycloheximide (CHX, 10 μg/ml). Induction of MMP-3 mRNA by bFGF was completely blocked by CHX (Fig. 5A, B). These results suggest that *de novo* protein synthesis is required for MMP-3 expression.

Recent studies showed that activation of mitogen-activated protein (MAP) kinase pathways is required for bFGF-dependent MMP-9 expression in mouse osteoclasts (19). To clarify whether and which MAP kinase pathway mediates MMP-3 expression in PDL cells exposed to bFGF, we used several MAP kinase inhibitors (Fig. 6). PD98059 is a specific inhibitor of MEK1, and partially inhibits MEK2, which inhibits ERK1 activation. U0126 is a specific inhibitor of MEK1/2 which inhibits ERK1/2 activation. SB203580 is a specific inhibitor of p38 MAP kinase which acts as a competitive inhibitor. Expression of MMP-3 by bFGF was completely blocked by U0126, however PD98059 and SB203580 showed no significant effect on the MMP-3 expression (Fig. 7A, B). These findings suggest that activation of MEK2 in the ERK/MAP kinase pathway is

required for bFGF-dependent MMP-3 expression.

To confirm whether bFGF activates ERK1/2 in PDL cells and whether U0126 inhibits the phosphorylated ERK1/2 induced by bFGF, the phosphorylation of ERK1/2 was monitored by Western blotting. bFGF up-regulated the phosphorylated ERK1/2 in 5 min with the maximal at 20 min, and the phosphorylated ERK1/2 were observed as 44 and 42 kDa bands (Fig. 8A). Furthermore, U0126 completely blocked the phosphorylation of ERK1/2 induced by bFGF (Fig. 8B). However PD98059 revealed a little inhibition in the phosphorylated ERK1/2. These findings suggest that bFGF activates the MEK2 in human PDL cells.

Discussion

Various hormones and growth factors are involved in the control of ECM synthesis and ECM degradation in PDL (2). bFGF has been shown to modulate ECM synthesis including type I collagen, laminin and tropoelastin (5, 20, 21). However, the role of bFGF on the cell-associated proteoglycans in PDL is unknown. In the present study, we found that bFGF was involved in the control of the cell-associated proteoglycans as well as proliferation of PDL cells. Incubation of PDL cells with bFGF increased the release of the cell-associated proteoglycans concentration-dependently. The increase in the release of proteoglycans by bFGF from the cell-matrix layer was not due to increased synthesis of proteoglycans. Interestingly, bFGF increased MMP-3 mRNA expression in PDL cells within 12 h, bFGF at 10 ng/ml also increased the production of MMP-3 protein by PDL cells within 24 h when analyzed by casein-zymography (data not shown). Furthermore, bFGF increased the release of proteoglycans and MMP-3 expression at similar concentrations in PDL cells. Thus, bFGF may stimulate the release of proteoglycans via the increase in MMP-3 production. Moreover, this rapid response of MMP-3 expression after bFGF treatment suggests that bFGF signaling may directly regulate MMP-3 expression in PDL cells. However, this action by bFGF required newly synthesized proteins. It remains unknown what newly synthesized protein(s) are involved in the bFGF action on MMP-3 expression.

FGF receptors activate multiple intracellular signaling, including the MAP kinase pathway in several cells (19, 22, 23). Three groups of MAP kinase pathways have been identified; the ERK/MAP kinase pathway, the JNK/SAPK pathway, and the p38 pathway (24, 25). All three groups of the MAP kinase pathways are activated by several growth factors and inflammatory cytokines, and medicate various cellular events including growth, differentiation, and stress responses. However, the activation of each type of MAP kinase depends on the types of the cells and the stimulus. Recently, it was demonstrated that mouse osteoclasts require activation of the p38 MAP kinase pathway for bFGF-dependent MMP-9 expression (19), whereas human primary fibroblasts require the activation of both the ERK/MAP and p38 MAP kinase pathways for bFGF-dependent MMP-1 expression (22). Furthermore, mouse osteoblastic cells require the activation of ERK/MAP kinase pathway, but not the p38 MAP kinase pathway for bFGF-dependent release of vascular endothelial growth factor (23). Several MAP kinase inhibitors are used for analyzing these signaling pathways, such as U0126, PD98059, and SB203580 (25-27). In the present study, U0126 abrogated bFGF-dependent MMP-3 expression in RT-PCR, and the phosphorylation of ERK1/2 by Western blotting in PDL cells. However, PD98059 showed little effect in both experiments, although U0126 and PD98059 are the inhibitors in ERK/MAP kinase pathway. PD98059 is a more selective inhibitor and mainly binds to nonactivated MEK1 and inhibits the MEK phosphorylation by Raf (25). U0126 is a significantly higher affinity

inhibitor in the ERK/MAP kinase pathway. U0126 inhibits both the phosphorylation of MEK1/2 by inhibiting Raf binding, and the phosphorylation of ERK1/2 by inhibiting the phosphorylation of MEK1/2 (27, 28). Therefore, the findings by RT-PCR and Western blotting using U0126 and PD98059 demonstrated that the bFGF signaling was mediated through the phosphorylation of MEK2, and the phosphorylation of MEK2 appears to be essential for the MMP-3 expression in PDL cells.

Previous studies have reported that bFGF increases MMP-1 (collagenase) synthesis in rat bone cells (20) and human smooth muscle cells (29), and MMP-9 (gelatinase B) synthesis in rabbit chondrocytes (30) and mouse osteoclasts (19). bFGF may enhance MMP-3 expression in PDL cells, but not in other cell types. PDL cells may have distinct characteristics compared with the other connective tissue cells, including gingival and lung tissues (31, 32). PDL cells show very rapid turnover. The rapid turnover of the ECM in PDL may offer advantages for regulating tooth eruption and the physiological movement of teeth in the jaw.

Although bFGF induced the release of proteoglycans and the expression of MMP-3 in human PDL cells, it is still uncertain wherther MMP-3 is the major enzyme degrading proteoglycans in PDL cells. There are several candidates in MMP family for digesting HSPGs. Stromelysin family is composed of stromelysin-1 (MMP-3), stromelysin-2 (MMP-10), and stromelysin-3 (MMP-11), and has broader specificity and can degrade proteoglycans, basement membrane collagens, and matrix glycoproteins (33). This family has been observed in carcinomas and inflamed synovial tissues in rheumatoid arthritis (34, 35). They play a role in connective tissue destruction associated with cancers and inflammatory diseases. Nevertheless, no information is available on oral tissues *in vitro* or *in vivo*, except for MMP-3. MMP-3 distributes in inflamed periodontal tissues and is detected in the gingival crevicular fluid of periodontitis (13). Matrilysin (MMP-7) degrades various non-collagenous ECMs and basement membrane components, and can activate several other latent collagenolytic MMPs (36). Matrilysin increases the localization in inflamed gingival tissue in adult periodontitis, and the gingival crevicular fluid in adult periodontitis contains high amounts of matrilysin (36). However, it remains unclear whether bFGF induces these MMPs in PDL cells.

PDL cells decrease the expression levels of FGF receptors and syndecans during the differentiation process (6). Differentiated PDL cells show a lower response to bFGF. The reductions in these molecules in PDL cells may be important events leading to the differentiated state. Furthermore, digesting HSPGs in PDL cells inhibited the mitogenic effect of bFGF (6). These findings suggested that the changes in HSPGs modulate the action of bFGF on PDL cells, and HSPGs exist as a functional regulatory molecule, not just as ECM components in PDL cells. It is unknown wherther MMP-3 degrades syndecans and other HSPGs in PDL cells. If MMP-3, induced by bFGF, could degrade

syndecans and other HSPGs, MMP-3 may play a role in negative feedback regulation on bFGF-FGFR interactions. Recently, Whitelock *et al.* reported that MMPs degrade HSPGs and induce the release of bFGF from HSPGs (37). Thus, MMP-3, induced by bFGF in PDL cells, may be part of a mechanism that releases active bFGF from HSPGs-bFGF complex.

In conclusion, we found that bFGF markedly enhanced the expression of MMP-3 and the release of the cell-associated proteoglycans in human PDL cell cultures. bFGF may be involved in the control of the cell-associated proteoglycans as well as proliferation in PDL cells, and may have a crucial role in periodontal regeneration. The information obtained in the present study will be useful for understanding the role of FGF in PDL.

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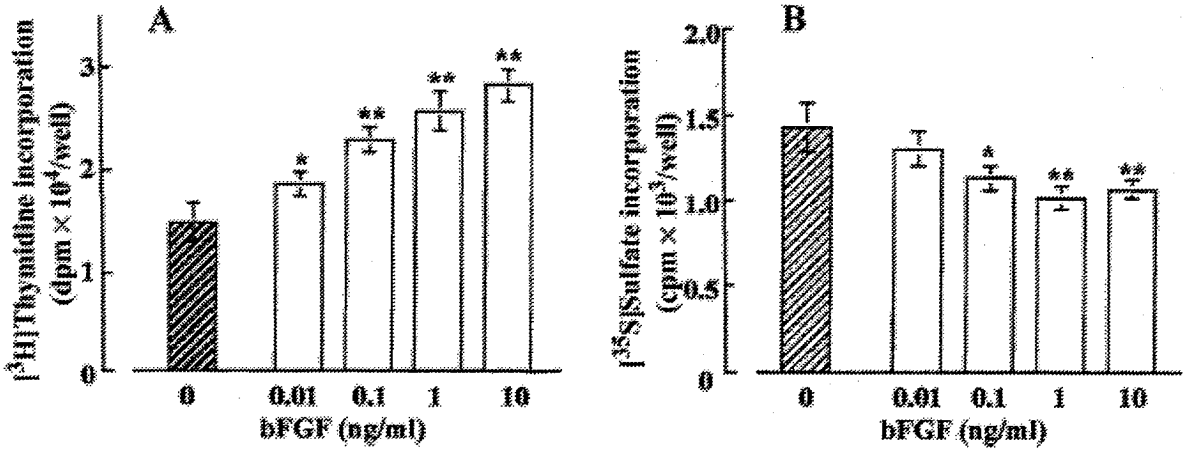

Figure 1. Effects of bFGF on DNA and proteoglycan syntheses in PDL cells. (A) PDL cells were cultured in 16 mm wells of a 24-well plate in □-MEM supplemented with 10% FCS until confluence. After confluence, the cells were further cultured with serum free □-MEM for 24 h. Then the cells were exposed to various concentrations (0.01 - 10 ng/ml) of bFGF for 24 h and labeled with [^{3}H]-thymidine for the final 4 h, and the incorporated radioactivity was determined. (B) PDL cells were labeled with [^{35}S]-sulfate for 24 h, and proteoglycan synthesis was determined by measurement of [^{35}S]-sulfate incorporation into material precipitated with cetylpridinium chloride after pronase E treatment. Points and bars are means ± SEM for four cultures. Similar results were obtained in three independent experiments. * $p<0.05$, ** $p<0.01$, significantly different from control cultures.

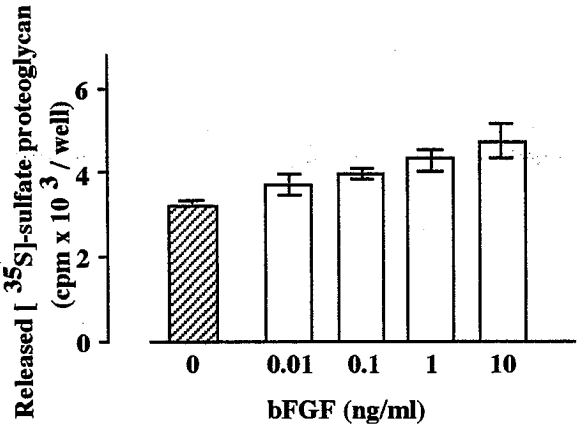

Figure 2. Effect of bFGF on the release of proteoglycans from the cell matrix layer in PDL cells.

PDL cells were labeled with [^{35}S]-sulfate for 48 h, washed, and then incubated for 48 h with various concentrations of bFGF. After 48 h incubation, released proteoglycans into the medium were determined by measurement of [^{35}S]-sulfate incorporation into material precipitated with cetylpridinium chloride. Points and bars are means ± SEM for four cultures. Similar results were obtained in three independent experiments. ** $p<0.01$, significantly different from control cultures.

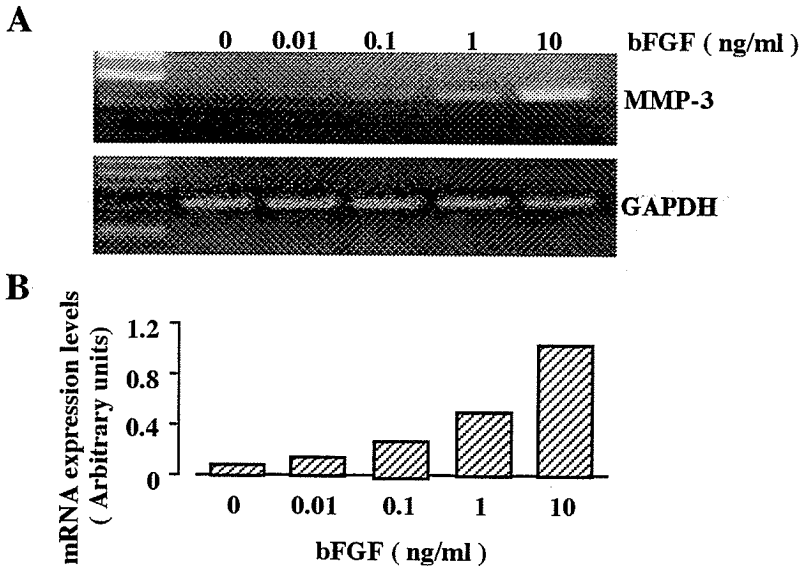

Figure 3. Effect of bFGF on the expression of MMP -3 mRNA in PDL cells. (A) PDL cells were exposed to various concentrations (0.01 - 10 ng/ml) of bFGF, and total RNAs isolated from cells treated at each concentration were subjected to RT-PCR analysis. The PCR products were separated on 2% agarose gels containing ethidium bromide, and then observed on an ultraviolet transilluminator. (B) The expression of each molecule shown in Fig. 3 (A) was quantitated by image analysis. Results are presented as the ratio of the mRNA level to GAPDH in each molecule.

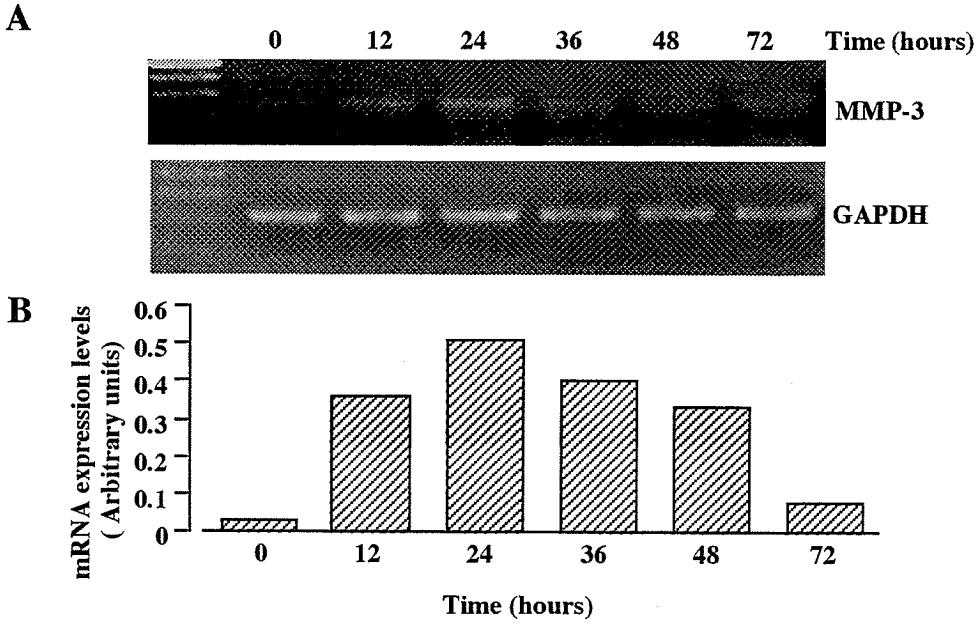

Figure 4. Expression of MMP-3 mRNA in PDL cells after stimulating with bFGF.

(A) PDL cells were treated with 10 ng/ml of bFGF for 0, 12, 24, 36, 48 or 72 h. Total RNAs were isolated at the indicated time points, and subjected to RT-PCR analysis. The PCR products were separated on 2% agarose gels containing ethidium bromide, and then observed on an ultraviolet transilluminator. (B) The expression of each molecule shown in Fig. 4 (A) was quantitated by image analysis. Results are presented as the ratio of the mRNA level to GAPDH in each molecule.

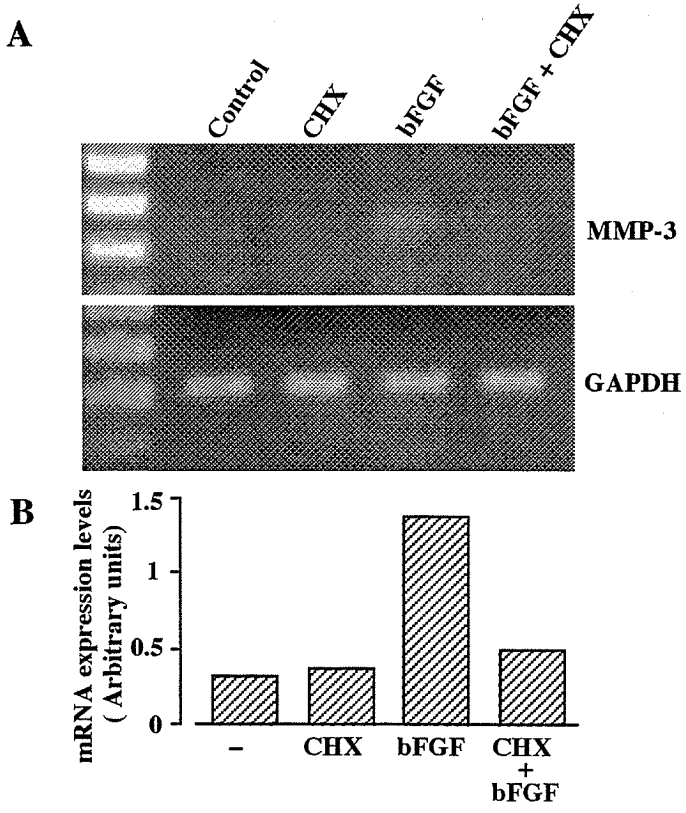

Figure 5. Effect of CHX on MMP-3 expression in PDL cells exposed to bFGF.

(A) PDL cells were treated with bFGF for 6 h. CHX (10 μg/ml) was added 30 min before the addition of bFGF. Total RNAs isolated from each treated cell were subjected to RT-PCR analysis. The PCR products were separated on 2% agarose gels containing ethidium bromide, and then observed on an ultraviolet transilluminator. (B) The expression of each molecule shown in Fig. 5 (A) was quantitated by image analysis. Results are presented as the ratio of the mRNA level to GAPDH in each molecule.

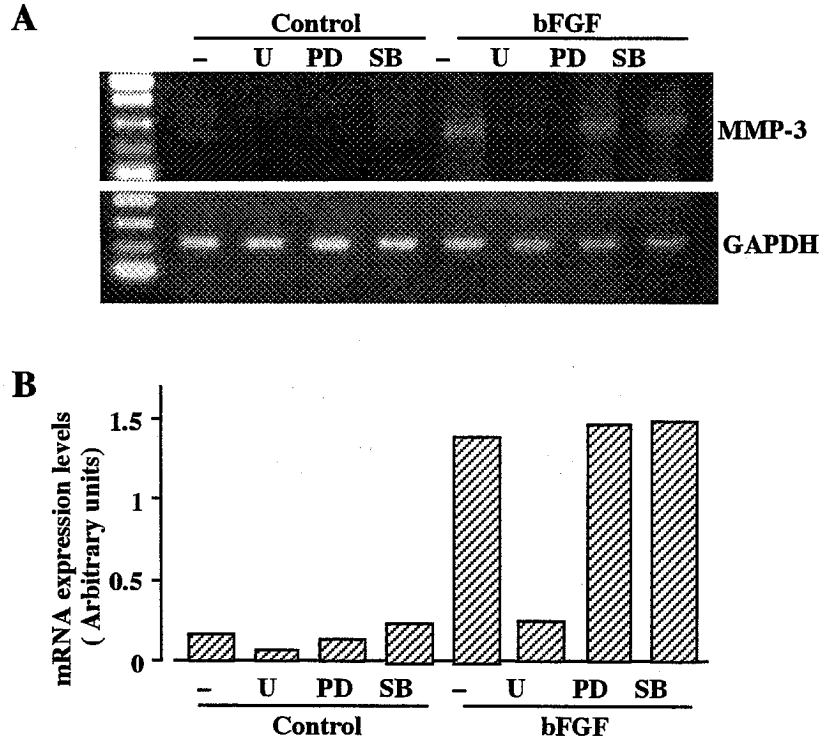

Figure 7. Effect of various MAP kinase inhibitors on MMP-3 expression in PDL cells exposed to bFGF.

(A) PDL cells were treated with bFGF for 6 h. A specific inhibitor of MAP kinase either PD98059 (PD), U0126 (U) or SB203580 (SB), was added 30 min before the addition of bFGF. Total RNA isolated from each treated cell was subjected to RT-PCR analysis. The PCR products were separated on 2% agarose gels containing ethidium bromide, and then observed on an ultraviolet transilluminator. (B) The expression of each molecule shown in Fig. 7 (A) was quantitated by image analysis. Results are presented as the ratio of the mRNA level to GAPDH in each molecule.

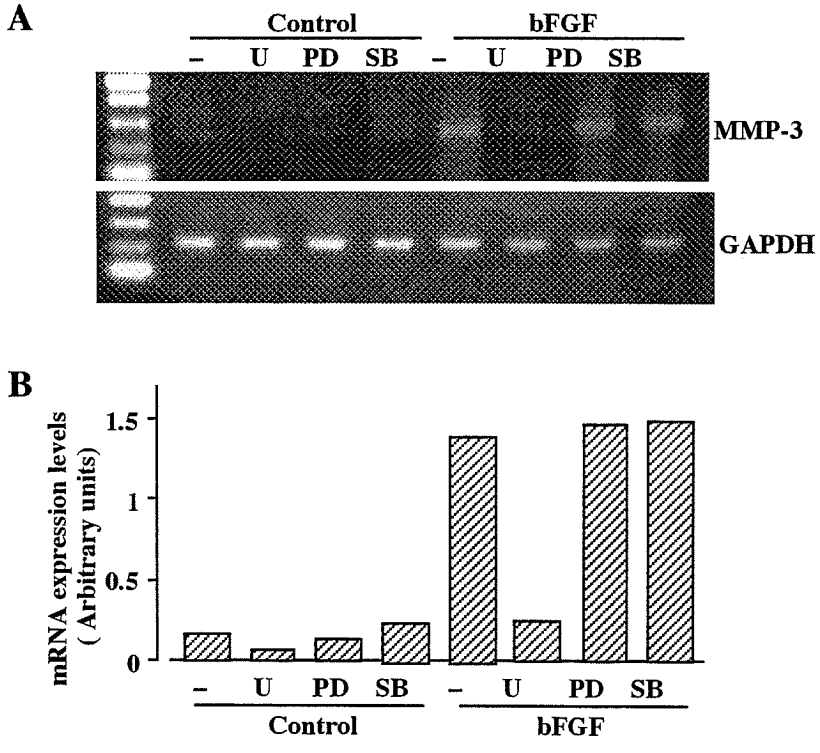

Figure 7. Effect of various MAP kinase inhibitors on MMP-3 expression in PDL cells exposed to bFGF.

(A) PDL cells were treated with bFGF for 6 h. A specific inhibitor of MAP kinase either PD98059 (PD), U0126 (U) or SB203580 (SB), was added 30 min before the addition of bFGF. Total RNA isolated from each treated cell was subjected to RT-PCR analysis. The PCR products were separated on 2% agarose gels containing ethidium bromide, and then observed on an ultraviolet transilluminator. (B) The expression of each molecule shown in Fig. 7 (A) was quantitated by image analysis. Results are presented as the ratio of the mRNA level to GAPDH in each molecule.

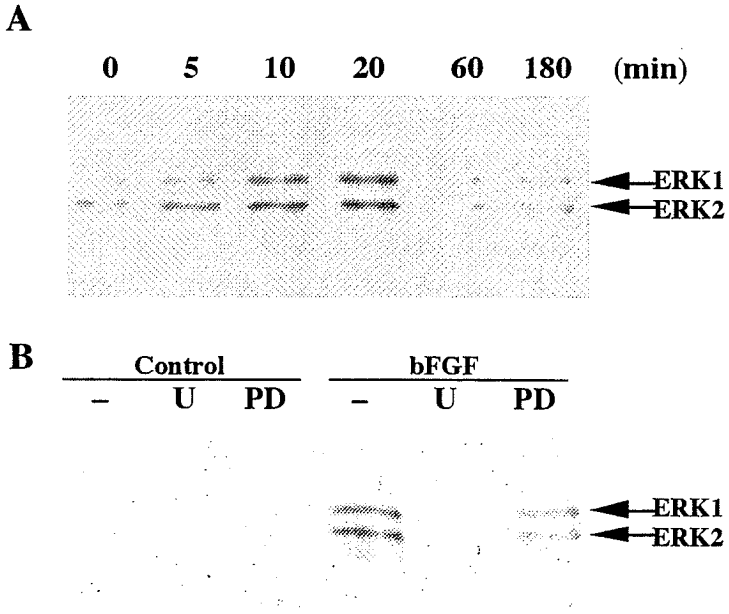

Figure 8. Effect of bFGF on ERK1/2 phosphorylation in PDL cells.
(A) PDL cells were treated with bFGF for the indicated times. Then 40 μg samples from cell layers were subjected to 10% SDS-PAGE, and the gels were subsequently electrotransferred onto PVDF membranes. The membranes were incubated with polyclonal rabbit antibody against phosphorylated ERK1/2 and phosphorylation of ERK1/2 was detected. (B) PDL cells were treated with bFGF for 20 min. A specific inhibitor of MAP kinase either U0126 (U) or PD98059 (PD) was added 60 min before the addition of bFGF.