Expression of Syndecan-2, -4 and Fibroblast Growth Factor Receptor Type 1 in Human Periodontal Ligament Fibroblasts and Down-Regulation of These Membrane Proteins During Maturation in Culture

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Syndecans are transmembrane heparan sulfate proteoglycans. They are known to interact with basic fibroblast growth factor (bFGF) and it has been suggested that they play important roles in the growth, morphology and migration of a variety of cell types. We examined the expression of syndecans and fibroblast growth factor receptor type 1 (FGFR1) in periodontal ligament (PDL) cells, because these membrane proteins may play roles in the control of growth and differentiation during regeneration of PDL. Reverse transcription-polymerase chain reaction (RT-PCR) showed that PDL cells expressed syndecan-2 and -4 mRNAs. This was confirmed by sequence analysis of the PCR products. When PDL cells were maintained for 25 days, alkaline phosphatase (ALPase) activity gradually increased and reached a maximal level on day 20. Northern blotting analysis showed that PDL cells expressed 2.3 kb syndecan-2, 2.6 kb syndecan-4 and 2.8 kb FGFR1 mRNAs throughout the entire culture period, whereas no syndecan-1 mRNA was detectable by this method. Maximal levels of syndecan-2, -4 and FGFR1 mRNAs were observed on day 5. However, their levels were markedly decreased on days 20 and 25. Accordingly, the inhibitory effect of bFGF on ALPase activity was less on day 20 than on day 5. When PDL cells were pretreated with heparitinase, a mitogenic response of PDL cells to bFGF was decreased. These observations indicate that PDL cells express syndecan-2, -4 and FGFR1 mRNAs, and that those levels are changed with the increase in ALPase activity in culture. The reductions in syndecan-2, -4 and FGFR1 levels may be involved in the control of growth and differentiation of PDL cells during development and regeneration.

Keywords; periodontal ligament (PDL), syndecan, heparan sulfate, fibroblast growth factor receptor (FGFR), basic fibroblast growth factor (bFGF).

Introduction

Periodontal ligament (PDL) cells secrete various extracellular matrix (ECM) components, and the ECM molecules play roles in maintaining the tissues, regulating tooth eruption and allowing physiological movement of teeth in the jaw (Hakkinen *et al.*, 1993; Ogata *et al.*, 1995; Oksala *et al.*, 1997; Takano-Yamamoto *et al.*, 1994). Proteoglycans are ECM components composed of core protein and various types of glycosaminoglycan side chains including heparan sulfate and chondroitin sulfate (Hakkinen *et al.*, 1993; Oksala *et al.*, 1997). These molecules are widely distributed in gingival tissue, PDL and alveolar bone (Bartold, 1990; Hakkinen *et al.*, 1993; Ogata *et al.*, 1995; Oksala *et al.*, 1997). Their distributions seem to vary depending upon growth, differentiation and inflammation status. The amounts of these proteoglycans decrease in inflamed gingival tissues, and the degraded components are detected in gingival crevicular fluid from the periodontal pockets (Last *et al.*, 1985; Oksala *et al.*, 1997).

Syndecans are a family of transmembrane proteoglycans which have heparan sulfate in their structure. This family comprises four members; syndecan-1 (syndecan), syndecan-2 (fibroglycan), syndecan-3 (N-syndecan) and syndecan-4 (ryudocan, amphiglycan) (Bernfield *et al.*, 1992; Elenius and Jalkanen, 1994; Jalkanen *et al.*, 1992). Syndecans bind to collagen, tenascin and fibronectin through their heparan sulfate chains (Bernfield *et al.*, 1992; Elenius and Jalkanen, 1994). They also interact with acidic and basic fibroblast growth factors (aFGF and bFGF) and function as low affinity receptors for these growth factors (Bernfield *et al.*, 1992; Elenius and Jalkanen, 1994; Jalkanen *et al.*, 1992).

Information concerning the syndecan family in oral tissues is limited. Syndecans are detected in the mesodermal condensation of the tooth bud (Thesleff *et al.*, 1990). Expression of syndecan-1 is limited to the presumptive pulp and odontoblastic tissues, whereas that of syndecan-2 is detected in the dental sac mesenchyme (Bai *et al.*, 1994; Thesleff *et al.*, 1990). In adult tissues, syndecan-1 is immunolocalized in epithelial cells and infiltrating lymphocytes, but not in fibroblastic tissues in the periodontium (Oksala *et al.*, 1997).

In the present study, we examined the expression of the syndecan family in adult PDL cells using reverse transcription-polymerase chain reaction (RT-PCR) and northern blotting analysis. In addition, we examined the changes in expression levels of the syndecan family and fibroblast growth factor receptor type 1 (FGFR1) during maturation of PDL cells in culture. Further, the biological consequences of changes in these molecules and functional roles of heparan sulfate in PDL were investigated.

Materials and methods

Cell cultures

Human PDL cells (P-1, P-2, P-3 and P-4) were separately isolated from healthy periodontal ligaments of the first premolar of individuals undergoing tooth extraction for orthodontic treatment in accordance with the method of Somerman (Somerman *et al.*, 1989). All patients gave their informed consent before extractions. The human subject protocols were approved by the Committee on Investigations Involving Human Subjects, Hiroshima University School of Dentistry. Healthy periodontal tissue was removed from the middle third of the root surface and then transferred to 10 cm plastic culture dishes (Corning, Corning, NY). The explants were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (FCS; GIBCO), 100 units/mL of penicillin and 100 μg/mL of streptomycin (GIBCO) in a humidified atmosphere of 95% air and 5% CO2 at 37 °C. Medium was changed every other day. When the cells growing from the explants became confluent, they were harvested with 0.125% trypsin in phosphate buffered saline (PBS), and transferred to plastic culture dishes at a 1:3 split ratio. For experiments, the cells were trypsinized and seeded at 2 x 10^6 cells per 10 cm culture dish or 2 x 10^5 cells per 16 mm well of 24-well plates (Corning) in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid. Experiments were carried out with cells from the fourth to tenth passaged cultures, and all cell lines provided similar results in each experiment.

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

PDL cells (P-1, P-2, P-3 and P-4) from the fourth passaged cultures were seeded at 2 x 10^6 cells per 10 cm culture dish in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid, and were maintained for 5 days until the cultures became confluent. Total RNAs were isolated from these PDL cells by the guanidinium isothiocyanate method (Chomczynski and Sacchi, 1987). The primers used for RT-PCR were designed based on the published sequence data for corresponding human syndecan cDNAs (Table 1) (David *et al.*, 1992; Mali *et al.*, 1990; Marynen *et al.*, 1989). Aliquots of 1 μg of total RNAs were reverse transcribed using AMV reverse transcriptase (Life Sciences, St. Petersburg, FL) with these 3'-specific primers. Amplification was performed for 25 cycles at 94 °C for 45 sec, 64 °C for 45 sec and 72 °C for 90 sec using *Taq* DNA polymerase (TaKaRa, Kyoto, Japan) with the 3' and 5'- specific primers. The PCR products were separated in 1% agarose gels containing ethidium bromide, and then observed on an ultraviolet transilluminator.

syndecan-1	5'-HSYN	5'-TCT GAC AAC TTC TCC GGC TC
	3'-HSYN	5'-CCA CTT CTG GCA GGA CTA CA
syndecan-2	5'-HFIBRO	5'-GGA GCT GAT GAG GAT GTA GA
	3'-HFIBRO	5'-CAC TGG ATG GTT TGC GTT CT

Preparation of cDNA probes

The PCR products were separated in 1% low melting agarose gels, purified with a DNA fragments extraction kit (QIAGEN, Hilden, Germany) and cloned into the pGEM-5Zf(+) vector (Promega, Madison, WI). Sequencing was carried out by the single primer extension method with an Applied Biosystems 377 Sequencer (Perkin-Elmer, Foster City, CA). The identities of the cloned DNAs were confirmed by comparison with sequences in the GenBank/EMBL/DDBJ database in RIKEN Life Science Center (Tsukuba, Japan). The cloned DNAs were named pASHSYN1 encoding human syndecan-1 nucleotides 326-537, pASHFIBRO encoding human syndecan-2 nucleotides 759-1153 or pASHRYUDO encoding human syndecan-4 nucleotides 120-464. A human FGFR1 cDNA for use as a probe was provided by Dr. Rei Asakai (Tokyo Medical and Dental University, Tokyo, Japan).

Southern blotting analysis

PCR products were separated in 1% agarose gels. The gels were denatured in 1.5 M NaCl, 0.5 M NaOH for 15 min twice, then neutralized in 1.5 M NaCl, 0.5 M Tris-HCl (pH 7.2), 0.005 M EDTA for 15 min twice according to the manufacturer's instructions (Amersham, Buckinghamshire, UK). The gels were capillary blotted onto Hybond-N nylon membranes (Amersham) and subjected to hybridization with ^{32}P-labeled probes. Hybridization was performed at 46 °C in 50% formamide, 6x SSC, 5x Denhardt's solution, 1% SDS and 100 μg/mL denatured fragmented salmon sperm DNA for 16 h in a Micro-4 rotary hybridization oven (Hybaid, Middlesex, UK). Blots were washed at high stringency (0.1x SSC, 0.4% SDS at 65 °C) and exposed to Kodak Bio-Max film (Kodak, Rochester, NY) at room temperature.

Measurement of ALPase activity

PDL cells (P-1) from the fifth passaged cultures were seeded at 2 x 10^6 cells per 10 cm culture dish, and maintained in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid for 5 - 25 days. In some experiments, PDL cells on days 5 and 20 were incubated with various concentrations (0.03 - 3 ng/mL) of bFGF

for 3 days. ALPase activity in the cell layers was measured by a modification of the method of Bessey with *p*-nitrophenyl phosphate (*p*NP) as a substrate (Bessey *et al.*, 1946; Iwamoto *et al.*, 1991) and normalized by DNA contents determined by the Hoechst 33258 (bisbenzimidazole) method (Labarca and Paigen, 1980).

Northern blotting analysis

PDL cells (P-1, P-2, P-3 and P-4) from the fifth passaged cultures were seeded at 2 x 10^6 cells per 10 cm culture dish in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid, and maintained for 5 - 25 days. Total RNAs from PDL cells were denatured by glyoxalation, electrophoresed in 1% agarose gels and transferred onto Hybond-N membranes (Amersham) by capillary blotting as described previously (Shimazu *et al.*, 1996). In some studies, PDL cells (P-1) from the fourth passaged cultures were passaged several times at a 1:3 split ratio when the cultures became confluent. Total RNAs were isolated from the fourth, sixth, eighth and tenth passaged cultures. Blots were stained with 0.04% methylene blue, 0.5 M sodium acetate (pH 5.2) to verify that each RNA sample had been transferred efficiently. Blots were then hybridized with ^{32}P-labeled cDNA probes at 46 °C overnight in a rotary hybridization oven in 6x SSC, 5x Denhardt's solution, 1% SDS, 100 μg/mL sheared denatured salmon sperm DNA and 50% formamide. Blots were washed at high stringency (0.1x SSC/ 0.4% SDS at 60 °C) and exposed to X-ray film at -80 °C. Blots were quantified using an image processing and analysis software (NIH Image, National Institutes of Health, Bethesda, MD).

[^{3}H]Thymidine incorporation

PDL cells (P-1) from the sixth passaged cultures were seeded at 2 x 10^5 cells per 16 mm well of 24-well plates in DMEM supplemented with 10% FCS. When the cultures became confluent, they were preincubated for 24 h in 0.5 mL of DMEM supplemented with 0.3% FCS in the presence or absence of heparitinase (0.03 - 3 mU/mL). The cells were then replaced with 0.5 mL of the same medium supplemented with or without bFGF (0.03 - 1 ng/mL). The cells were incubated for 24 h and labeled with [6-^{3}H]thymidine (Japan Atomic Energy Institute, Tokyo, Japan ; final concentration, 10 μCi/mL) for the last 4 h as described previously (Kato and Iwamoto, 1990). 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 0.1N NaOH, the solution was neutralized with 6N HCl and radioactivity was measured in a liquid scintillation spectrometer (Aloka, Tokyo, Japan).

Statistical analysis

Data were analyzed using the unpaired *t* - test by a statistical software (StatView, Abacus Concepts, Berkeley, CA).

Results

Detection of syndecan mRNAs in PDL cells by RT-PCR

We performed RT-PCR for syndecans using the primers shown in Table 1. We detected PCR products of the expected sizes for syndecan-2 (395 bp) (Fig. 1A) and syndecan-4 (345 bp) (Fig. 2A) in four different PDL cell lines. The DNA sequences of these cloned PCR products were identical to those of the appropriate core proteins (data not shown). These results were confirmed by Southern blotting analysis (Figs. 1B and 2B).

Changes in expression levels of syndecan mRNAs in PDL cells during a long culture period

When PDL cells were maintained in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid for 5 - 25 days, ALPase activity gradually increased during the culture period and reached a maximal level on day 20 (Fig. 3). Northern blotting analysis showed that PDL cells expressed syndecan-2 and -4 mRNAs (Fig. 4A). The size of syndecan-2 mRNA was 2.3 kb, while that of syndecan-4 was 2.6 kb. These sizes are similar to those of other cell types (David *et al.*, 1992; Grover and Roughley, 1995; Lories *et al.*, 1992). Densitometric analysis showed that the levels of syndecan-2 and -4 were high on day 5, decreasing gradually thereafter until day 20-25 (Fig. 4B). No syndecan-1 mRNA was detected by northern blotting analysis, although it was detected in some PDL cells by RT-PCR (data not shown). In addition, FGFR1 expression was detected by northern blotting analysis (Fig. 4A). The level of FGFR1 mRNA was high on day 5, but had decreased on day 10-25 (Fig. 4B). Similar results were obtained with all PDL cells (P-2, P-3 and P-4) examined in this study (Fig. 5, A and B).

Figure 6 shows that PDL cells expressed syndecan-2, -4 and FGFR1 mRNAs on day 5 in the fourth, sixth, eighth and tenth passaged cultures at similar levels. This finding suggests that PDL cells do not lose the ability to synthesize syndecan and FGFR1 mRNAs during passage.

Effects of bFGF on ALPase activity on days 5 and 20

The syndecan and FGFR1 mRNA levels gradually decreased during the whole culture period. We examined whether this decrease affected the responsiveness of PDL cells to bFGF. bFGF inhibited ALPase activity in PDL cells on days 5 and 20 dose-dependently (Fig. 7A and B). However, the effect of bFGF on ALPase activity was much less on day 20 than on day 5. These findings supported the correlation between the decrease in syndecan and FGFR1 mRNA levels during the culture period and the decrease in the responsiveness of PDL cells to bFGF.

Possible role of heparan sulfate in PDL cells

PDL cells were treated with bFGF and/or heparitinase, and [^{3}H]thymidine

incorporation into cell layers was measured. bFGF increased [^{3}H]thymidine incorporation into PDL cells dose-dependently (Fig. 8). This stimulatory effect was detected at the concentration of 0.03 ng/mL with the maximum effect at 0.3 ng/mL. However, this effect of bFGF was partially inhibited by addition of heparitinase at a concentration of 0.03 mU/mL (Figs. 8 and 9). Heparitinase alone decreased [^{3}H]thymidine incorporation to some degree (Fig. 9). This may be due to the inhibition of the effect of endogenous bFGF derived from PDL cells. Chondroitinase and hyaluronidase had little effect in [^{3}H]thymidine incorporation into PDL cells (data not shown).

Discussion

The purpose of the present study was to examine whether PDL cells expressed syndecan mRNAs and to determine how the levels of these core proteins changed with increased ALPase activity in PDL cells in culture. We demonstrated that human PDL cells expressed some members of the syndecan family. In addition, the expression levels of syndecans correlated with ALPase activity of PDL cells.

PDL cells expressed syndecan-2 and -4 mRNAs as detected by RT-PCR (Figs. 1 and 2) and northern blotting analysis (Figs. 4 and 5). It is not known whether human PDL cells express syndecan-3 mRNA, because the sequence of human syndecan-3 has not yet been published. Syndecan-1 mRNA was not detected in PDL cells by northern blotting analysis, perhaps because of very low expression levels, although we did detect this transcript in some PDL cells by RT-PCR (data not shown). This expression pattern of the syndecan family in PDL cells was similar to those in osteoblasts, chondrocytes, skin fibroblasts and lung fibroblasts. These cells express syndecan-2 and -4 mRNAs at high levels, and syndecan-1 mRNA only at very low levels (David *et al.*, 1992; Grover and Roughley, 1995; Lories *et al.*, 1992). Syndecan-1 is usually detected in cells of epithelial origin, although it is transiently expressed in the condensing mesenchyme during tooth development (Vainio and Thesleff, 1992).

Recent studies suggest that heparan sulfates derived from syndecan-1, -3 and -4 bind to bFGF (Chernousov and Carey, 1993; Kojima *et al.*, 1996). aFGF and bFGF are heparin-binding growth factors that have various effects on a variety of cell types (Canalis *et al.*, 1988; Iwamoto *et al.*, 1991; Kato and Iwamoto, 1990; Okamoto *et al.*, 1997; Takayama *et al.*, 1997; Terranova *et al.*, 1989). Heparan sulfate functions as a low affinity receptor for FGFs and stores FGFs in the ECM for protection against proteolytic degradation (Bashkin *et al.*, 1989; Turnbull *et al.*, 1992). Furthermore, aFGF and bFGF require heparan sulfate for their biological activity, and form complexes with heparan sulfate on the high affinity tyrosine kinase receptors, FGF receptors (Turnbull *et al.*, 1992; Yayon *et al.*, 1991). The FGF receptor family comprises four members, named FGFR1 (*flg*), FGFR2 (*beg*), FGFR3 and FGFR4 (Coutts and Gallagher, 1995). These receptors have two or three immunoglobulin-like structures in the extracellular domains and bind both aFGF and bFGF. Previous studies have shown that PDL cells express bFGF and FGFR1 mRNAs as detected by RT-PCR (Ohgi and Johnson, 1996; Okamoto *et al.*, 1997), and that bFGF increases the cell number and inhibits ALPase activity in these cells (Okamoto *et al.*, 1997; Takayama *et al.*, 1997). In addition, bFGF is immunolocalized in PDL tissue *in vivo* (Gao *et al.*, 1996). In the present study, we detected FGFR1 mRNA in PDL cells by northern blotting analysis (Figs. 4 and 5). bFGF increased [^{3}H]thymidine incorporation dose-dependently in the PDL cells (Fig. 8). However, removing heparan sulfates from the cells resulted in a decrease in the stimulatory effect of bFGF (Figs. 8 and 9). Mali *et al.* showed that overexpression of syndecan-1 in

fibroblasts inhibits bFGF-induced proliferation (Mali *et al.*, 1993), perhaps because of the interference of bFGF binding to its receptor by excess amounts of heparan sulfates. These observations suggest that heparan sulfates exist as a functional regulatory molecule, not just as ECM components in these fibroblasts and PDL cells.

Previous studies have shown that PDL cells form mineral nodules and express some bone markers (Basdra and Komposch, 1997; Nohutcu *et al.*, 1997; Somerman *et al.*, 1990). PDL cells have been hypothesized to be capable of differentiating into either cementoblast- and/or osteoblast-like cells. Some of their phenotypic expressions, increases in ALPase activity, osteocalcin and mineralization levels during maturation in culture (Basdra and Komposch, 1997; Morishita *et al.*, 1998), are similar to the expressions of osteoblasts. In any case, induction of ALPase activity is a necessary event for calcification of tissues, such as cartilage and bone, during the differentiation process (Iwamoto *et al.*, 1994; Iwamoto *et al.*, 1991; Jikko *et al.*, 1993; Kato and Iwamoto, 1990). ALPase gene knock-out mice show abnormal bone mineralization and abnormal osteoblast shape (Narisawa *et al.*, 1997). PDL cells express this activity during differentiation (Ogata *et al.*, 1995; Okamoto *et al.*, 1997; Takayama *et al.*, 1997). In our study, ALPase activity in PDL cells gradually increased and reached a maximal level on day 20 (Fig. 3). In contrast, the expression levels of syndecan-2, -4 and FGFR1 mRNAs decreased during the long culture period (Fig. 4). The levels of heparan sulfate proteoglycan, syndecan-3 and FGF receptor have been shown to decrease during terminal differentiation of chondrocytes (Chintala *et al.*, 1995; Iwamoto *et al.*, 1991; Shimazu *et al.*, 1996). The reductions in these molecules in PDL cells observed in the present study may also be important events leading to the differentiated state.

To examine the biological consequences of the reductions in syndecan and FGFR1 mRNAs during maturation of PDL cells in culture, we examined the responsiveness of PDL cells to bFGF on days 5 and 20. The effect of bFGF on ALPase activity was much less on day 20 than on day 5 (Fig 6). This could be due to the decreased numbers of syndecans and FGF receptor even in the presence of bFGF at high levels. It is noteworthy that the down-regulation of syndecan mRNAs in PDL cells on days 20 and 25 was more prominent than that of FGFR1 (Figs. 4 and 5). We also observed that digesting heparan sulfate in PDL cells inhibited the mitogenic effect of bFGF (Figs 8 and 9). These findings suggest that the changes in syndecans modulate the action of bFGF on PDL cells.

In conclusion, we demonstrated that PDL cells express syndecan-2, -4 and FGFR1 mRNAs, and that the levels of these molecules change with increased ALPase activity suggesting an association with cell differentiation. The reductions in the levels of syndecans and FGFR1 may be involved in the control of growth and differentiation of PDL cells during development and regeneration.

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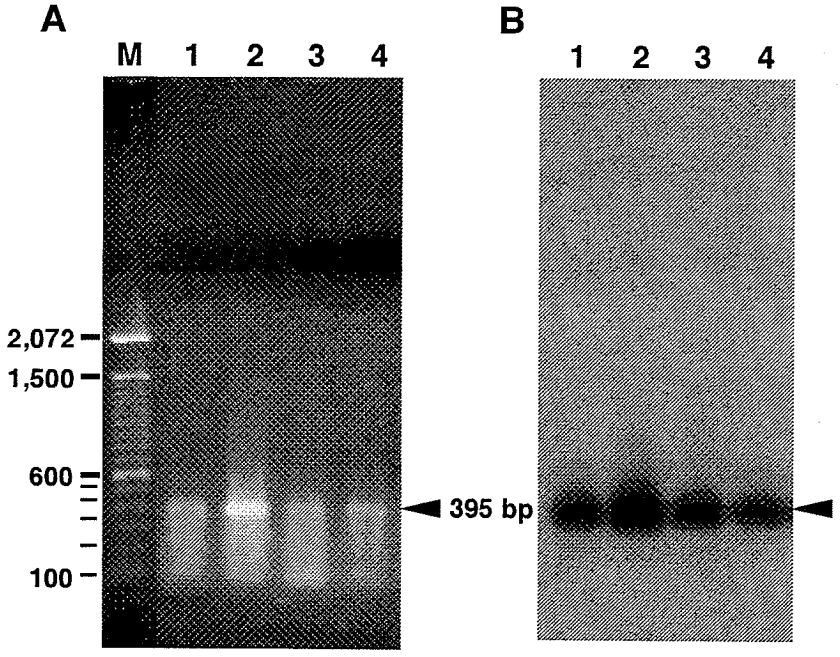

Figure 1. Analysis of syndecan-2 mRNA expression by RT-PCR and Southern blotting analysis.

PDL cells (P-1, P-2, P-3 and P-4) from the fourth passaged cultures were seeded at 2 x 10^6 cells per 10 cm culture dish in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid, and maintained for 5 days until the cultures became confluent. Total RNAs extracted from these cells were analyzed by RT-PCR (A) for syndecan-2 mRNA, and the PCR products were separated in a 1% agarose gel containing ethidium bromide. Lanes: M, 100 base pair ladder marker; 1, P-1; 2, P-2; 3, P-3; 4, P-4. Then, the PCR products were transferred onto a nylon membrane and hybridized with syndecan-2 cDNA. (B) lanes: 1, P-1; 2, P-2; 3, P-3; 4, P-4.

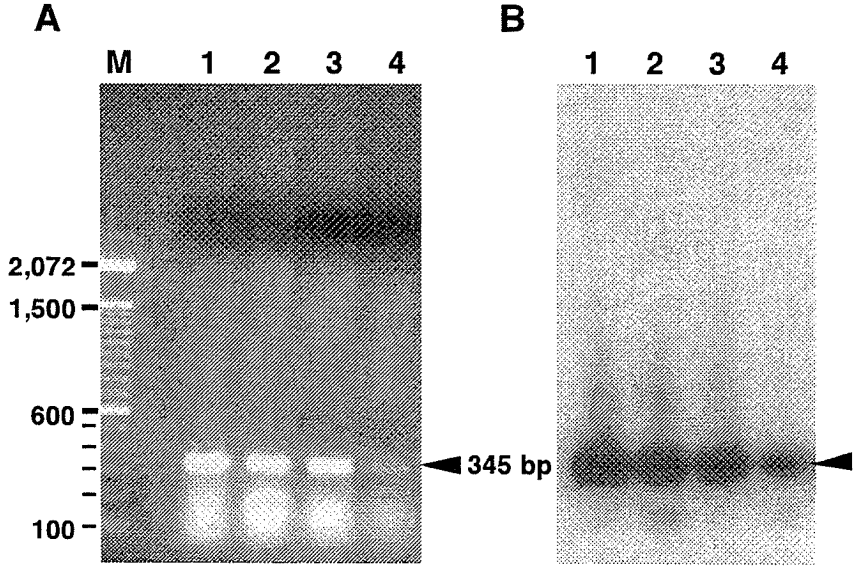

Figure 2. Analysis of syndecan-4 mRNA expression by RT-PCR and Southern blotting analysis.

PDL cells (P-1, P-2, P-3 and P-4) from the fourth passaged cultures were seeded at 2 x 10^6 cells per 10 cm culture dish in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid, and maintained for 5 days until the cultures became confluent. Total RNAs extracted from these cells were analyzed by RT-PCR (A) for syndecan-4 mRNA, and the PCR products were separated in a 1% agarose gel containing ethidium bromide. Lanes: M, 100 base pair ladder marker; 1, P-1; 2, P-2; 3, P-3; 4, P-4. Then, the PCR products were transferred onto a nylon membrane and hybridized with syndecan-4 cDNA. (B) lanes: 1, P-1; 2, P-2; 3, P-3; 4, P-4.

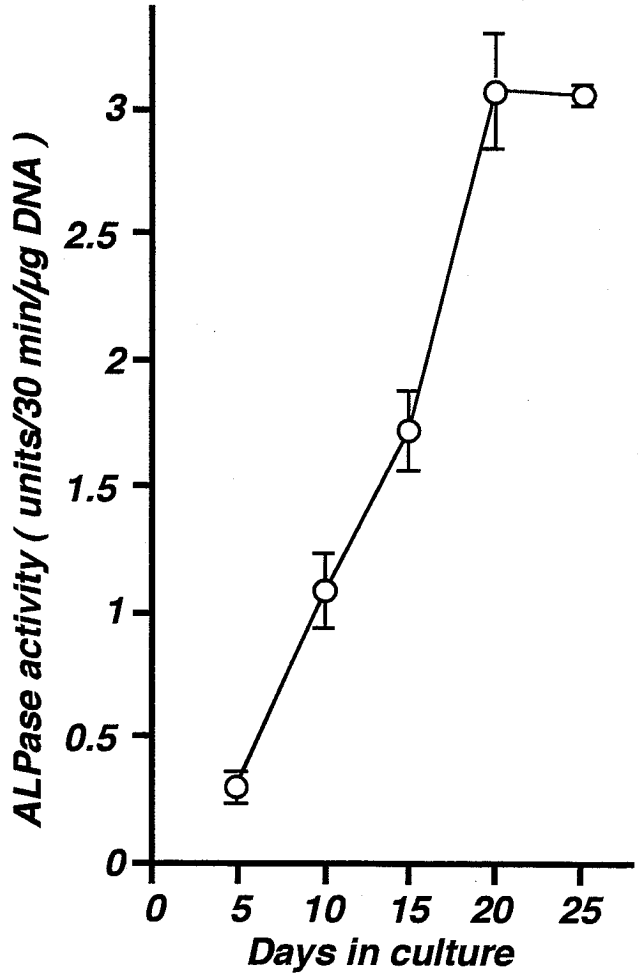

Figure 3. ALPase activity in PDL cells during 25 days in culture.
PDL cells (P-1) from the fifth passaged cultures were seeded at 2 x 10^6 cells per 10 cm culture dish, and maintained in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid for 5 - 25 days. ALPase activity in cell layers was measured and normalized by DNA contents. The date represents the means ± SD for four cultures from one out of three independent experiments.

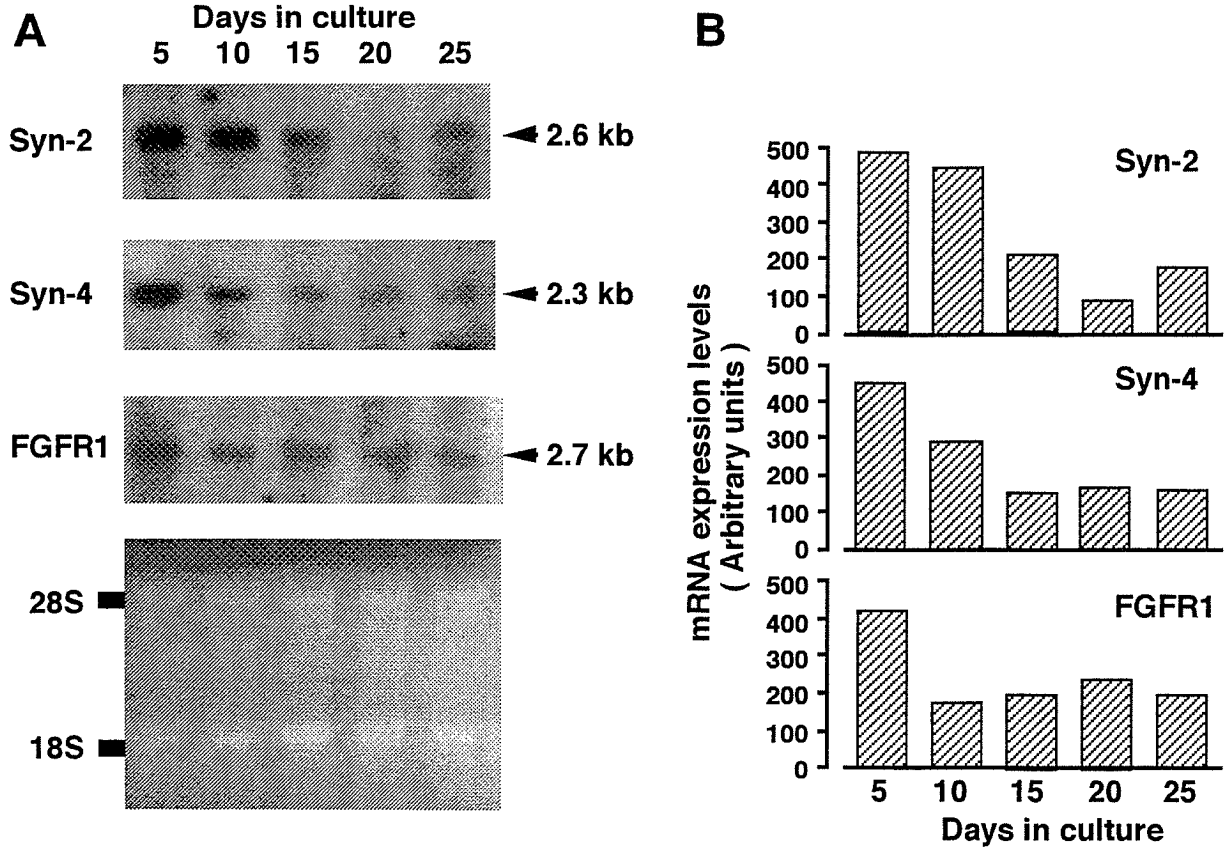

Figure 4. Expression of syndecan-2, -4 and FGFR1 during a long culture period.

(A) PDL cells (P-1) from the fifth passaged cultures were seeded at 2 x 10^6 cells per 10 cm culture dish in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid, and maintained for 5 - 25 days. Total RNAs were isolated and denatured by glyoxalation, electrophoresed in 1% agarose gels and transferred onto nylon membranes by capillary blotting. Blots were then hybridized to ^{32}P-labeled cDNA probes at 46 °C overnight in a rotary hybridization oven. Blots were washed and exposed to X-ray film. (B) Blots were quantified using an imaging software (NIH Image). The results were normalized to the ethidium bromide staining of 28S ribosomal RNA. Syn-2, syndecan-2; Syn-4, syndecan-4; FGFR1, fibroblast growth factor receptor type 1.

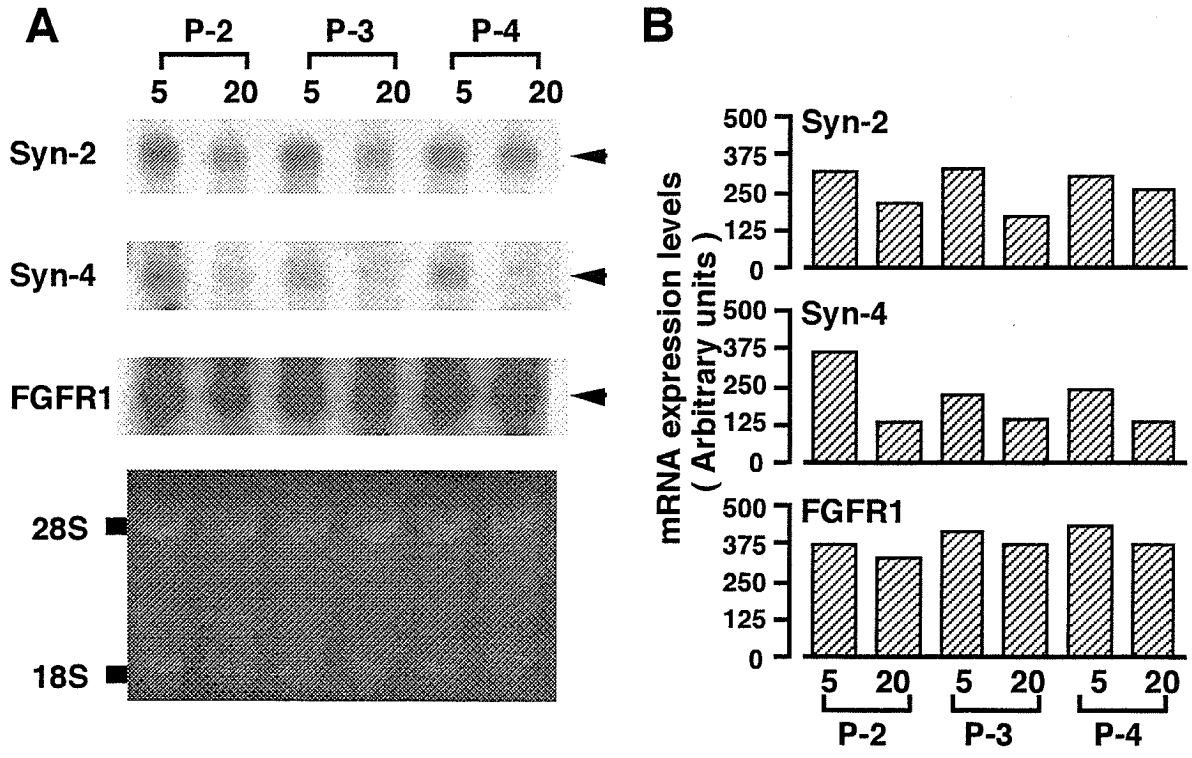

Figure 5. Expression of syndecan-2, -4 and FGFR1 mRNAs on days 5 and 20 in three lines of PDL cells.

(A) PDL cells (P-2, P-3 and P-4) from the fifth passaged cultures were seeded at 2 x 10^6 cells per 10 cm culture dish in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid, and maintained for 5 - 20 days. Total RNAs isolated on days 5 and 20 were denatured by glyoxalation, electrophoresed in 1% agarose gels and transferred onto nylon membranes by capillary blotting. Blots were then hybridized to ^{32}P-labeled cDNA probes at 46 °C overnight in a rotary hybridization oven. Blots were washed and exposed to X-ray film. (B) Blots were quantified using an imaging software. The results were normalized to the ethidium bromide staining of 28S ribosomal RNA.

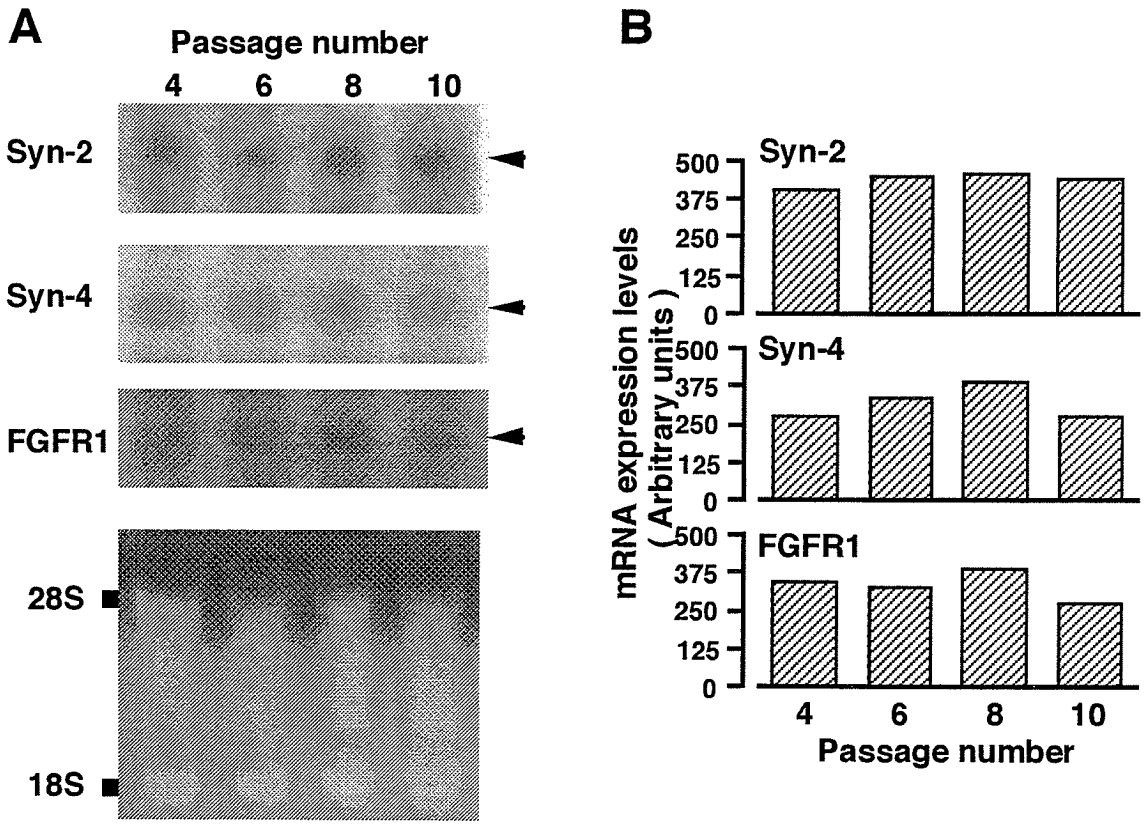

Figure 6. Expression of syndecan-2, -4 and FGFR1 mRNAs by PDL cells after passage in culture.

(A) PDL cells (P-1) from the fourth passaged cultures were passaged several times at a 1:3 split ratio when the cultures became confluent. Total RNAs were isolated from the fourth, sixth, eighth and tenth passaged cultures, and denatured by glyoxalation, electrophoresed in 1% agarose gels and transferred onto nylon membranes by capilLary blotting. Blots were then hybridized to ^{32}P-labeled cDNA probes at 46 °C overnight in a rotary hybridization oven. Blots were washed and exposed to X-ray film. (B) Blots were quantified using an imaging software. The results were normalized to the ethidium bromide staining of 28S ribosomal RNA.

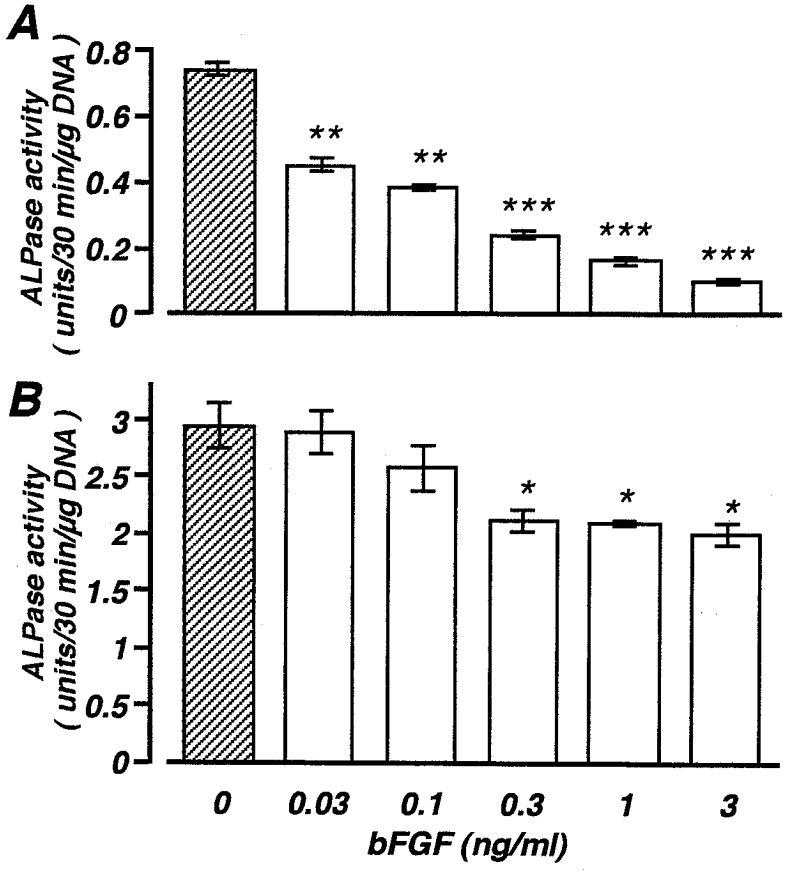

Figure 7. Effect of bFGF on ALPase activity in PDL cells on days 5 and 25 in culture.

PDL cells (P-1) from the fifth passaged cultures were maintained in DMEM supplemented with 10% FCS and 50 μg/mL ascorbic acid. PDL cells on days 5 (A) and 20 (B) were incubated with bFGF (0.03 - 3 ng/mL) for 3 days. ALPase activity in these cells was measured and normalized by DNA contents. The date represents the means ± SD for four cultures from one out of three independent experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, Significantly different from cultures without bFGF.

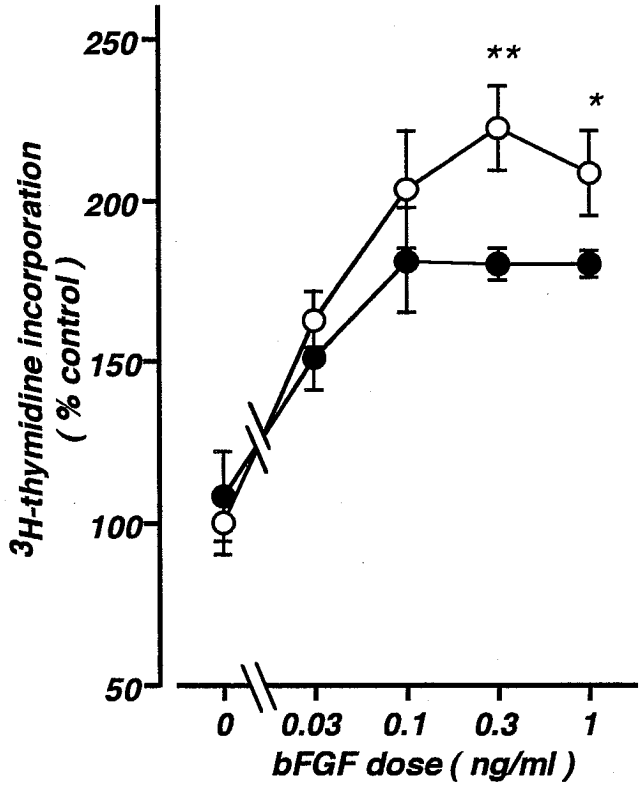

Figure 8. Effects of heparitinase treatment on DNA synthesis in PDL cells in the presence and absence of bFGF.

PDL cells (P-1) from the sixth passaged cultures were seeded at 2 x 10^5 cells per 16 mm well of 24-well plates in DMEM supplemented with 10% FCS. When the cultures became confluent, they were preincubated for 24 h in 0.5 mL of DMEM supplemented with 0.3% FCS in the presence (□) or absence (O) of 0.03 mU/mL of heparitinase. The medium was then replaced with 0.5 mL of the same medium supplemented with bFGF (0.03 - 1 ng/mL). The cells were incubated for 24 h and labeled with [^{3}H]thymidine for the last 4 h, and incorporated radioactivity was determined. Points and bars are means ± SD for four cultures. Similar results were obtained in three independent experiments. * $p<0.05$, ** $p<0.01$, Significantly different from heparitinase-treated cells.

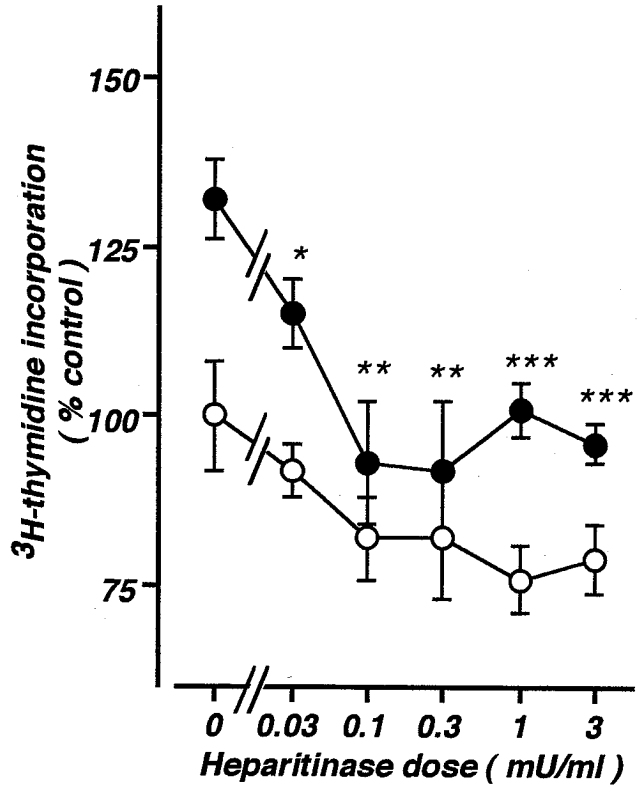

Figure 9. Effects of various concentrations of heparitinase on DNA synthesis in PDL cells in the presence (□) and absence (O) of bFGF.
PDL cells (P-1) from the sixth passaged cultures were seeded at 2 x 10^5 cells per 16 mm well of 24-well plates in DMEM supplemented with 10% FCS. When the cultures became confluent, they were preincubated in 0.5 mL of DMEM supplemented with 0.3% FCS in the presence or absence of heparitinase (0.03 - 3 mU/mL). The medium was then replaced with 0.5 mL of the same medium supplemented with (□) or without (O) 0.03 ng/mL of bFGF. The cells were incubated for 24 h and labeled with [^{3}H]thymidine for the last 4 h, and incorporated radioactivity was determined. Points and bars are means ± SD for four cultures. Similar results were obtained in three independent experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, Significantly different from cells treated with 0.03 ng/mL bFGF alone.