BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **240,** 21–26 (1997)
ARTICLE NO. RC977588

Basic Fibroblast Growth Factor Induces Apoptosis in Myofibroblastic Cells Isolated from Rat Palatal Mucosa

Noriko Funato,*,† Keiji Moriyama,† Hitoyata Shimokawa,‡ and Takayuki Kuroda†

**Department of Maxillo-Facial Orthognathics, Division of Life Science of Maxillo-Facial Systems, Graduate School of Dentistry, †2nd Department of Orthodontics, and ‡Department of Biochemistry, Faculty of Dentistry, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113, Japan*

Received September 24, 1997

The effect of basic fibroblast growth factor (bFGF) on apoptosis in normal rat palatal fibroblasts and rat palatal scar fibroblasts was examined by the TUNEL method in order to clarify the mechanism of apoptosis induction in myofibroblasts during the scar formation process. A percentage of scar fibroblasts undergoing apoptosis was significantly higher than that of palatal fibroblasts when they were treated with bFGF succeeding to serum starvation. Palatal fibroblasts, phenotypically modulated into myofibroblasts by the pretreatment with transforming growth factor-β1 (TGF-β1), similarly showed a higher level of apoptosis induction by bFGF-treatment. TGF-β1 elevated protein and mRNA level of FGF receptor (FGFR) in palatal fibroblasts. Tyrosine autophosphorylation of FGFR upon stimulation by bFGF was significantly higher in scar fibroblasts than in normal palatal fibroblasts. These findings suggested that bFGF may be a potential stimulator of apoptosis in myofibroblasts during palatal scar formation and that FGFR may be responsible for this process.

Wound healing is a highly organized and well coordinated process that involves inflammation, cell proliferation, matrix deposition and tissue remodeling. During the granulation tissue formation, many fibroblasts acquire morphological and biochemical features as contractile cells. These cells have been called myofibroblasts and are considered to be responsible for the granulation tissue contraction (1). We have previously reported that scar fibroblasts isolated from the rat palatal immature scar tissue manifested myofibroblastic characteristics, and that palatal fibroblasts isolated from the normal rat palatal mucosa underwent the transition to myofibroblastic cells by transforming growth factor-β1 (TGF-β1) *in vitro* (2). As the wound becomes re-epithelized and the scar forms, there is a striking decrease in cellularity, accompanying the disappearance of typical myofibroblasts and small blood vessel cells. Recently, apoptosis has been reported to be responsible for the disappearance of myofibroblasts during the wound healing process which might, in turn, evolve granulation tissue into scar (3). However, the question of which mechanisms are implicated in apoptosis of myofibroblasts during the wound healing process is still unknown. A series of studies indicates that growth factor signals are key modulators of apoptosis induction (4). Therefore, we hypothesize that growth factors and their signaling pathways may have a relationship with the apoptosis induction in myofibroblasts.

Among a wide variety of growth factors which are implicated in the wound healing process, basic fibroblast growth factor (bFGF), a prototype member of the FGF family, is a potent accelerator of wound healing (5). The scarless fetal wound healing, in which myofibroblasts do not appear at early gestation (6), shows no bFGF, while it is observed in the adult or neonatal wound (7). Other studies have also indicated that bFGF acts as a lethal factor inducing apoptotic cell death in cultured chick embryonic neural retina cells (8) or in oligodendrocytes (9).

In this study, we have examined the effect of bFGF on cultured palatal fibroblasts and scar fibroblasts in order to further elucidate the mechanism of apoptosis induction in myofibroblasts during the palatal wound healing process.

METHODS

Growth factors and antibodies. Porcine TGF-β1 and bovine bFGF were purchased from R&D Systems, Inc. (Minneapolis, MN). The monoclonal FGFR antibodies (VBS-7), which recognize both FGFR1 and FGFR3, were purchased from ZYMED Laboratories, Inc. (San Francisco, CA), and anti-phosphotyrosine specific monoclonal antibodies (4G10) were purchased from Upstate Biotechnology (Lake Placid, NY).

Cell culture. Palatal fibroblasts and scar fibroblasts were obtained by the explant culture of the normal oral palatal mucosa and

0006-291X/97 $25.00

the immature scar tissue (4 weeks after wounding) of 12-week-old male Sprague-Dawley rats. Both cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS). We have previously reported that palatal fibroblasts undergo phenotypic modulation into α-smooth muscle (α-SM) actin-expressing myofibroblasts on treatment with 5 ng/ml TGF-β1 for 48 h, which are similar to scar fibroblasts (2).

Detection of apoptosis. For the detection of apoptosis, the procedure is based upon the method described by Oberhammer et al. (10). To investigate the response of growth-arrested cells to bFGF, cultures reaching 70-80 % confluence in the complete media were serum-starved for 36 h in D-MEM supplemented with 0.25% FBS, then 5 ng/ml of bFGF was applied with the simultaneous recovery of serum. It is widely accepted that a great majority of fibroblasts are growth-arrested within 24 h under a serum-starved condition (11, 12). Apoptosis induction was detected *in situ* by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) method (13), using the ApopTag system (Oncor, Gaithersburg, MD) according to the manufacturer's protocol. The apoptotic cell percentage was calculated for the further statistical evaluation.

Determination of FGFR1 mRNA expression by reverse transcriptase-polymerase chain reaction (RT-PCR). FGFR1 mRNA expression was evaluated by RT-PCR method in a semi-quantitative manner (14, 15) on the basis of G3PDH mRNA expression as an internal control. Aliquots of total RNA (1.0 μg) were transcribed with 50 units of MMLV reverse transcriptase (RT) (Perkin-Elmer Cetus Corp., Norwalk, CT) in a buffer containing final concentrations of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 20 units of RNase inhibitor, 2.5 mM of random primers, and 4 mM of each dNTP at 42 °C for 15 min, then at 99 °C for 5 min and 5 °C for 5 min.

Primers for PCR amplifications were designed according to the published partial sequence of the rat FGFR1 cDNA (16) and rat G3PDH (17, 18). Primers used for the amplification of FGFR1 and G3PDH are listed below.

(a) FGFR1 (forward) 5′-TGCCGTATGTCCAGATCC-3′ (size = 278 bp), (reverse) 5′-CTTGTAGATGATGACGGAGC-3′
(b) G3PDH (forward) 5′-ACCACAGTCCATGCCATCAC-3′ (size = 452 bp), (reverse) 5′-TCCACCACCCTGTTGCTGTA-3′.

One twentieth of the RT reaction mixture was used in each PCR reaction in a PCR buffer containing 0.2 mM of each set of primers, 0.5 units of Taq polymerase (Perkin-Elmer Cetus Corp., Norwalk, CT), 10 mM Tris (pH 8.3), 2 mM $MgCl_2$, and 50 mM KCl. The PCR mixtures were incubated in a thermal cycler (GeneAmp PCR System 2400, Perkin-Elmer Cetus Corp., Norwalk, CT) for 29 cycles (in the case of FGFR1 amplification) or 28 cycles (in the case of G3PDH) using the following profile: an initial denaturation step at 94 °C for 7 min, then repeated cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 90 s. The samples were finally incubated at 72 °C for 7 min, then stored at 4 °C. Eight μl of the PCR samples was run on 5.0% acrylamide gels stained with 0.2 μg/ml ethidium bromide, and the PCR products were visualized with a uv transilluminator and then photographed using Polaroid films (667, ISO3000, Hertfordshire, U.K.). The photographic images of the gel staining were scanned with a color image scanner (GT-6000, SEIKO-EPSON, Nagano, JAPAN). The band intensities were quantitated by image analyzing computer software, NIH Image. The relative intensity value, the intensity of the FGFR1 band divided by that of the G3PDH band, was calculated for the standardization of FGFR1 mRNA expression.

Western blot analysis for FGFR. Cell lysates were prepared from subconfluent palatal fibroblasts and scar fibroblasts, using RIPA buffer (19) containing 0.12 TIU/ml aprotinin, 50 μg/ml leupeptin, 1 mM sodium orthovanadate, and 1 mM phenylmethylsulfonylfluoride. Cell lysates were electrophoresed in 7.5% SDS-polyacrylamide gels and transblotted onto PVDF membranes (Immobilon-P, Millipore Co., Bedford, MA). The membranes were blocked with 5% BSA and incubated with anti-FGFR monoclonal antibodies diluted at 1:1000, followed by incubation with goat anti-mouse Ig (G+A+M) conjugated with horseradish peroxidase (Cappel Research Products., Durham, NC.) diluted at 1:2000. The membranes were washed and developed by the ECL system (Amersham International plc., Buckinghamshire, England).

Detection of tyrosine autophosphorylation of FGFR. Cells were serum-starved for 36 h and then treated with 100 ng/ml of bFGF for 5 min in the presence of 10% FBS. Cell lysates were prepared, incubated with monoclonal antibodies to FGFR (VBS-7) for 2 h at 4 °C and with agarose-bound protein G (Immuno Pure Plus Immobilized Protein G; Pierce, Rockford, IL) for 2 h at 4 °C, then centrifuged at 13,000 g for 3 min at 4 °C. The immunoprecipitates were subjected to 7.5% SDS-PAGE and immunoblotted with anti-phosphotyrosine specific monoclonal antibodies. Tyrosine autophosphorylation of the FGFR was detected by the ECL system.

Statistical analysis. Apoptosis was expressed as the percentage of apoptotic cells. The data represented the mean of the apoptotic cell percentage counted in ten randomly selected fields in each triplicate experiment. Statistical analyses of significance were made by a one-way analysis of variance (ANOVA), using the Fisher's PLSD test.

RESULTS

Apoptosis induction in palatal and scar fibroblasts by bFGF in vitro. As the first attempt to examine the effects of growth factors on palatal and scar fibroblasts, we observed the cell detachment from the culture dish by employing phase-contrast microscopy (FIG. 1). Scar fibroblasts and TGF-β1-pretreated palatal fibroblasts, both of which were reported to display myofibroblastic features (2), showed a marked increase in the cell detachment on treatment with bFGF following serum starvation, while no significant cell detachment was observed in normal palatal fibroblasts by the same treatment (FIG. 1). To discriminate between apoptosis and necrosis, we examined the DNA fragmentation of growth factor-treated myofibroblasts by the TUNEL method. As shown in FIG. 1, a number of scar fibroblasts and TGF-β1-pretreated palatal fibroblasts underwent apoptosis. By contrast, apoptotic cells in palatal fibroblasts were extremely rare even after the bFGF treatment. Either cell detachment or apoptosis induction was hardly observed in these cells only by serum starvation or the succeeding FBS recovery without bFGF treatment. Neither did they occur only with bFGF treatment without serum starvation (data not shown). The analysis of the apoptotic cell percentage, as shown in FIG. 2, indicated that only 0.62±0.34% of palatal fibroblasts underwent apoptosis on treatment with bFGF after serum starvation, while approximately a 9-fold increase in the apoptotic cell percentage was observed in scar fibroblasts (5.49±0.85%) or TGF-β1-pretreated palatal fibroblasts (5.40±1.49%) with the same treatment, both of which were significantly higher than that in palatal fibroblasts ($p<0.05$). Neither tumor necrosis factor (TNF)-α nor epidermal growth factor (EGF) induced apoptosis in these cells (data not shown).

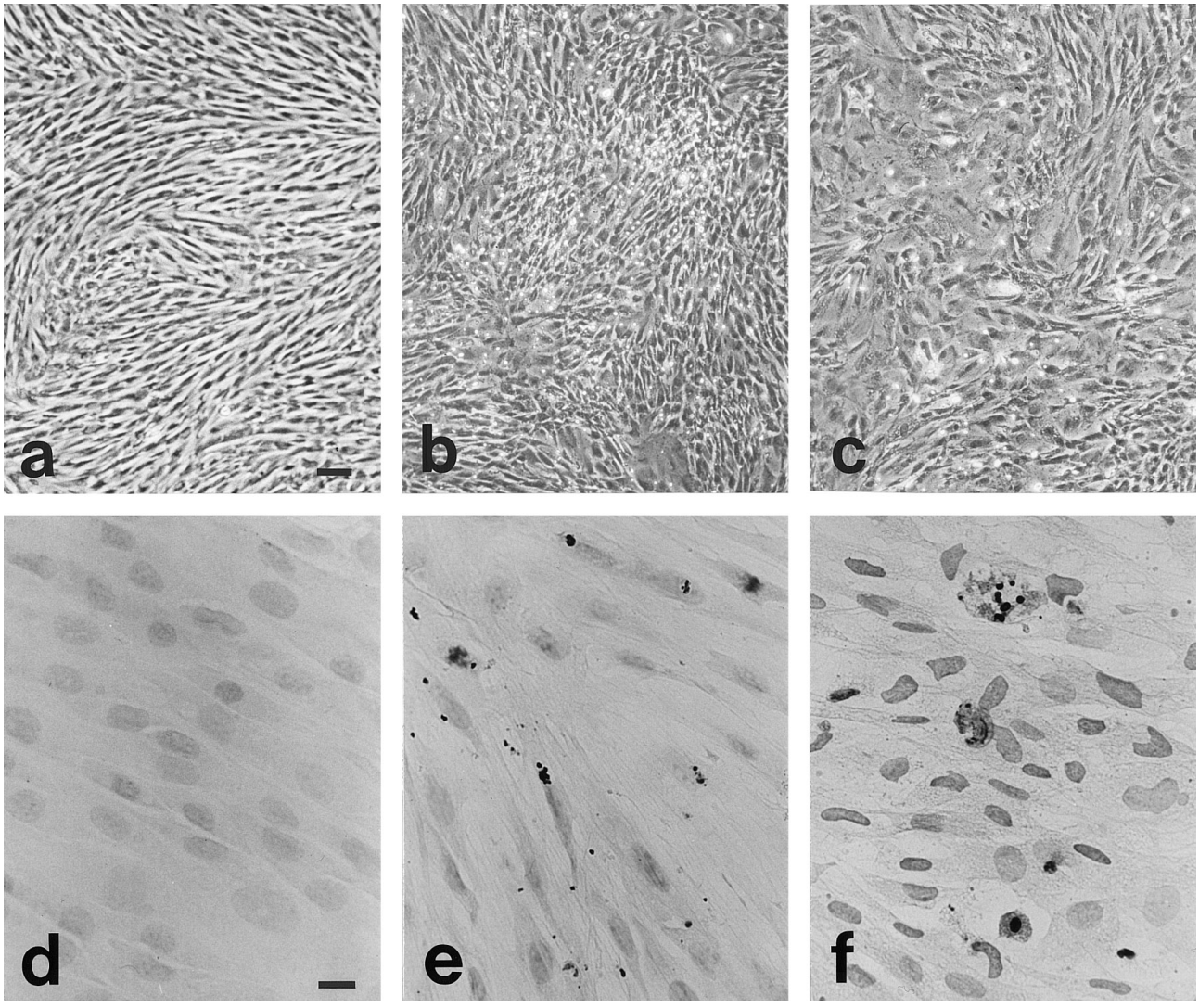

FIG. 1. Determination of apoptotic fibroblasts by the TUNEL method *in vitro.* Palatal fibroblasts (PF; a, d), scar fibroblasts (SF; b, e), and phenotypically modulated palatal fibroblasts into myofibroblasts by the treatment with TGF-β1 (TGF-β1 pretreated PF; c, f) were treated with bFGF after 36 h of serum starvation. Representative cell morphology was determined by phase-contrast microscopy (a, b, c). Apoptosis induction of cells was determined by the TUNEL method (d, e, f). Although apoptosis was very scarce in palatal fibroblasts treated with bFGF (a, d), a larger enhancement of apoptosis induction was also observed by bFGF in scar fibroblasts (b, e) and phenotypically modulated palatal fibroblasts into myofibroblasts by treatment with TGF-β1 (c, f). Bars: a = 100 μm; d = 15 μm.

Effect of TGF-β1 on FGFR1 mRNA expression in palatal and scar fibroblasts. According to the above finding that bFGF leads TGF-β1-pretreated palatal fibroblasts and scar fibroblasts to apoptosis, we next performed RT-PCR in order to see the effect of TGF-β1 on FGFR1 mRNA expression in palatal fibroblasts. Linear amplification was obtained within 25-35 PCR cycles for FGFR1 and within 25-30 PCR cycles for G3PDH cDNA (data not shown), when the relative intensity values were plotted against the cycle numbers. Therefore, 29 cycles for FGFR1 and 28 cycles for G3PDH were utilized for semi-quantitative amplification in this study. RT-PCR showed that FGFR1 mRNA expression was significantly stimulated in palatal fibroblasts by 48 h of TGF-β1 treatment (5 ng/ml) and that scar fibroblasts spontaneously expressed a higher level of FGFR1 mRNA compared with non-treated palatal fibroblasts (FIG. 3).

Effect of TGF-β1 on FGFR expression in palatal fibroblasts. We next investigated the effect of TGF-β1 on FGFR expression by Western blot analysis using monoclonal antibodies which recognize FGFR1 and FGFR3. The increase in FGFR expression was obvious in 12 h of the treatment with TGF-β1 (5 ng/ml), and became more evident time-dependently up to 24 h (FIG. 4A). An increase in FGFR expression was observed on treatment with 5 ng/ml of TGF-β1 for 48 h and became more evident dose-dependently up to 20 ng/ml (FIG.

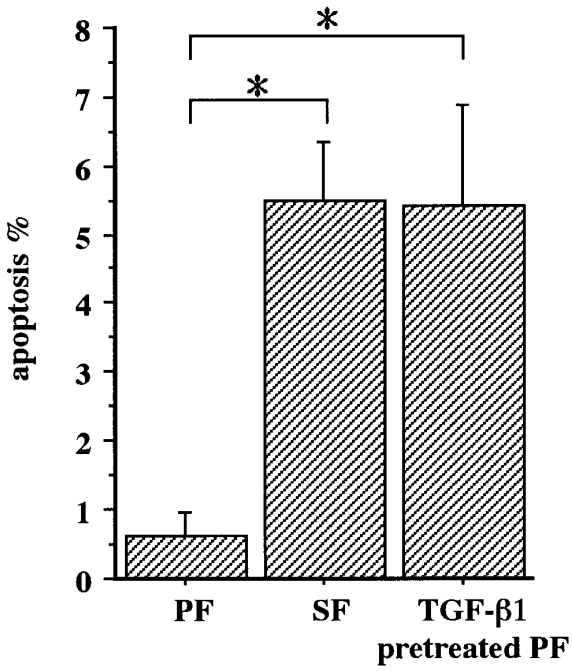

FIG. 2. Graphic representation of apoptotic cell percentage of palatal fibroblasts (PF), scar fibroblasts (SF), and phenotypically modulated palatal fibroblasts into myofibroblasts by treatment with TGF-β1 (TGF-β1 pretreated PF). The data represent the mean of the apoptotic cell percentage counted in ten randomly selected fields in each triplicate experiment. Statistical analyses of significance were made by a one-way analysis of variance (ANOVA), using Fisher's PLSD test. *: $p < 0.05$.

4B). Serum starvation of palatal fibroblasts for 36 h had no effect on the protein level of FGFR (FIG. 4C). This may imply that the absence of apoptosis induction in normal palatal fibroblasts by the treatment with bFGFR is not due to the reduction in FGFR level by serum starvation.

Tyrosine autophosphorylation of FGFR in palatal and scar fibroblasts. FGFR is a glycoprotein with intrinsic protein tyrosine kinase activity (20). After 5 min of bFGF (100 ng/ml) treatment following serum starvation, tyrosine autophosphorylation of FGFR in palatal and scar fibroblasts was recognized by Western blot with anti-phosphotyrosine monoclonal antibodies. Stimulation of tyrosine autophosphorylation of FGFR was more significant in scar fibroblasts compared with that of palatal fibroblasts (FIG. 5). Tyrosine autophosphorylation of FGFR without serum starvation prior to bFGF stimulation was also measured. The tyrosine phosphorylation level of FGFR in scar fibroblasts was higher than that of palatal fibroblasts; however, no significant increase in autophosphorylation of FGFR by bFGF treatment was recognized without serum starvation possibly due to the constitutively high autophosphorylation level due to the effect of serum (data not shown).

DISCUSSION

In the present study, we have demonstrated that bFGF leads to apoptosis of scar fibroblasts and TGF-β1-pretreated palatal fibroblasts *in vitro*. This indicates that bFGF, which is well-known to be mitogenic for fibroblasts, acts at the same time as a factor which is involved in the apoptosis induction in differentiated myofibroblastic cells under a growth-arrested condition. The condition that has been identified in this work as essential to the bFGF apoptosis signal is the imposition of G_0 cell cycle arrest. In this experiment, the quiescent cells maintained in low-serum D-MEM were found to be more sensitive to the cytocidal effect of bFGF compared to cells grown in serum-containing medium. Various fibroblasts respond to serum deprivation by reversible growth arrest in the G_0 phase (11, 12, 21, 22). This may indicate that the G_0 phase of the cell cycle was the susceptible phase to apoptosis (23, 24) or that growth factor-dependent apoptosis is concerned with inability to transit the G_1/S checkpoint (24).

We have also shown that TGF-β1 increased the protein and mRNA levels of bFGFR in rat palatal scar

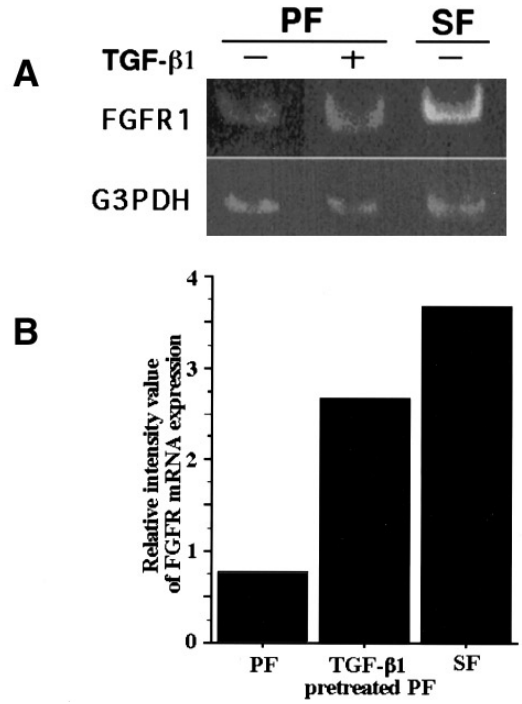

FIG. 3. RT-PCR of FGFR1 mRNA in palatal and scar fibroblasts. (A) RT-PCR showed that FGFR1 mRNA expression was significantly stimulated in palatal fibroblasts (PF) by 48 h of TGF-β1 treatment (5 ng/ml) and that scar fibroblasts (SF) simultaneously expressed a higher level of FGFR1 mRNA compared with non-treated PF. No significant difference was observed in control G3PDH gene expression among the three samples. (B) The FGFR1 mRNA level was calculated by dividing the intensity of the FGFR1 band (A, upper) by the intensity of the G3PDH band (A, lower) as determined by densitometric scanning.

fibroblasts. These data corroborate earlier findings that TGF-β1 increased the levels of bFGF and FGFR mRNAs in myofibroblastic liver cells (25) and induced the expression of tyrosine kinase-containing FGFR1 transcripts in endothelial cells (26). In support of the *in vitro* data, we have also observed that FGFR protein was localized in the α-SM actin-expressing myofibroblasts during palatal wound healing *in vivo* (data not shown).

In the present study, bFGF stimulated tyrosine autophosphorylation of FGFR significantly higher in scar fibroblasts than that in palatal fibroblasts. This may be because scar fibroblasts expressed higher FGFR than palatal fibroblasts. Other studies also indicated that TGF-β1 synergistically increased bFGF-induced osteoblast mitogenicity (27) and that incubation of cells with TGF-β prior to bFGF addition substantially increased the responsiveness of adult skin fibroblasts to the latter cytokine (28). Combined with these data, it is suggested that TGF-β1 regulates the expression of FGFR and possibly its activation.

In vivo, surgical wounds are characterized by a rapid and early angiogenic environment that is mediated in part by bFGF, suggesting that tissue or platelet stores of bFGF may initiate wound repair (29). bFGF treatment resulted in an acceleration of wound healing in almost the same dose range regardless of impairment causes or animal species used (30). For example, treatment with bFGF restored the inflammatory response in wound healing of diabetic mice (31). Moreover, bFGF decreases the ability of fibroblasts to contract collagen fibers (32). Preliminary results have shown that bFGF decreases the expression of α-SM actin in cultured fibroblasts (1). The

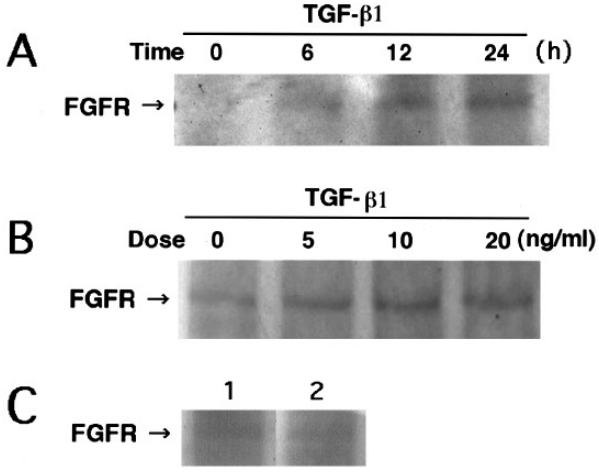

FIG. 4. Western blot analysis for FGFR in palatal fibroblasts (PF). FGFR in PF was significantly increased by TGF-β1 treatment time- (A) and dose- (B) dependently. No significant difference in the FGFR protein levels in PF before (lane 1; C) and after serum starvation (lane 2; C).

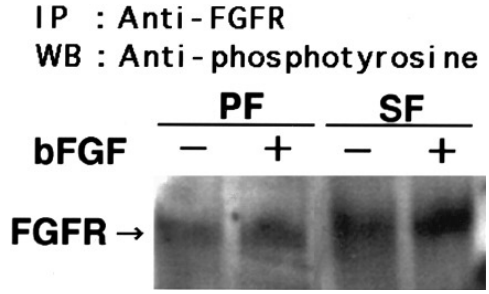

FIG. 5. Tyrosine autophosphorylation of FGFR upon stimulation by bFGF in palatal fibroblasts (PF) and scar fibroblasts (SF). After 5 min of bFGF (100 ng/ml) treatment following to serum starvation, tyrosine autophosphorylation of FGFR was analyzed by immunoprecipitation (IP) with monoclonal FGFR antibodies followed by Western blot (WB) with anti-phosphotyrosine monoclonal antibodies. Stimulation of tyrosine autophosphorylation of FGFR was more significant in SF as compared with that of PF.

beneficial effects of bFGF on wound healing were possibly due to its potent angiogenesis and granulation tissue formation activities, leading to a rapid reepithelialization of the wound. In addition, bFGF may promote the induction of apoptosis in myofibroblasts during wound healing. The properties of bFGF associated with its ability to inhibit fibroblasts contraction and induce apoptosis in myofibroblasts may be of interest in the management of wound healing.

In conclusion, we showed that 1) bFGF induced apoptosis of scar fibroblasts and TGF-β1-pretreated myofibroblastic palatal fibroblasts in culture. 2) TGF-β1 elevated both the protein level and the mRNA expression level of FGFR1 in palatal fibroblasts. 3) Tyrosine phosphorylation of bFGFR upon stimulation by bFGF was significantly higher in scar fibroblasts compared with palatal fibroblasts.

These findings have suggested that bFGF is a possible inducer of apoptosis in myofibroblasts during palatal scar formation and that FGFR may be responsible for apoptosis induction.

ACKNOWLEDGMENTS

We are indebted to Dr. Yoshiyuki Baba for his helpful comments throughout these studies. This study was supported by Grants-in Aid for Scientific Research from the Japanese Ministry of Education Nos. 08877308, 08771942, and 09672094 and by the "Research for the Future" Program from the Japan Society for Promotion of Science, JSPS-RFTF 96I00205.

REFERENCES

1. Desmoulière, A., and Gabbiani, G. (1996) The Molecular and Cellular Biology of Wound Repair (Clark, R. A. F., Ed.), pp. 403, Plenum Press, New York, NY.
2. Yokozeki, M., Moriyama, K., Shimokawa, H., and Kuroda, T. (1997) *Exp. Cell Res.* **231,** 328–336.
3. Desmoulière, A., Redard, M., Darby, I., and Gabbiani, G. (1995) *Am. J. Pathol.* **146,** 56–66.

4. Chen, M. W., Levin, R. M., and Buttyan R. (1995) *World J. Urol.* **13,** 344–348.
5. Abraham, J. A., and Klagsbrun, M. (1996) The Molecular and Cellular Biology of Wound Repair (Clark, R. A. F., Ed.), pp. 195–248, Plenum Press, New York, NY.
6. Estes, J. M., Vande, B. J., Adzick, N. S., MacGillivray, T. E., Desmoulière, A., and Gabbiani, G. (1994) *Differentiation* **56,** 173–181.
7. Whitby, D. J., and Ferguson, M. W. (1991) *Dev. Biol.* **147,** 207–215.
8. Yokoyama, Y., Ozawa, S., Seyama, Y., Namiki, H., Hayashi, Y., Kaji, K., Shirama, K., Shioda, M., and Kano, K. (1997) *J. Neurochem.* **68,** 2212–2215.
9. Muir, D. A., and Compston D. A. (1996) *J. Neurosci. Res.* **44,** 1–11.
10. Oberhammer, F., Bursch, W., Parzefall, W., Breit, P., Erber, E., Stadler, M., and Schulte H. R. (1991) *Cancer Res.* **51,** 2478–2485.
11. Evan, G. I., Wyllie, A. H., Gilbert, C. S., Littlewood, T. D., Land, H., Brooks, M., Waters, C. M., Penn, L. Z., and Hancock D. C. (1992) *Cell* **69,** 119–128.
12. Olashaw, N. E., Van, W. J., and Pledger, W. J. (1987) *Am. J. Physiol.* **253,** C575–C579.
13. Gavrieli, Y., Sherman, Y., and Ben, S. S. (1992) *J. Cell Biol.* **119,** 493–501.
14. Gilliland, G., Perrin, S., Blanchard, K., and Bunn H. F. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 2725–2729.
15. Becker, A. M., and Hahlbrock K. (1989) *Nucleic Acids Res.* **17,** 9437–9446.
16. Wanaka, A., Johnson, E. J., and Milbrandt, J. (1990) *Neuron* **5,** 267–281.
17. Nasrin, N., Ercolani, L., Denaro, M., Kong, X. F., Kang, I., and Alexander M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5273–5277.
18. Arcari, P., Martinelli, R., and Salvatore, F. (1984) *Nucleic Acids Res.* **12,** 9179–9189.
19. Yoneda, T., Niewolna, M., Lowe, C., Izbicka, E., and Mundy G. R. (1993) *Mol. Endocrinol.* **7,** 1313–1318.
20. Jaye, M., Schlessinger, J., and Dionne, C. A. (1992) *Biochim. Biophys. Acta* **1135,** 185–199.
21. Lau, L. F., and Nathans D. (1985) *EMBO J.* **4,** 3145–3151.
22. Aaronson, S. A. (1991) *Science* **254,** 1146–1153.
23. Luciano, L., Hass, R., Busche, R., von Engelhardt, W., and Reale, E. (1996) *Cell Tissue Res.* **286,** 81–92.
24. Kim, H. R., Upadhyay, S., Li, G., Palmer, K. C., and Deuel, T. F. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 9500–9504.
25. Rosenbaum, J., Blazejewski, S., Preaux, A. M., Mallat, A., Dhumeaux, D., and Mavier P. (1995) *Gastroenterology* **109,** 1986–1996.
26. Zhao, X. M., Frist, W. H., Yeoh, T. K., and Miller, G. G. (1994) *J. Clin. Invest.* **94,** 992–1003.
27. Kasperk, C. H., Wergedal, J. E., Mohan, S., Long, D. L., Lau, K. H., and Baylink D. J. (1990) *Growth Factors* **3,** 147–158.
28. Kikuchi, K., Yamakage, A., Smith, E. A., LeRoy, E. C., and Trojanowska, M. (1992) *J. Invest. Dermatol.* **99,** 201–205.
29. Nissen, N. N., Polverini, P. J., Gamelli, R. L., and DiPietro, L. A. (1996) *Surgery* **119,** 457–465.
30. Okumura, M., Okuda, T., Nakamura, T., and Yajima, M. (1996) *Arzneimittel Forschung* **46,** 547–551.
31. Tanaka, E., Ase, K., Okuda, T., Okumura, M., and Nogimori, K. (1996) *Biol. Pharm. Bull.* **19,** 1141–1148.
32. Imaizumi, T., Jean, L. F., Dubertret, M. L., Bailly, C., Cicurel, L., Petchot, B. J., and Dubertret, L. (1996) *J. Dermatol. Sci.* **11,** 134–141.