TGF-β1 Enhances the Expression of α–Smooth Muscle Actin in Cultured Human Pulpal Fibroblasts: Immunochemical and Ultrastructural Analyses

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Abstract

Transforming growth factor–beta 1 (TGF-β1) has been related to induce the expression of α–smooth muscle actin (α-SMA) in fibroblasts during repair. Because pulpal fibroblasts seem to be somewhat different from other fibroblasts, the present study investigated in vitro whether TGF-β1 enhances the expression of α-SMA in human pulpal fibroblasts. TGF-β1 was added in doses between 5–10 ng/mL to cultures of both dental pulp and gingival human fibroblasts. The expression of α-SMA was analyzed by immunofluorescence and Western blotting, whereas the ultrastructure was evaluated by electron microscopy. In addition, the expression of tenascin, osteonectin, and vimentin was also investigated. Both cell types were immunoreactive for α-SMA even without TGF-β1. When TGF-β1 was added to cell cultures, the expression of α-SMA increased dramatically in pulpal fibroblasts, independent of the concentration used. It was confirmed by the Western blotting analysis. Ultrastructure revealed myofilaments and indented nuclei in both fibroblasts treated with TGF-β1. Tenascin and osteonectin were only immunolabeled in pulpal fibroblasts treated or not with TGF-β1. Both fibroblast types were positive for vimentin. The present findings showed that TGF-β1 up-regulated the expression of α-SMA, thus inducing pulpal fibroblasts to acquire the myofibroblast phenotype. *(J Endod 2007;33:1313–1318)*

Key Words

α-SMA, human dental pulp, myofibroblast phenotype, pulpal fibroblasts, TGF-β1

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The dental pulp is the soft connective tissue surrounded by dentin that fills the center of the root canals of teeth. It consists of 3 distinct zones: the peripheral odontoblast layer, the subodontoblastic layer formed by the cell-free zone and the cell-rich zone, and the pulp core. The pulp core is a typical loose connective tissue in which fibroblasts are the most abundant cells, besides undifferentiated ectomesenchymal cells, macrophages, lymphocytes, and other immunocompetent cells (1). The pulp core extracellular matrix is composed of a number of macromolecules, as collagen, other proteins, and glycoconjugates that seem to play a role in gene expression (2). Pulpal fibroblasts exhibit some particularities in relation to fibroblasts present in other connective tissues (1) such as the expression of tenascin and osteonectin, 2 mineralized tissue-related extracellular matrix proteins (3, 4). Some cytokines and growth factors modulate cell proliferation and differentiation and/or matrix synthesis in dental pulp (5). In conjunction with the extracellular matrix, the transforming growth factors (TGFs) expressed in ectomesenchymal cells, odontoblasts, and enamel organ cells are involved in proliferation of ectomesenchymal cells and dentin formation (6).

Specialized cells known as myofibroblasts have been related to play a role in formation of granulation tissue and scar shrinking in connective tissues. Myofibroblasts possess intermediary morphologic and biochemical characteristics between fibroblasts and smooth muscle cells (7, 8). The expression of the isoform α–smooth muscle actin (α-SMA), a cytoskeletal protein that is the major constituent of the contractile system of smooth muscle cells, is considered as the myofibroblastic phenotype marker (9, 10). TGF-β is probably the most important growth factor for induction of α-SMA in fibroblasts (11), mainly during tissue repair and regeneration after injury (12).

The dental pulp is a potential source of stem cells in adults (13, 14). Most in vitro studies on dental pulp cells have focused on their capability to form mineralized matrix by adding to the culture medium some mineralizing factors such as ascorbic acid, β-glycerolphosphate (15, 16), and dexamethasone (17, 18), in some occasions in conjunction with TGF-β (19, 20). In addition, cultured dental pulp cells might express small amounts of α-SMA, which have been related to their possible origin from the undifferentiated cells known as pericytes (13, 18, 21). Because dental pulp cells possess multiple type I and type II receptors for the TGF-β superfamily (22), the possible influence of these growth factors on pulpal fibroblasts needs to be investigated.

The purpose of this in vitro study was to test the hypothesis that TGF-β1 might enhance the expression of α-SMA in human pulpal fibroblasts, ie, in dental pulp cells cultured in the absence of mineralizing factors. The expression of this cytoskeletal protein was analyzed by immunofluorescence and Western blotting. The ultrastructural aspects of human pulpal fibroblasts in comparison with those of human gingival fibroblasts were also examined. In addition, the expression of 2 mineralized tissue-related extracellular matrix proteins (tenascin and osteonectin) in both fibroblast types was evaluated to observe their commitment to mineralization before and after TGF-β1 stimulation.

Materials and Methods

Cell Cultures

Human dental pulp cells were obtained from the pulp of impacted third molar teeth that were extracted for orthodontic reasons and gingival cells from explants of healthy attached human gingiva provided by periodontal surgery conducted for crown

lengthening, each one from 3 different donors. This study was conducted following the approval of the Ethical Committee of the University of São Paulo, Brazil (Protocol # 728/06).

The obtained cells were cultured in Dulbecco modified Eagle medium (DMEM) (Sigma, St Louis, MO) supplemented with 1% antimycotic-antibiotic solution (10,000 units of penicillin, 10 mg of streptomycin, and 25 μg of amphotericin B per mL in 0.9% sodium chloride; Sigma), containing 10% of donor calf serum (DCS; GIBCO, Buffalo, NY), plated in 60-mm diameter plastic culture dishes and incubated under standard cell culture conditions (37°C, 100% humidity, 95% air, and 5% CO_2). The cells were treated with TGF-β1 (Sigma #T7039-2 μg) in doses between 5–10 ng/mL (23). When the cell growth from pulp and gingival tissue had reached confluence, the cells were detached with 0.05% trypsin and subcultured at a density of 20,000 cells/well ($\sim$110 cells/mm^2). The cells were used at subculture levels 3 or 4. Medium was removed 24 hours after plating, and cells were incubated for 7 days, in the same conditions, with TGF-β1.

Immunofluorescence

Cells grown on coverslips were fixed in methanol for 6 minutes at 20°C, rinsed in phosphate-buffered saline (PBS), followed by blocking with 1% bovine albumin in PBS for 30 minutes at room temperature. The primary monoclonal and/or polyclonal antibodies are described in Table 1. All cell lines were stained with an anti-vimentin antibody to identify their mesenchymal origin. Control staining reaction was performed by using PBS as non-immune immunoglobulin Gs (IgGs) at the same dilution used for the primary antibody.

The secondary antibodies used were biotinylated anti-mouse IgG (Vector Laboratories Inc, Burlingame, CA) to cells incubated with tenascin, vimentin, and α-SMA, and biotinylated anti-rabbit IgG (Vector) for cells incubated with osteonectin. Fluorescein-streptavidin conjugated (Vector) was used for the second step.

After washing, preparations were mounted by using Vectashield DAPI-associated (4′-6-diamidino-2-phenylindole) (Vector) and observed on a Zeiss Axiophot 2 conventional fluorescence microscope (Carl Zeiss, Oberkechen, FRG) equipped with 63× Plan Apochromatic 1.4NA and 100× Plan Apochromatic 1.4NA objectives in standard conditions (Carl Zeiss).

Western Blotting

Lysates were prepared by homogenization at 4°C and centrifuged at 15,000*g* for 15 minutes at 4°C. The protein concentration was measured by BCA assay (Pierce, Rockford, IL). Protein lysates were separated on 10% sodium dodecylsulfate–polyacrylamide gels, electrotransferred onto polyvinylidene difluoride membranes (Hybond; Amersham Biosciences, Piscataway, NJ), and probed with anti–α-SMA (1:500; Dako, Glostrup, Denmark) and β-actin (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA) as primary antibodies for 1 hour. After incubation with a mouse-monoclonal secondary antibody, the reaction was revealed with Bio-Rad Laboratories (Hercules, CA) Western blotting chemiluminescent detection reagents (Opti-4CN). Optical density measurements were made with NIH Image 1.37 (National Institutes of Health, Bethesda, MD) for scanned membranes.

TABLE 1. Primary Antibodies

Antibody	Host	Dilution	Sources
Anti-tenascin, human	Mouse	1:100	Chemicon
Anti-osteonectin, human (polyclonal)-LF37	Rabbit	1:100	L. W. Fisher (24)
Anti–α-SMA	Mouse	1:50	Dako
Anti-vimentin (clone V9)	Mouse	1:300	Dako

α-SMA, α-smooth muscle actin.

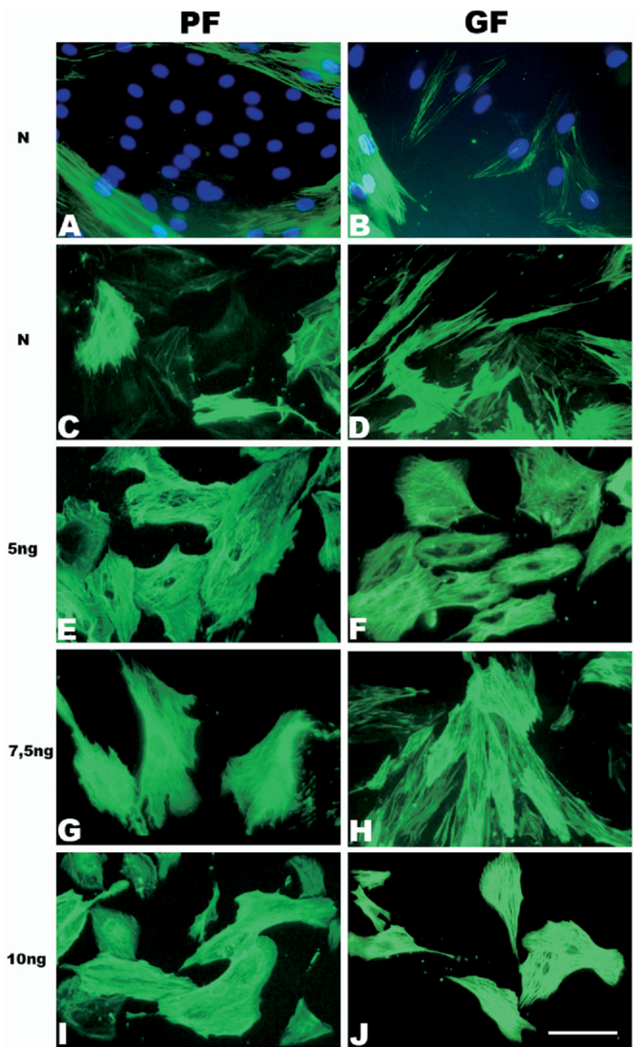

Figure 1. Immunostaining for α-SMA on pulpal (PF) and gingival (GF) fibroblasts. *(A–D)* Numerous untreated cells (N) could be identified through their nuclei labeled with DAPI (in blue), without cytoplasmic immunoreactivity for α-SMA, especially pulpal fibroblasts *(A, B)*. After treatment with 5–10 ng/mL TGF-β1, both cell lineages exhibited strong immunoreactivity for α-SMA *(E-J)*. Bar, 10 μm.

Transmission Electron Microscopy

After confluence, cell lines were fixed in 0.1% glutaraldehyde and 4% formaldehyde (freshly prepared from paraformaldehyde) in 0.1 mol/L sodium cacodylate buffer (pH, 7.4) for 2 hours at room temperature. They were rinsed in the same buffer and post-fixed in 1% osmium tetroxide for 1 hour, dehydrated in a graded series of ethanol and acetone, and embedded in Spurr resin. Semithin sections were cut in a Micron HM360 microtome with glass knives made in a knife maker LKB 7800-B and stained with 0.25% toluidine blue. Representative areas were selected for 80-nm-thick ultrathin sections by using a Leica (Leica Instruments GmbH, Nussloch, Germany) Ultracut R ultramicrotome with diamond knife and examined with a JEM 1010 electron microscope (Jeol USA Inc, Peabody, MA) operating at 80 kV.

Results

Pulpal and gingival fibroblasts were immunoreactive for α-SMA even in the absence of TGF-β1. However, numerous pulpal fibroblasts could be identified through their nuclei labeled with DAPI, without

cytoplasmic immunoreactivity for α-SMA. In contrast, only a few gingival fibroblasts appeared free of intracytoplasmic immunolabeling (Fig. 1*A, B*). In general, approximately 75% of gingival fibroblasts were positive for the α-SMA antibody, whereas around 50% of pulpal fibroblasts showed that labeling. When both untreated pulpal and gingival fibroblasts were positive for α-SMA, the immunolabeling exhibited a filamentous appearance (Fig. 1*C, D*).

In cell cultures treated with 5 ng/mL TGF-β1, the immunoreactivity for α-SMA increased in pulpal fibroblasts when they were compared with gingival fibroblasts labeled with this antibody (Fig. 1*E, F*). The treatment of cell cultures with 7.5 and 10 ng/mL TGF-β1 did not show any alteration in the aspect observed with 5 ng/mL TGF-β1 (Fig. 1*G–J*).

Ultrastructural examination of cell cultures without TGF-β1 treatment revealed typical fibroblasts containing well-developed rough endoplasmic reticulum and Golgi apparatus (Figs. 2*A* and 3*A*), some of them exhibiting a peripheral layer of myofilaments. These cells also displayed a notched (indented) nucleus with granular chromatin and nucleoli typical of myofibroblastic phenotype (Figs. 2*B* and 3*B*). The presence of myofilaments (Figs. 2*C–F* and 3*C–F*) and indented nuclei (Figs. 2*E* and 3*D, F*) were more frequent findings in both cell types when concentrations of 5–10 ng/mL TGF-β1 were added to cultures than in untreated cells.

The Western blotting data confirmed the significant increase in the α-SMA expression in pulpal fibroblasts when TGF-β1 was added to cell cultures, independently of the concentration used. In relation to gingival fibroblasts, the effect of TGF-β1 was not discerned, because even the cells without treatment showed high amounts of α-SMA. Although the distinct intensity of immunoreactivity between bands was evident, the quantitative evaluation carried out by means of the densitometric analysis confirmed the increased expression of α-SMA in the stimulated pulpal fibroblasts (Fig. 4).

Tenascin and osteonectin were immunoexpressed by pulpal cells independent of TGF-β1 stimulation. Both proteins appeared as a diffuse reticular network throughout the cytoplasm of pulpal fibroblasts (Fig. 5*A–D*). In contrast, gingival fibroblasts showed no immunoreactivity for tenascin and osteonectin, even when TGF-β1 was added to cultures (data not shown).

When cell cultures were incubated with the anti-vimentin antibody, a strong immunoreactivity was detected, independent of the TGF-β1 treatment. Vimentin was present as a reticular network into the cytoplasm of both pulpal and gingival fibroblasts (Fig. 6).

Discussion

The present findings showed that TGF-β1 enhances the expression of α-SMA in human pulpal fibroblasts in vitro, as assessed by immunologic and morphologic approaches. Although α-SMA is an actin isoform abundant in smooth muscle cells, it is also present in connective cells such as those of the fibroblastic type, especially in the fibroblast variant known as myofibroblasts. Pulpal fibroblasts are believed to be a con-

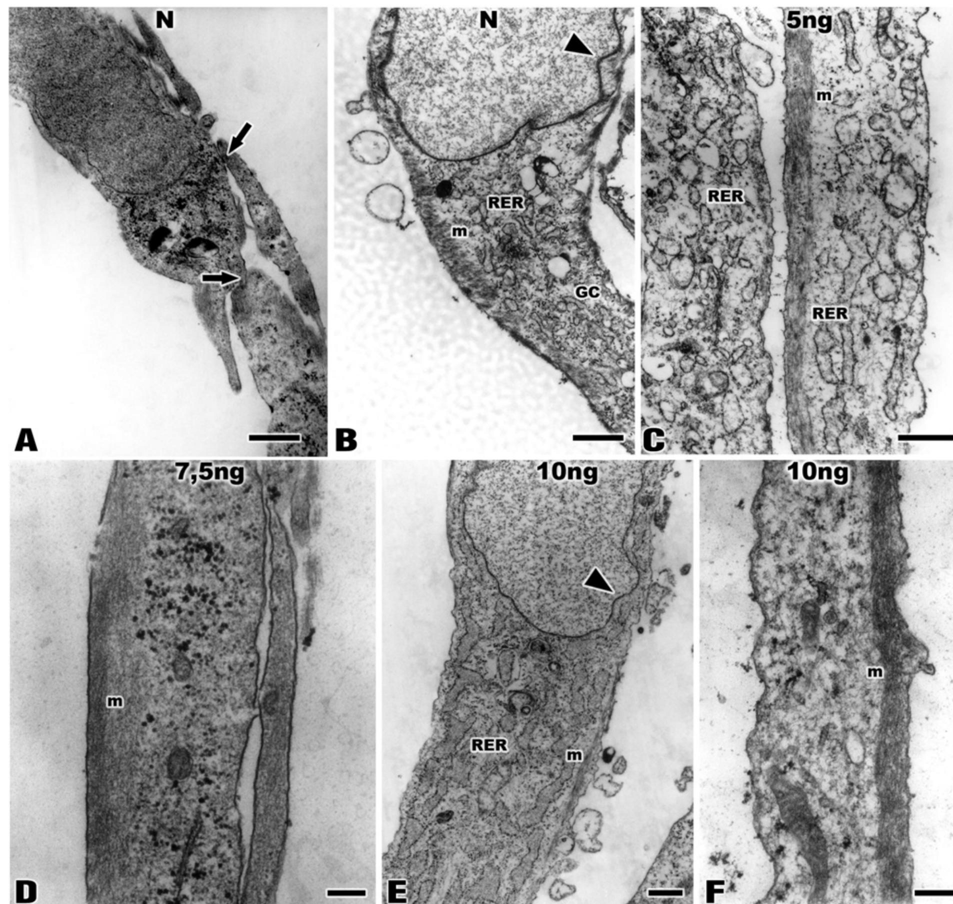

Figure 2. Transmission electron micrographs showing pulpal fibroblasts untreated (N) and treated with 5–10 ng/ml TGF-β1. *(A)* Portions of pulpal fibroblasts with some areas of intercellular contact (arrows). *(B)* A cell exhibiting its nucleus indented (arrowheads), well-developed rough endoplasmic reticulum (RER), and Golgi apparatus (GC), as well as peripheral myofilaments (m). *(C–F)* The peripheral myofilaments (m) are evident. Bars, 0.25 μm *(D)*; 0.5 μm *(F)*; 1 μm *(A, E)*; 2 μm *(B, C)*.

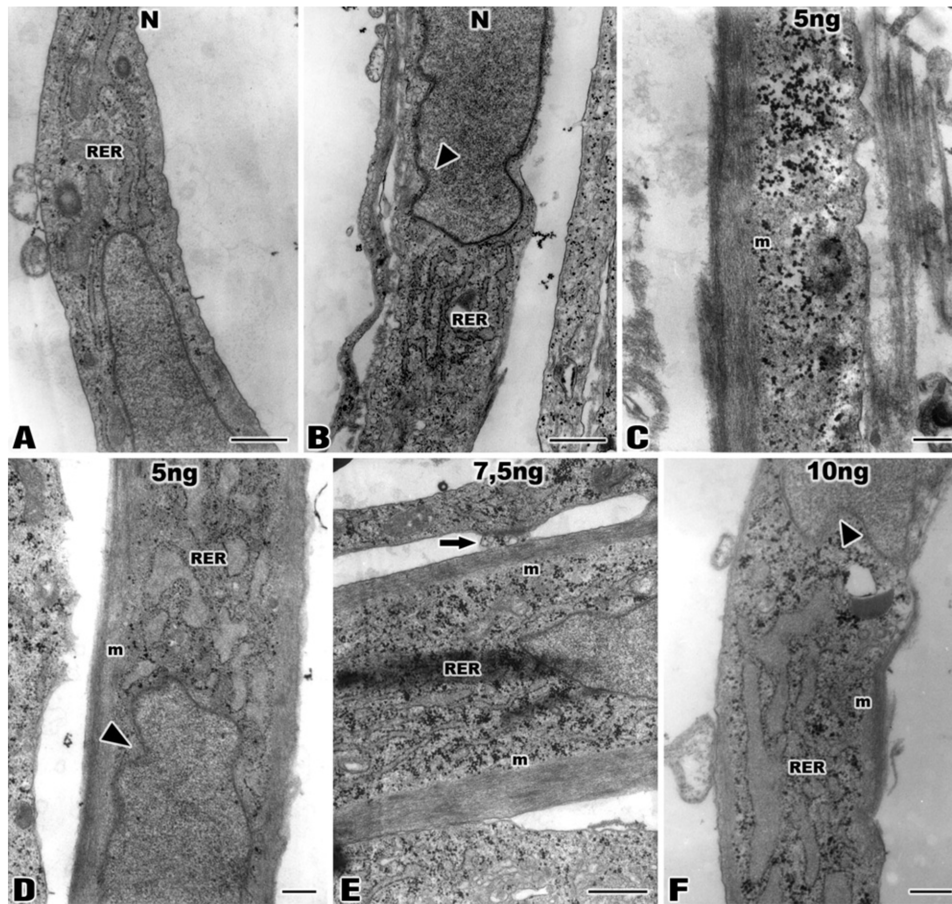

Figure 3. Transmission electron micrographs showing gingival fibroblasts untreated (N) and treated with 5–10 ng/mL TGF-β1. *(A)* A gingival fibroblast with well-developed rough endoplasmic reticulum (RER). *(B)* Portions of gingival fibroblasts; one of them exhibits an indented nucleus (arrowhead). *(C–F)* The peripheral myofilaments (m) are evident. An area of intercellular contact (arrow) is clearly seen in *(E)*. Bars, 0.5 μm *(C, D, F)*; 1 μm *(A, B, E)*.

spicuous fibroblast type that also expresses α-SMA in small amounts. The present findings suggest that human pulpal fibroblasts could acquire in vitro the myofibroblastic phenotype under the influence of TGF-β1.

Although fibroblasts are the most predominant cell type in dental pulp, undifferentiated ectomesenchymal cells are also present, especially in the subodontoblastic cell-rich zone (2, 25). In addition, some authors have recently claimed that dental pulp is one of the adult body sources for obtaining stem cells (13, 14). Undifferentiated dental pulp cells have been shown to differentiate in vitro into odontoblast-like cells with the addition of some mineralizing factors such as ascorbic acid, β-glycerolphosphate (15, 16), and dexamethasone (17, 18). In the present study, these factors were not added to the cultures, with the purpose to have a homogeneous fibroblastic population that did not form mineral deposits and appeared spindle-shaped, different from the columnar shape exhibited by odontoblast-like cells (16). In addition, the ultrastructural examination of pulpal fibroblasts revealed relatively few synthesis and secretion organelles and no discernible differences from gingival fibroblasts.

A large number of studies have focused on the major effect of TGF-β1 as an inductor of odontoblast differentiation (19, 20); however, the present findings constitute an additional effect of this growth factor. Indeed, some pulp cells have been observed to differentiate into odontoblasts in the presence of TGF-β1 during reparative dentinogenesis (26). However, these studies added mineralizing agents, which are necessary for in vitro odontoblast differentiation (27). No mineral nodules have been detected in our cultured dental pulp cells, indicating that dental pulp cells cultured without mineralizing agents remain as fibroblasts, despite the presence of TGF-β1. This is coincident with a recent study in which dental pulp cells stimulated with TGF-β1 in a medium devoid of mineralizing factors did not express dentin matrix proteins (28).

TGF-β1 up-regulated the expression of α-SMA in pulpal fibroblasts. They clearly expressed less α-SMA than gingival fibroblasts in control cultures. Expression of this smooth muscle protein in pulpal fibroblasts was initially weak but increased dramatically when TGF-β1 was added to cultures, independent of the concentration used. It indicates that the presence of TGF-β1 in small amounts is sufficient to trigger the increase of α-SMA expression in pulpal fibroblasts. This was also noted in fibroblasts obtained from human gingiva (29, 30), breast skin (23), as well as in adult stem cells (31). It is possible that concentrations of TGF-β1 lower than those used herein could show a dose-dependent effect on the expression of α-SMA in the studied cells, as shown by Sobral et al (32) in gingival fibroblasts.

The immunofluorescence assay confirmed the reactivity of both types of fibroblasts to α-SMA. In addition, the ultrastructural analysis showed that they contained peripheral myofilaments that appeared to increase in the presence of TGF-β1. They were, however, less evident than the findings obtained by Western blotting suggesting that the increase of α-SMA would reach a threshold from which morphologic differences can no longer be observed. Nevertheless, the ultrastructural examination showed myofilaments that are known as stress fibers with

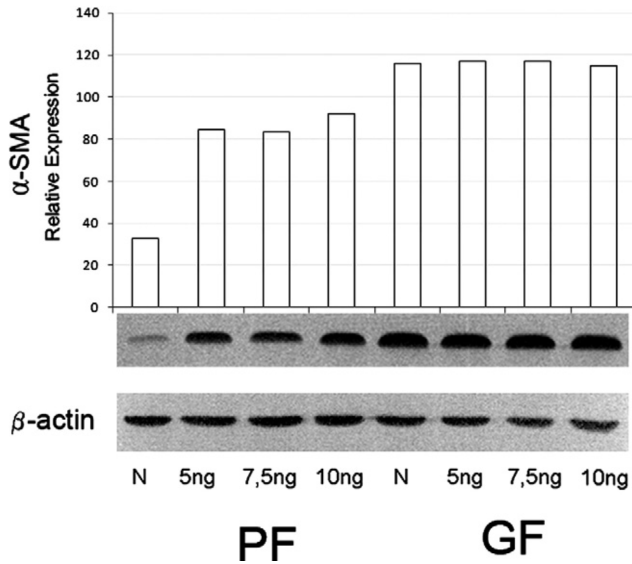

Figure 4. Western blotting analysis of α-SMA in pulpal (PF) and gingival fibroblasts (GF) untreated (N) or treated with 5–10 ng/mL TGF-β1. Note that expression of α-SMA was clearly weak in untreated pulpal fibroblasts. Human β-actin was used as a control to confirm similar loading in each sample.

dense bodies running parallel to the long axis through the fibroblast cytoplasm that render morphologic features common to the myofibroblast fibronexus (21, 33). Moreover, such cells also displayed an indented nucleus with granular chromatin typical of myofibroblastic phenotype, besides occasional intercellular contacts between them. They also exhibited a well-developed rough endoplasmic reticulum and Golgi apparatus, suggesting their active involvement in synthetic activities (33).

Pulpal fibroblasts expressed tenascin and osteonectin independent of having added TGF-β1 to the medium, whereas gingival fibroblasts did not. Expression of both proteins by pulpal fibroblasts was expected because they appear to be associated with hard tissue formation. Because TGF-β1 did not induce gingival fibroblasts to express these proteins, our findings confirm the hypothesis that pulpal and gingival fibroblasts are cells with 2 different commitments and that growth factors such as TGF-β1 do not modify their phenotype. Indeed,

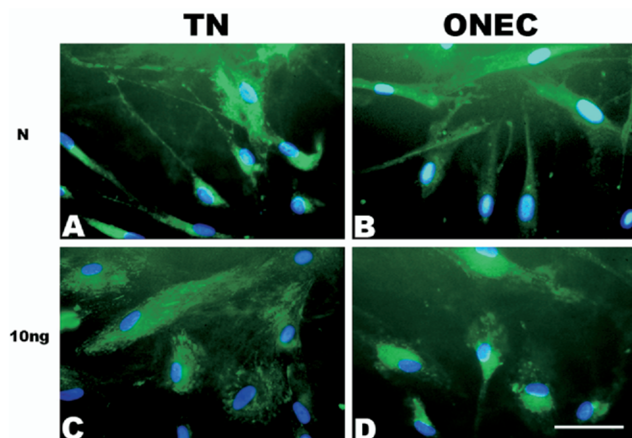

Figure 5. Immunostaining for tenascin (TN) and osteonectin (ONEC) on pulpal fibroblasts. Observe that all untreated (N) or treated with 10 ng/mL TGF-β1 cells (10 ng) are immunoreactive for both antibodies. Nuclei stained with DAPI appear in blue. Bar, 10 μm.

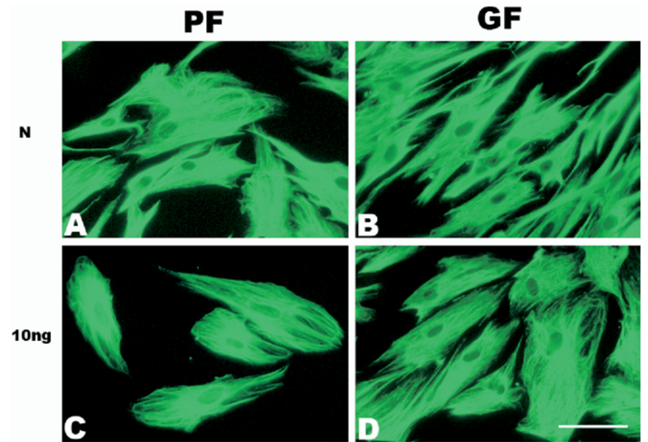

Figure 6. Immunostaining for vimentin on pulpal (PF) and gingival (GF) fibroblasts. Observe that all untreated (N) or treated with 10 ng/mL TGF-β1 cells (10 ng) are immunoreactive for this antibody. Bar, *10* μm.

this was never seen in our cultures, despite it had been suggested that gingival fibroblasts cultured in the presence of TGF-β1 could express osteonectin (34). However, gingival fibroblasts were cultured in the study of Wrana et al (34) with α-minimum essential medium (α-MEM) that contains ascorbic acid, which is a mineralizing factor, in addition to sodium ascorbate. Thus, the fibroblast phenotype could be changed, whereas any mineralizing factor was avoided in our study to leave the cell phenotype unchanged. On the other hand, tenascin is overexpressed in several cells under the influence of growth factors such as TGF-β1 (35).

Cultures in the present study were free of other cell types such as epithelial cells, as it has been shown by immunoreaction of both pulpal and gingival cells against vimentin, a cytoskeletal protein that forms intermediate filaments in cells from mesenchymal origin such as fibroblasts (36). Cultured pulpal fibroblasts expressed vimentin also after the influence of TGF-β1, when they overexpressed α-SMA.

In summary, our findings showed that TGF-β1 induces in vitro pulpal fibroblasts to acquire a myofibroblast phenotype by the higher expression of α-SMA and by their morphologic appearance.

Acknowledgments

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References

1. Cohen S, Burns RC. Pathways of the pulp. St Louis: Mosby, 2002.
2. Nanci A. Dentin-pulp complex. In: Nanci A, ed. Ten Cate's oral histology. St Louis: Mosby, 2003:192–239.
3. Martinez EF, Souza SOM, Correa L, Araujo VC. Immunohistochemical localization of tenascin, fibronectin, and type III collagen in human dental pulp. J Endod 2000;26:708–11.
4. Martinez EF, Araujo VC. *In vitro* immunoexpression of extracellular matrix proteins of pulpal and gingival mucosa fibroblasts. Int Endod J 2004;37:749–55.
5. Shiba H, Fujita T, Doi N, et al. Differential effects of various growth factors and cytokines on the syntheses of DNA, type I collagen, laminin, fibronectin, osteonectin/secreted protein, acidic and rich in cysteine (SPARC), and alkaline phosphatase by human pulp cells in culture. J Cell Physiol 1998;174:194–205.
6. Jepsen S, Schiltz P, Strong DD, Scharla SH, Snead ML, Finkelman RD. Transforming growth factor-beta1 mRNA in neonatal ovine molars visualized by in situ hybridization: potential role for the stratum intermedium. Arch Oral Biol 1992;37:645–53.

7. Gabbiani G, Ryan GB, Majno G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia 1971;27:549–50.
8. Gabbiani G, Le Lous M, Bailey AJ, Bazin S, Delaunay A. Collagen and myofibroblasts of granulation tissue: a chemical, ultrastructural and immunologic study. Virchows Arch B Cell Pathol 1976;21:133–45.
9. Vaughan MB, Howard EW, Tomasek JJ. Transforming growth factor-beta 1 promotes the morphological and functional differentiation of the myofibroblast. Exp Cell Res 2000;25:180–9.
10. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast. one function, multiple origins. Am J Pathol 2007;170:1807–16.
11. Desmoulière A. Factors influencing myofibroblast differentiation during wound healing and fibrosis. Cell Biol Int 1995;19:471–6.
12. Chaponnier C, Gabbiani G. Pathological situations characterized by altered actin isoform expression. J Pathol 2004;204:386–95.
13. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. Proc Natl Acad Sci USA 2000;97:13625–30.
14. Miura M, Gronthos S, Zhao M, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA 2003;100:5807–12.
15. Kasugai S, Adachi M, Ogura H. Establishment and characterization of a clonal cell line (RPC-C2A) from dental pulp of the rat incisor. Arch Oral Biol 1988;33:887–91.
16. Couble ML, Farges JC, Bleicher F, Perrat-Mabillon B, Boudelle M, Magloire H. Odontoblasts differentiation of human dental pulp cells in explant cultures. Calcif Tissue Int 2000;66:129–38.
17. Kasugai S, Shibata S, Suzuki S, Susami T, Ogura H. Characterization of a system of mineralized-tissue formation by rat dental pulp cells in culture. Arch Oral Biol 1993;38:769–77.
18. Alliot-Lich B, Bluteau G, Magne D, et al. Dexamethasone stimulates differentiation of odontoblast-like cells in human dental pulp cultures. Cell Tissue Res 2005;321: 391–400.
19. Melin M, Joffre-Romeas A, Farges JC, Couble ML, Magloire H, Bleicher F. Effects of TGFβ1 on dental pulp cells in cultured human tooth slices. J Dent Res 2000;79: 1689–96.
20. Lucchini M, Romeas A, Couble ML, Bleicher F, Magloire H, Farges JC. TGF beta 1 signaling and stimulation of osteoadherin in human odontoblasts in vitro. Connect Tissue Res 2002;43:345–53.
21. Alliot-Licht B, Hurtrel D, Gregoire M. Characterization of α-smooth muscle actin positive cells in mineralized human dental pulp cultures. Arch Oral Biol 2001; 46:221–8.
22. Toyono T, Nakashima M, Akamine A. Expression of TGF-β superfamily receptors in dental pulp. J Dent Res 1997;76:155–60.
23. Desmoulière A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-β1 induces α-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol 1993;122:103–11.
24. Fisher LW, Stubbs JT, Young MF. Antisera and cDNA probes to human and certain animal model bone matrix noncollagenous proteins. Acta Orthop Scand 1995;266: 61–5.
25. Arana-Chavez VE, Massa LF. Odontoblasts: the cells forming and maintaining dentine. Int J Biochem Cell Biol 2004;36:1367–73.
26. Magloire H, Bouvier M, Joffre A. Odontoblast response under carious lesions. Proc Finn Dent Soc 1992;99:257–74.
27. Lopez-Cazaux S, Bluteau G, Magne D, Lieubeau B, Guicheux J, Alliot-Licht B. Culture medium modulates the behaviour of human dental pulp-derived cells: technical note. Eur Cell Mater 2006;11:35–42.
28. Luisi SB, Barbachan JJD, Chies JAB, Filho MS. Behavior of human dental pulp cells exposed to transforming growth factor-beta1 and acidic fibroblast growth factor in culture. J Endod 2007;33:833–5.
29. Premdas J, Tang J-B, Warner JP, Murray M, Spector M. The presence of smooth muscle actin in fibroblasts in the torn human rotator cuff. J Orthop Res 2001;19: 221–8.
30. Fang Y, Svoboda KKH. Nicotine inhibits myofibroblast differentiation in human gingival fibroblasts. J Cell Biochem 2005;95:1108–19.
31. Kinner B, Zaleskas JM, Spector M. Regulation of smooth muscle actin expression and contraction in adult human mesenchymal stem cells. Exp Cell Res 2002;278:72–83.
32. Sobral LM, Montan PF, Martelli-Junior H, Graner E, Coletta RD. Opposite effects of TGF-β1 and IFN-γ on transdifferentiation of myofibroblast in human gingival cell cultures. J Clin Periodontol 2007;34:397–406.
33. Eyden B. The myofibroblast: an assessment of controversial issues and a definition useful in diagnosis and research. Ultrastruct Pathol 2001;25:39–50.
34. Wrana JL, Overall CM, Sodek J. Regulation of the expression of a secreted acidic protein rich in cysteine (SPARC) in human fibroblasts by transforming growth factor β. Eur J Biochem 1991;197:519–28.
35. Chiquet-Ehrismann R. Tenascins. Int J Biochem Cell Biol 2004;36:986–90.
36. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular biology of the cell. New York: Garland Science, 2002.