Basic Fibroblast Growth Factor Regulates Gene and Protein Expression Related to Proliferation, Differentiation, and Matrix Production of Human Dental Pulp Cells

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Abstract

Introduction: Basic fibroblast growth factor (bFGF) plays differential effects on the proliferation, differentiation, and extracellular matrix turnover in various tissues. However, limited information is known about the effect of bFGF on dental pulp cells. The purposes of this study were to investigate whether bFGF influences the cell differentiation and extracellular matrix turnover of human dental pulp cells (HDPCs) and the related gene and protein expression as well as the role of the mitogen-activated protein kinase (MEK)/extracellular-signal regulated kinase (ERK) signaling pathway. The expression of fibroblast growth factor receptors (FGFRs) in HDPCs was also studied. **Methods:** The expression of FGFR1 and FGFR2 in HDPCs was investigated by reverse-transcription polymerase chain reaction. HDPCs were treated with different concentrations of bFGF. Cell proliferation was evaluated using the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. Cell differentiation was evaluated using alkaline phosphatase (ALP) staining. Changes in messenger expression of cyclin B1 and tissue inhibitor of metalloproteinase (TIMP) 1 were determined by reverse-transcription polymerase chain reaction. Changes in protein expression of cdc2, TIMP-1, TIMP-2, and collagen I were determined by Western blotting. U0126 was used to clarify the role of MEK/ERK signaling. **Results:** HDPCs expressed both FGFR1 and FGFR2. Cell viability was stimulated by 50–250 ng/mL bFGF. The expression and enzyme activities of ALP were inhibited by 10–500 ng/mL bFGF. At similar concentrations, bFGF stimulates cdc2, cyclin B1, and TIMP-1 messenger RNA and protein expression. bFGF showed little effect on TIMP-2 and partly inhibited collagen I expression of pulp cells. U0126 (a MEK/ERK inhibitor) attenuated the bFGF-induced increase of cyclin B1, cdc2, and TIMP-1. **Conclusions:** bFGF may be involved in pulpal repair and regeneration by activation of FGFRs to regulate cell growth; stimulate cdc2, cyclin B1, and TIMP-1 expression; and inhibit ALP. These events are partly associated with MEK/ERK signaling. *(J Endod 2017;■:1–7)*

Key Words

Alkaline phosphatase, basic fibroblast growth factor, cell proliferation, dental pulp cells, proliferation, signal transduction, tissue inhibitor of metalloproteinase 1

Significance

bFGF is crucial for pulpal healing/repair and regeneration via stimulation of cell proliferation, cdc2, cyclin B1, and TIMP-2 expression, possibly via activation of FGF receptor 1 and 2 and the MEK/ERK signaling. bFGF can be used with cells/scaffold for tissue engineering.

During the repair processes of dental hard tissues, fibroblast growth factors (FGFs) play a crucial role in the control of cartilage and bone development (1–3). They stimulate the proliferation of growth plate chondrocytes and osteoblasts and modulate their phenotypic expression. However, little is known about the roles of FGFs in tooth development and pulp/dentin repair. Recent studies have shown that basic FGFs (bFGF [FGF2]) are present in peridental and dental basement membranes, dental mesenchyme, and stellate reticulum. Acidic FGF (aFGF [FGF1]) is localized in the dental lamina, enamel organ, ameloblasts, predentin, dentin, and stratum intermedium. Both bFGF and aFGF are also shown to be expressed in some dental pulp cells (4). Moreover, transcripts of FGF receptor 1 (FGFR1) were present in the odontoblast layer and the underlying dental papilla mesenchyme *in situ* (5). However, these observations did not directly prove the involvement of FGFs in odontogenic processes. Limited information is known about the expression

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of FGFRs in cultured dental pulp cells and their downstream signal transduction pathways responsible for bFGF-induced events. bFGF plays a key role in extracellular matrix remodeling by controlling a number of proteolytic activities in various cell types and affects the orchestration of matrix enzyme interaction. Extracelluar matrix turnover is tightly regulated by the expression of collagen, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs). A previous study has shown a dose-dependent effect of bFGF administration on the levels of gene expression of collagen I and III and MMP-1 in periodontal ligament cells (6). Whether bFGF affects the matrix turnover of dental pulp through its regulation on type I collagen and TIMPs is an intriguing issue for pulpal repair and regneration.

In the past, there were several studies regarding the effects of bFGFs on dental tissue, such as immature or mature dental pulp cells and mesenchymal stem cells of dental pulp (7, 8). *In vitro* studies have found that bFGF suppressed alkaline phosphatase (ALP) activity, osteonectin synthesis, and calcium content (9); decreased the formation of type I collagen and laminin; and abolished the calcification of the extracellular matrix (10). Moreover, bFGF inhibited the expression of the odontoblast phenotype. Nevertheless, bFGF increased the DNA content and proliferation of pulp cells (11, 12). Whether bFGF induces the proliferation of human dental pulp cells (HDPCs) via stimulation of cell cycle–related gene/protein (cdc2/cyclin B1) expression is awaiting confirmation.

During tooth development, bFGF is present on the basement membrane between the oral epithelium and the mesenchymal tissue. bFGF may regulate the differentiation of odontoblasts and ameloblasts and induce the differentiation of preodontoblasts to odontoblasts in combination with transforming growth factor beta 1 and insulin-like growth factor 1. Therefore, bFGF may be crucial for cytodifferentiation of odontoblasts in the formation of dentin. In a recent report, the localization of bFGF in dentin was observed. It was suggested that bFGF embedded in the heparan sulfate matrix may be released from the injured dentin by bacterial, chemical, or mechanical stimuli (13) and might play a role in the wound healing and regeneration of the pulpodentin complex (14). After injury to dental pulp, the inflammatory process and tissue repair may occur, including inflammatory cell accumulation and migration of cells into the wound area through the interaction of the chemotactic factors and the extracellular matrix. Dental pulp cells may be exposed to bFGF and undergo activation by that, but the downstream signaling pathways by bFGF on FGFRs in HDPCs are not well clarified. In the experimental 3-wall bony defect (15), bFGF induced prominent tissue regeneration. bFGF potentiated the cell activity of periodontal tissue and alveolar bone by its effects on cell migration, proliferation, and differentiation. On the basis of these observations, bFGF may play an important role in the proliferation, migration, and wound healing of dental pulp tissue.

Considering the role of bFGF in pulpal development, repair, and regeneration as well as its potential use with other biomaterials for pulpal tissue engineering, the aims of this study were to investigate whether HDPCs express various FGFRs and whether bFGF influences the cell proliferation and matrix turnover of HDPCs *in vitro* via mitogen-activated protein kinase (MEK)/extracellular-signal regulated kinase (ERK) signaling.

Materials and Methods

Culture of HDPCs

After approval from the Ethics Committee, National Taiwan University Hospital, Taipei, Taiwan, human third molars or premolars were extracted after obtaining proper informed consent from the patients. Human dental pulp tissues were obtained by splitting the teeth with a hammer. These pulp tissues were cut into small pieces and cultured using a tissue explant technique in Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS; Gibco Life Technologies, Grand Island, NY) and 1× penicillin/streptomycin in 10-cm culture dishes; the medium was changed every 2 or 3 days. The cultured HDPCs were maintained at 37°C in a humidified atmosphere with 95% air and 5% CO_2. They were characterized to express osteocalcin, osteonectin (data not shown), and ALP activities as described previously (16). Experiments were performed with cells from the 3rd to 8th passages.

Viable Cell Assay: 3-(4,5-Dimethyl-Thiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide Assay

In short, 1×10^5 HDPCs were incubated in DMEM with 10% FBS. After 24 hours, the medium was replaced by fresh medium containing different concentrations of bFGF (0, 1, 10, 50, and 250 ng/mL; Peprotech Inc, Rocky Hill, NJ). The cells were further cultured for 5 days. Then, 20 μL 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (final 0.5 mg/mL) was added into each well and incubated at 37°C for 2 hours. The cultured medium was decanted, and the produced formazan was dissolved in 300 μL dimethyl sulfoxide. The amount of dissolved formazan was monitored by the readings against the blank sample (dimethyl sulfoxide) at optical density at a wavelength of 540 nm using a microwell plate reader (Dynatech, Edgewood, NY) (17).

Reverse-transcription Polymerase Chain Reaction

Briefly, 1.5×10^6 HDPCs were cultured onto 10-cm dishes with 10 mL DMEM containing 10% FBS for 24 hours. The culture medium was replaced by fresh medium and exposed to different concentrations of bFGF (0, 1, 10, 50, and 250 ng/mL) for 24 hours. The total cellular RNA of dental pulp cells was extracted using an RNA isolation kit (Macherey-Nagel Inc, Easton, PA) and used for reverse-transcription polymerase chain reaction (RT-PCR) using the SuperScript TM III First-Strain DNA synthesis system and polymerase chain reaction kits (Invitrogen, Carlsbad, CA). Specific primers for this experiment included ALP, TIMP-1, cyclin B1, cdc2, and β-actin as the control. The sequence of primer pairs was shown in Table 1 (18–23). At first, complementary DNA samples were denatured at 94°C for 5 minutes and run for 20 to 35 cycles. Each cycle was composed of 94°C for 1 minute (denaturing), 55°C for 1 minute (annealing), and 72°C for another 1 minute (elongation) followed by a final extension at 72°C for 7 minutes.

The polymerase chain reaction product mixtures were loaded into the wells of the ethidium bromide. After the electrophoresis process was completed, the gels were removed from the holder for ultraviolet photography and quantified using the AlphaEaseFC software program (Alpha Innotec Corp, San Leandro, CA). The total RNA isolated from DPSCs incubated in fresh medium containing 10% FBS for 24 hours was also used for analysis of FGFR1 and FGFR2 expression using specific primers by RT-PCR as described previously.

Western Blot

Briefly, 1.5×10^6 HDPCs were cultured onto 10-cm dishes with 10 mL DMEM containing 10% FBS for 24 hours. The culture medium was replaced by fresh medium and exposed to different concentrations of bFGF (0, 1, 10, 50, and 250 ng/mL) for 24 hours. Cell lysate proteins were extracted from cells after a phosphate-buffered saline (PBS) wash using lysis buffer (Tris-HCl 50 mmol/L, NaCl 150 mmol/L, sodium dodecyl sulfate 0.1%, NP-40 0.5%, and protease inhibitor cocktail [Sigma-Aldrich, St Louis, MO]). After quantification, equal amounts of proteins (determined by the Bradford method) were resolved on 12% sodiumdodecyl sulfate–polyacrylamide gel electrophoresis gel,