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ORIGINAL ARTICLE

Effects of fibroblast growth factor-2 on cell proliferation of cementoblasts

Hui-Chieh Yu [a], Fu-Mei Huang [b], Shiuan-Shinn Lee [c], Cheng-Chia Yu [b], Yu-Chao Chang [a,b*]

[a] School of Dentistry, Chung Shan Medical University, Taichung, Taiwan
[b] Department of Dentistry, Chung Shan Medical University Hospital, Taichung, Taiwan
[c] School of Public Health, Chung Shan Medical University, Taichung, Taiwan

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KEYWORDS
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Abstract *Background/purpose:* Fibroblast growth factor (FGF)-2 is known as a signaling molecule that induces tissue regeneration. Little is known about the effect of FGF-2 on cementoblasts for periodontal and periapical regeneration. The aim of this study was to investigate the effects of FGF-2 on murine immortalized cementoblast cell line (OCCM.30).
Materials and methods: Cell growth and proliferation was judged by using alamar blue reduction assay. Flow cytometry analysis was used to evaluate Stro-1 positive cells expression with or without FGF-2. Western blot was used to evaluate the expression of phosphorylated serine–threonine kinase Akt (p-Akt) and extracellular signal-regulated protein kinase (p-ERK) in cementoblasts.
Results: FGF-2 was found to increase cell growth in a dose-dependent manner ($P < 0.05$). The concentration of 10 ng/mL FGF-2 enhanced cell proliferation in a time-dependent manner ($P < 0.05$). In addition, 10 ng/mL FGF-2 significantly increased the number of Stro-1 positive cells in the first 24 hours ($P < 0.05$). Moreover, 10 ng/mL FGF-2 was found to upregulate p-Akt and p-ERK in a time-dependent manner ($P < 0.05$).
Conclusion: Taken together, FGF-2 could increase cementoblast growth, proliferation, and Stro-1 positive cells. These enhancements are associated with the upregulation of p-Akt and p-ERK expression. The application of FGF-2 may provide benefit for periodontal and periapical regeneration during the early phase of wound healing.

* Corresponding author. School of Dentistry, Chung Shan Medical University, 110, Section 1, Chien-Kuo North Road, Taichung 404, Taiwan.
E-mail address: cyc@csmu.edu.tw (Y.-C. Chang).

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Introduction

Traditional periodontal and endodontic therapy can be predictably used to arrest mild to moderate periodontal and periapical bony defects; however, it may be inadequate for the treatment of disease characterized by wide circumferential defects. Surgical procedures could provide better access in these situations. Recently, many techniques and materials have become available to promote periodontal and periapical tissue regeneration.[1] Growth factors are responsible for the wound healing process by stimulating and regulating numerous cell activities such as mitogenesis, chemotaxis, metabolism, and differentiation.[2] These may play a pivotal role in bone remodeling, osteogenesis, and cementogenesis in periodontal and periapical tissue regeneration.

Fibroblast growth factor-2 (FGF-2), a signaling peptide that binds heparin and heparan sulfate, can modulate the expression of the main components of connective tissue including glycosaminoglycans, proteoglycans, collagen, and noncollagenous protein.[3] FGF-2 is considered to be involved in the early stage of the wound healing process by the stimulation of growth, migration, and proliferation on the cells derived from pulp[4] and periodontal ligament.[5–7] In addition, FGF-2 has been reported to be effective in human periodontal regeneration procedure.[8,9]

Cementum, which has similar composition and properties to bone, is a mineralized tissue that is synthesized by cementoblasts during tooth root formation and plays an essential role in anchoring teeth to surrounding alveolar bone. Cementoblasts play a critical role in the healing of periodontal ligament and cementum in periapical portions.[10] However, little is known about the precise mechanisms of FGF-2 on cementoblasts, hampering the establishment of more effective therapies for periodontal and periapical regeneration. The aim of this study was to investigate FGF-2 on murine immortalized cementoblast cell line (OCCM.30) by measuring cell growth, proliferation, Stro-1 positive cells, and the phosphorylated proteins Akt and ERK expression.

Materials and methods

Cell culture

Immortalized murine cementoblasts (OCCM.30) was a generous gift from Dr Somerman's laboratory (University of Washington, Seattle, WA, USA).[11] The cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Gaithersburg, MD, USA), supplemented with 10% fetal calf serum (FCS) and antibiotics (Gibco BRL, Gaithersburg, MD, USA). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Cell proliferation assay

Cells were seeded at a 2×10^4 cell density per well into 96-well culture plates for 24 hours. The culture medium was replaced with fresh DMEM with 0.1 ng/mL, 1 ng/mL, 10 ng/mL, 50 ng/mL, and 100 ng/mL FGF-2 for 24 hours. In addition, cells were incubated with 10 ng/mL FGF-2 for 0 hours, 24 hours, 48 hours, and 72 hours, respectively. Cell proliferation was tested using the alamar blue dye (BUF012A/B alamarBlue; AbD serotec, Oxford, UK) as described previously.[12]

Stro-1 staining by flow cytometry

Cells were stained with anti-Stro-1 antibody (R&D Systems, Minneapolis, MN, USA) and phycoerythrin-conjugated goat antimouse immunoglobulin G antibody (Miltenyi Biotech., Auburn, CA, USA), with labeling according to the manufacturer's instructions. Red (> 650 nm) fluorescence emission from 10,000 cells illuminated with blue (488 nm) excitation light was measured with a FACSCalibur (Becton Dickinson, Franklin Lakes, NJ, USA) using CellQuest software (Becton Dickinson, San Jose, CA, USA).

Western blot

Cells arrested in G_0 by serum deprivation (0.5% FCS; 48 hours) were used in the experiments.[13] Nearly confluent monolayers of cells were washed with serum-free DMEM and immediately thereafter exposed at the indicated incubation times to 10 ng/mL FGF-2. Cultures without FCS were used as negative control. Cell lysates were collected at 1 day, 2 days, and 3 days. The extraction of proteins from cells and immunoblotting analysis were performed as described previously.[14,15] p-Akt (phosphorylated serine—threonine kinase Akt) and p-ERK (extracellular signal-regulated protein kinase) antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA).

Statistical analysis

Triplicate experiments were performed throughout this study. All assays were repeated three times to ensure reproducibility. The significance of the results obtained

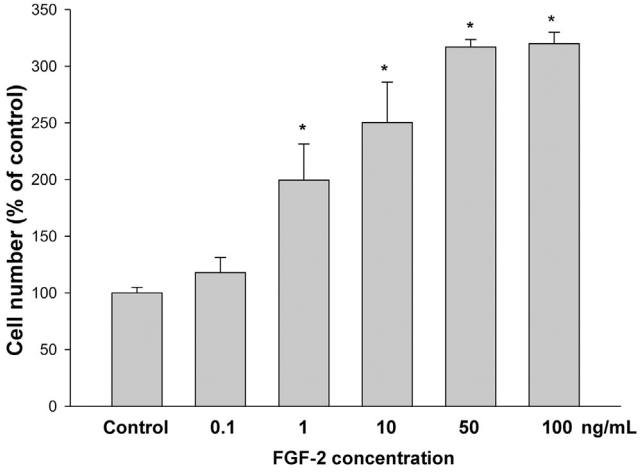

Figure 1 Effects of various concentrations (0.1 ng/mL, 1 ng/mL, 10 ng/mL, 50 ng/mL, and 100 ng/mL) of FGF-2 on cementoblasts as measured by alamar blue assay. Each point and bar represent mean ± SD. * Significant differences from control values with $P < 0.05$.