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Effect of follicular dendritic cell secreted protein on gene expression of human periodontal ligament cells

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ABSTRACT

Objective: The objective of this study was to investigate the specific roles of follicular dendritic cell secreted protein (FDC-SP), a protein exists in saliva, in the inhibition of calcium precipitation during periodontal regeneration, as well as affect phenotype expression of human periodontal ligament cells (hPDLCs) during the differentiation process.

Design: To investigate this, we applied microarray technology to identify gene expression changes in hPDLCs transfected with FDC-SP and then clustered them according to their biological functions.

Results: One hundred seventy-one genes were found differentially expressed by at least two-fold between FDC-SP -transfected and empty vector-transfected cells. Besides, genes encoding cell-cycle proteins, blood-related and cell differentiation-related proteins tended to be up-regulated after FDC-SP transfection, whereas cytokine/growth factors, signal transduction and metabolism-related genes tended to be down-regulated in hPDLCs overexpression FDC-SP.

Conclusions: The present study investigated FDC-SP's roles in hPDLCs' phenotype expression, via comparing the gene expression profiles between FDC-SP -transfected hPDLCs and empty vector-transfected cells upon microarray analysis. hPDLCs overexpression FDC-SP appear to display different gene expression patterns. In all, these observations showed a potential of FDC-SP in the maintenance of PDL homeostasis and its ultimate contribution to periodontal would-healing processes.

1. Introduction

Periodontal ligament (PDL) plays quite important roles in proprioception, tooth support and acts as a receptor of biting forces and shock absorber against the mastication impact (Lv et al., 2009; Ralph 1982; Poiate, de Vasconcellos, de Santana, & Poiate, 2009). It is an unmineralized connective tissue that connects cementum and alveolar bone, which located between the alveolar bone and the tooth. Periodontal ligament cells (PDLCs) are considered as an ideal cell type for periodontal regeneration including the restoration of periodontal ligament, cementum and alveolar bone, due to their mesenchymal stem cell-like properties (Gjertsen, Stothz, Neiva, & Pileggi, 2011; Ivanovski S, Haase, & Bartold, 2001; Ishikawa et al., 2009). Interestingly, the PDL always maintains its width unmineralized despite the mechanical stress in physiological conditions. Therefore, it has been hypothesized that PDLCs may have regulatory mechanisms to inhibit their osteogenesis (Kato et al., 2005).

Recently, follicular dendritic cell secreted protein (FDC-SP), a novel small protein, has been originally identified in primary follicular dendritic cells isolated from human tonsils (Al-Alwan et al., 2007; Marshall et al., 2002). Then, Nakamura et al. identified this novel protein in human PDL tissue (Nakamura et al., 2005). FDC-SP has been proved to prevent calcium precipitation in PDL tissue (Xiang, Ma, He, Wei, & Gong, 2014). However, the molecular mechanism of how FDC-SP regulates osteogenic activity of PDLCs has been less studied.

Nowadays, lentiviral vectors have been broadly used in functional genomics (Li et al., 2010; Logan, Lutzko, & Kohn, 2002). A series of studies have shown application of lentiviral vectors containing specific

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genes to obtain efficient and stable transgene expression in bone marrow and other tissue cells (Bu, Xin, Toneff, Li, & Li, 2009; Pawelczyk et al., 2009). In the present study, we firstly established a recombinant lentiviral vector containing FDC-SP and obtained safe and efficient FDC-SP overexpression in human periodontal ligament cells (hPDLCs). After that, we applied microarray technology to identify differentially expressed genes between empty vector-transfected hPDLCs and FDC-SP -transfected ones and then clustered them according to their biological functions. It is hypothesized that this information will provide insight into changes in cell phenotype expression during periodontal restoration, as well as the molecular process that FDC-SP regulates hPDLCs' differentiation.

2. Materials and methods

2.1. Cell culture

hPDLCs were isolated and cultured via standard techniques as previously described (Xiang et al., 2014). Briefly stated, premolars extracted from healthy voluntary donors with matching ages (12–14 years old) were used. Informed consent had been obtained from patients, and the study protocol was approved by the Ethics Committee of Sichuan University. Periodontal ligament were separated from only the middle third of the roots and were cut into small pieces. After a 30-min enzymatic digestion (0.05% trypsin and 0.15% collagenase; Sigma, St Louis, MO, USA) and centrifugation, single cells in suspension were obtained and then cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA), containing 20% fetal bovine serum (FBS; Gibco, Grand Island, NY), 200 mM L-glutamine (Invitrogen, Life Technologies Co.), 100 U/ml penicillin, and 100 mg/ml streptomycin sulfate, at 37 °C with 5% CO_2. Cell culture was continued with medium changes every three days until hPDLCs reached subconfluency. Cells at passage 2–4 were used in the following tests.

2.2. FDC-SP genes transfection

hPDLCs' transfection with FDC-SP was conducted as previously described (Xiang et al., 2014). Briefly, 2×10^5 hPDLCs were incubated in the mixture containing growth medium and viral supernatant (1:1) with 5 ug/ml polybrene (Sigma) for 10 h. The transfected cells were then washed twice with PBS, and cultured in normal DMEM medium containing 20% FBS for 96 h. Two different groups were designed for further research experiments, including the negative control group (transfection with empty lentiviral vector), and the experimental group (transfection with lentiviral vector containing FDC-SP). As for the transfection efficiency, we applied an inverted fluorescent microscope (OLYMPUS IX70, Japan) to analyze the expression of green fluorescence protein (GFP) 2 days later, and the transfection efficiency turned to be approximately 80%.

2.3. RNA extraction and microarray analysis

Agilent Whole Human Genome Oligo Microarray (4 × 44 K) (Agilent Technologies, Palo Alto, CA) was applied in the following experiments. Firstly, total RNA was extracted using TRIZOL Reagent (Life technologies, Carlsbad, CA, US) following the manufacturer's instructions and checked for a RIN number to inspect RNA integration by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US). Qualified total RNA was further purified by RNeasy mini kit (QIAGEN, GmBH, Germany) and RNase-Free DNase Set (QIAGEN, GmBH, Germany). Then, total RNA was amplified and labeled by Low Input Quick Amp Labeling Kit, One-Color (Agilent technologies, Santa Clara, CA, US), following the manufacturer's instructions. Labeled cRNA were purified by RNeasy mini kit (QIAGEN, GmBH, Germany). Each Slide was hybridized with 1.65 μg Cy3-labeled cRNA using Gene Expression Hybridization Kit (Agilent technologies, Santa Clara, CA, US) in Hybridization Oven (Agilent technologies, Santa Clara, CA, US). After 17 h hybridization, slides were washed in staining dishes (Thermo Shandon, Waltham, MA, US) with Gene Expression Wash Buffer Kit (Agilent technologies, Santa Clara, CA, US). Slides were scanned by Agilent Microarray Scanner (Agilent technologies, Santa Clara, CA, US) with default settings, Dye channel: Green, Scan resolution = 3 μm, 20bit. Data were extracted with Feature Extraction software 10.7 (Agilent technologies, Santa Clara, CA, US). Raw data were normalized by Quantile algorithm, Gene Spring Software 11.0 (Agilent technologies, Santa Clara, CA, US). The entire microarray experiments were performed at the National Engineering Center for Biochip at Shanghai, China. The differentiated genes were selected based on the criteria of $P < 0.05$ and a fold change of ≥ 2 of their expression values between the two groups. As for gene enrichment, GO analysis was applied to analyze the main function of the differential expression genes according to the Gene Ontology. Generally, Fisher's exact test and χ^2 test were used to classify the GO category, and the false discovery rate (FDR) was calculated to correct the P-value. The FDR was defined as $FDR = 1 - \frac{N_k}{T}$, where N_k refers to the number of Fisher's test P-values less than χ^2 test P-values. We computed P-values for the GOs of all the differential genes. Enrichment provides a measure of the significance of the function: as the enrichment increases, the corresponding function is more specific, which helps us to find those GOs with more concrete function description in the experiment. Within the significant category, the enrichment Re was given by: $Re = (n_f/n)/(N_f/N)$, where n_f is the number of differential genes within the particular category, n is the total number of genes within the same category, N_f is the number of differential genes in the entire microarray, and N is the total number of genes in the microarray.

The data were submitted to the GEO database (http://www.ncbi.nlm.nih.gov/geo/) with a series accession number GSE61384. All data are compliant with MIAME standards.

2.4. Real-time PCR

For real-time PCR, cDNA was synthesized using PrimeScript Reverse Transcriptase (Takara Bio Inc., Shiga, Japan) according to the manufacturer's protocol. Real-time PCR was carried out in triplicate and performed using an ABI PRISM 7300 Real-time PCR System (Applied Biosystems, Foster City, CA, USA), the cycling conditions were chosen according to the manufacturer's instructions. Expression levels of formin2 (FMN2), SRY (sex determining region Y)-box 12 (SOX12), early growth response 1 (EGR1) and insulin-like growth factor 1 (somatomedin C) (IGF1) were normalized to glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) house-keeping gene expression and performed according to the $2^{-\Delta\Delta Ct}$ method, then presented as fold increase relative to the control group.

2.5. Statistical analysis

All assays were performed in triplicate and each experiment was repeated at least three times. The results were presented as mean standard deviation (SD). The microarray bioinformatic analysis enrolled in this study applied Student's *t*-test to analyze the differential expression genes. In addition, P-value < 0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. Identification of robust and consistent differences in gene expression patterns

Differences in gene expression were analyzed via the SAS analysis system (Shanghai Biotechnology Co, Ltd). We compared all experimental samples to all control ones. This comparison of FDC-SP -transfected hPDLCs to empty vector-transfected cells identified 171