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ORIGINAL ARTICLE

Lipopolysaccharide extracted from *Porphyromonas gingivalis* induces DNA hypermethylation of runt-related transcription factor 2 in human periodontal fibroblasts

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Background/Purpose: Epigenetic alterations such as DNA methylation and histone acetylation are described as changes in the pattern of gene expression not involving the DNA sequence. Lipopolysaccharide (LPS) derived from *Porphyromonas gingivalis* has been shown to inhibit osteoblastic cell differentiation. We examined whether DNA hypermethylation was involved in the inhibitory effect of LPS on osteoblastic differentiation of fibroblasts derived from human periodontal ligament (HPDL).
Methods: The HPDL cells were incubated with LPS derived from *P. gingivalis* at a concentration of 10 μg/ml for 24 h. The cells were treated with DNA methyltransferase inhibitor 5-Aza-2'-deoxycytidine (5Aza). Untreated cells were used as a control. Cell viability was determined using cell proliferation reagent. DNA methyltransferase (DNMT1) and runt-related transcription factor 2 (RUNX2) mRNAs were evaluated by quantitative polymerase chain reaction (RT-PCR). Analysis of RUNX2 DNA methylation was performed using quantitative methylation-specific PCR.

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Results: The expression level of RUNX2 was significantly lower in the cells stimulated with LPS than the controls. The presence of 5Aza increased the expression of RUNX2 in cells stimulated with LPS. The expression levels of DNMT1 mRNA in the cells stimulated with LPS were significantly higher than in the control. The presence of 5Aza completely abolished the upregulated expression of DNMT1 in cells stimulated with LPS. The methylation of DNA at 0.1 kb and −1.9 kb in the cells stimulated with LPS was significantly higher than the control.
Conclusion: The results indicate that DNA hypermethylation may be involved in the inhibitory effect of LPS on osteoblastic differentiation in HPDL.

Introduction

Epigenetics is described as changes in the pattern of gene expression not involving the DNA sequence. Epigenetic events act through chemical modification of DNA and by selectively activating or inactivating genes to determine their expression during development.[1] DNA methylation and histone deacetylation are two major mechanisms of epigenetic alteration in human cells. DNA methylation is characterized by the addition of a methyl group to cytosines within CpG regions. Histone deacetylation is a mechanism that involves the removal of the acetyl group leading to alteration of the charge and packing of DNA around histones.[2] These epigenetic modifications are often observed in malignant and premalignant lesions.[3] It has recently been shown that epigenetic modifications may cause other common diseases such as diabetes, metabolic diseases, allergies, autoimmune diseases, and neurodegenerative diseases.[4] Epigenetic alterations have also recently been implicated in periodontal disease.[5] The expression profiles of several cytokines may be epigenetically modified.[6] Preliminary data suggest that expression of periodontal disease-related cytokines such as IL-6 may be altered by an epigenetic modification.[7] Methylation of the collagen-a1 gene has been observed in the periodontal ligament during the aging process.[8] Some local risk factors for periodontal disease such as tobacco smoke, alcohol and infectious agents can cause epigenetic modification.[9,10]

Epigenetic modification can be caused by certain types of bacteria and lipopolysaccharide (LPS).[11] For example, oral pathogens including *Porphyromonas gingivalis* and *Fusobacterium nucleatum* were recently shown to cause epigenetic modifications.[12] Epigenetic modifications such as DNA hypermethylation often induce downregulation of transcriptional levels.[13] LPS derived from *P gingivalis* has been demonstrated to inhibit osteoblastic differentiation of osteoprogenitor cells derived from fatal rat calvaria.[14] Runt-related transcription factor 2 (RUNX2) is a key transcription factor associated with osteoblast differentiation, and the inhibition of osteoblastic cell differentiation is accompanied by downregulated expression of RUNX2.[15] We hypothesized that the downregulated expression of RUNX2 is involved in DNA hypermethylation. Fibroblasts derived from periodontal tissues have the potential for osteoblastic differentiation. We examined whether LPS derived from *P gingivalis* caused downregulated expression of RUNX2 in fibroblasts derived from periodontal ligaments, and whether DNA hypermethylation is involved in the downregulated expression.

Methods

Cell culture

Human periodontal ligament (HPDL) cells from normal human periodontal ligament tissue were purchased from Lonza Walkersville (Basel, Switzerland) and cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (Gibco, Invitrogen Corporation, Carlsbad, CA, USA), 100 U/ml penicillin and 100 mg/ml streptomycin (Sigma-Aldrich) at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

HPDL cells were spread onto 60 mm tissue culture plates at a density of 4.0×10^5 cells/ml. After overnight incubation, the cells were cultured with *P gingivalis* LPS (LPS: InvivoGen, San Diego, CA, USA) at a concentration of 10 μg/ml, or with 5-Aza-2'-deoxycytidine (5Aza: Sigma), an inhibitor of DNA methyltransferase, at concentrations of 1μM, 10 μM, and 100 μM. Untreated cells were used as controls.

In vitro cytotoxicity assays

Cell viability was determined using cell proliferation reagent WST-1 (Roch Diagnostics, Mannheim, Germany). HPDL cells were seeded in 96-well plates in Dulbecco's modified Eagle's medium and cultured overnight. The cells were treated with different concentrations of LPS or 5Aza. After incubation for 12 and 24 h, 10 μl of WST-1 was added to each well and cultured for 1 hour. The absorbance at 450 nm was determined using an Infinite F200 microplate reader (Tecan, Männedorf, Switzerland).

RNA extraction and quantitative RT-PCR

Total RNA was extracted from HPDL cells using an RNeasy Mini Kit (Qiagen, Venlo, Netherlands), following the manufacturer's instructions. Total RNA was reversely transcribed into cDNA using an Omniscript RT Kit (Qiagen). Nucleotide contents were measured on a Nanodrop ND-1000 spectral photometer (Nanodrop Technologies, Wilmington, DE, USA). The cDNA levels were measured using the ABI 7500 Real-Time PCR System (Applied Biosystems, Foster