Porphyromonas gingivalis由来LPS長期刺激によるヒト歯根膜線維芽細胞におけるDNA高メチル化の網羅的解析 - 細胞外マトリックス関連および老化抑制関連の遺伝子について -

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ABSTRACT

DNA methylation analysis of Extracellular matrix-related and Aging-related genes in Human Periodontal Ligament Fibroblasts by long-term stimulus of Lipopolysaccharide derived from *Porphyromonas gingivalis*

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Objectives:

Although aberrant DNA methylations have been observed in periodontal diseases, the mechanisms by which periodontal pathogens cause them remain unclear. Aberrant DNA methylation may occur as chronic events stimulated by environmental factors during the development of disease. Lipopolysaccharide (LPS) derived from *Porphyromonas gingivalis* (*P. gingivalis*) is a major periodontal pathogen. In the present study, we developed a new in vitro model of LPS infection in human periodontal fibroblast cells (HPdLFs) over a prolonged period in order to simulate chronic LPS stimulation. We performed a genome-wide analysis of DNA methylation in the HPdLFs stimulated with LPS for 1 month. In addition, the hypermethylation status of extracellular matrix (ECM)-and aging-related genes was examined; thereafter, we assessed the effect of hypermethylation on the transcription levels of these genes.

Methods:

The HPdLFs were grown in Dulbecco's Modified Eagle's medium containing 10% fetal bovine serum. The culture was repeated, alternating every 3 days with and without LPS for 1 month. Untreated samples were used as controls. DNA was extracted from the cells and analyzed using human CpG island microarrays. Quantitative methylation-specific polymerase chain reaction (PCR) was carried out to confirm the reproducibility of the microarray data. The mRNA and protein expression levels of selected ECM and aging-related genes from the data were analyzed by quantitative reverse transcription (RT)-PCR and cell enzyme-linked immuno-sorbent assay, respectively. Cells with downregulated mRNA and protein expression following LPS stimulation were treated with a demethylating agent, 5-Aza-deoxycytidine (5-Aza), for 24 hour s in order to assess whether the expressions of these genes is regulated by DNA demethylation.

Results:

We found 25 ECM-related and four aging-related genes at the CpG islands of the

promoter region, which exhibited 4-fold greater hypermethylation than the controls. Among them, the hypermethylation of nine ECM-related genes (Fibronectin type III and ankyrin repeat domains 1, Collagen type IV alpha 1-alpha 2, Collagen type XII alpha 1, Collagen type XV alpha 1, Laminin alpha 5, Laminin beta 1, matrix metallopeptidase 25, Protein-O-mannosyltransferase 1 and Elastin microfibril interfacer 3), and one aging-related gene (Klotho) induced a significantly downregulated expression of their mRNAs. The expression levels of Fibronectin type III and ankyrin repeat domains 1, Collagen type XII alpha 1, and Klotho proteins were decreased following LPS stimulation, however, no decrease in the expression of these genes was observed following treatment with 5-Aza.

Conclusion:

These results indicate that LPS derived from *P. gingivalis* may cause DNA hypermethylation of ECM and aging-related genes in the HPdLFs, leading to the downregulated expression of these genes at the transcriptional level.