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Differential induction of collagens by mechanical stress in human periodontal ligament cells

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ABSTRACT

Objective: Excessive mechanical stress (MS) during hyperocclusion is known to result in destruction of periodontal tissues and alveolar bone, leading to occlusal trauma. Collagens are extracellular matrix components that are encoded by more than 30 different genes. They are classified into three types: fibril-forming, fibril-associated with interrupted triple helices (FACIT), and non-fibril forming collagens. Although MS is known to affect COL I, little is known about its effects on other types of collagens in the periodontal ligament (PDL). We hypothesised that MS could induce expression of the three different types of collagens, thus protecting against occlusal trauma.

Design: The aim of this study was to investigate intermittent uniaxial stretching-induced collagen expression in PDL cells using DNA microarray, polymerase chain reaction, and western blotting analysis. We compared changes in collagen expression caused by MS stimulation and osteogenic stimulation, and examined relationships between expression of collagen and their digestive enzymes, matrix metalloproteases (MMPs).

Results: Expression of both fibril-forming and FACIT collagens was transiently decreased in the initial phase after MS, while the expression of non-fibril-forming collagens was gradually increased. MS for 3–7 days resulted in gradual upregulation of all three types of collagen. Furthermore, the expression of fibril- and non-fibril-forming collagens was reciprocally related to expression of MMPs. In contrast, expression of all three types of collagen was slightly upregulated during osteogenesis.

Conclusion: The MS-induced expression patterns of fibril-forming and FACIT collagens suggest changes in the composition of the extracellular matrix to increase the resistance of PDL cells to hyperocclusal force.

1. Introduction

The periodontal ligament (PDL) is a band of dense connective tissue located between the tooth-root cementum and the alveolar bone.[1,2] PDL cells in healthy periodontal tissue are exposed to mechanical stress (MS) during occlusion and mastication.[3,4] PDL cells can differentiate into either osteoblasts or cementoblasts in response to MS.[4–8] The regulatory

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Abbreviations: MS, mechanical stress; COL, collagen; PDL, periodontal ligament; MMP, matrix metalloprotease; FACIT, fibril-associated with interrupted triple helices.

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role of MS is believed to be critical in mandibular bone remodeling.[9,10] However, excessive MS causes periodontal tissue damage, including bone resorption and tooth loss.[11]

Collagen is the most abundant extracellular matrix protein in vertebrates, accounting for 30% of total body proteins.[12] It is present in all tissues and organs of the body. Collagen is encoded by a family of at least 38 distinct genes, and more than 27 different types of collagen exist.[11,12] Furthermore, collagens can be classified into three major families based on their assembly modes and structural features. The extensive collagen family comprises proteins with several chain types, including fibril-forming collagens, fibril-associated collagens with interrupted triple helix (FACIT), and non-fibril forming collagens. Each type contains multiple isoforms.[12]

The collagen molecules in the PDL are known to respond to mechanical stimuli.[13,14] This is an essential event in the process of alveolar bone remodeling induced by MS.[15] PDL is known to contain all three types of collagen.[16] The fibril-forming collagens COL I, III, and V, the non-fibril-forming COL VI, and the FACIT collagens, COL XII and XIV are all found in the PDL.[17,18] The induction and turnover of collagen fibers in the PDL can occur very rapidly.[1,19]

Although several studies have demonstrated the effects of occlusal MS on the fibril-forming COL I in PDL cells,[20–22] little is known about the differential effects of MS on the expression of the three different types of collagen in PDL cells. This study therefore aimed to clarify the changes in expression of the three types of collagen in PDL cells, following MS. Because MS are known to be upregulated some collagens, especially COL I in fibril-forming collagens during osteoblastic differentiation,[20–22] we also examined the effects of osteogenic stimulation on the expression of the three different types of collagens, and compared these patterns with those of MS-induced collagens.[11–15]

2. Materials and methods

The protocol for the present study was reviewed and approved by the Fukuoka Dental College Research Ethics Committee, Fukuoka, Japan (Approved No. 116). Informed consent was obtained from all volunteers.

2.1. Cell culture

Human PDL cells were obtained from three third molars extracted from three healthy donors (two 20 years persons, 40 years person), as described previously.[23] The sections of PDL were scraped off with a surgical knife form the mid-third of each root, to exclude contamination by gingival and dental pulp. Three different clonal PDL cells derived from three donors were used in each experiment. Cells that grew from the extracts were used in the present experiments, after 4–8 passages. The PDL cells were cultured in silicon rubber chambers in α-minimal essential medium (MEM) containing 10% fetal bovine serum (FBS). In some experiments, PDL cells were cultured with osteogenic medium (α-MEM + 10% FBS including 10 mM β-glycerophosphate and 50 μg/ml ascorbic acid) for 14–20 days. Similar results were obtained in PDL cells derived from different donors and/or after different passages.

2.2. Intermittent MS loading

PDL cells were removed from the dish using 0.01% EDTA-0.02% trypsin and re-cultured in 50 cm^2 silicon chambers coated with 50 μg/ml COL I at a density of 5×10^5 cells/cm^2. The silicon chamber was attached to a stretching apparatus (STB-140; Strex Co. Ltd., Osaka, Japan) driven by a computer-controlled stepping motor. Cells were allowed to attach to the chamber base for 1–7 days, after which uniaxial sinusoidal stretching (conditions: 60 s/returns; resting time: 29 s; stretch length: 1.6 mm; stretch ratio: 105%) was applied at 37 °C, 5% CO_2 (Appendix Fig. 1).

2.3. Gene chip analysis

PDL cells were cultured in the presence of MS loading using the stretching apparatus. After 48 h, total RNA was extracted using an RNeasy mini kit (QIAGEN K.K, Tokyo, Japan). Total RNA (15 μg) was utilised for cDNA synthesis, followed by synthesis of biotinylated cRNA by *in vitro* transcription. Following cRNA fragmentation, it was hybridised with Gene Chip Human Genome U133 plus 2.0 arrays (Agilent Technologies, Santa Clara, CA, USA) displaying probes for 47,000 human genes/expressed sequence tags, according to the manufacturer's protocol. Chips were washed, stained with streptavidin-phycoerythrin, and analysed using the Affymetrix GeneChip scanner and GeneSpring software (Agilent Technologies).

2.4. RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from PDL cells using TRIzol reagent. First-strand cDNA was synthesised from 1 μg total RNA using SuperScript II reverse-transcriptase, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). To detect mRNA expression of collagens and their digestive enzymes, matrix metalloproteases (MMPs), we selected specific primers based on the nucleotide sequence of the cDNA (Appendix Table 1). The cDNA was amplified by PCR under the following conditions: 1 min denaturation at 95 °C, 1 min annealing 60 °C, and 1 min extension at 72 °C, for 23 cycles. PCR products were subjected to electrophoresis on 2% agarose gels and visualised after staining with ethidium bromide.

2.5. Western blot analysis

Cells were lysed in buffer containing 20 mM Tris–HCl, pH 7.5, 200 mM NaCl, 1% Triton-X, 1 mM dithiothreitol, and protease inhibitors (Roche, Basel, Switzerland). The protein content of the samples was measured using a protein assay kit, according to the manufacturer's protocol (Pierce, Hercules, CA, USA). Protein samples of 20 μg were subjected to 5–20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the proteins were then electrophoretically transferred to a PVDF membrane at 100 V for 1 h at 4 °C. The membrane was then incubated with dilutions of antibodies against collagens IA1 (Santa Cruz Biotechnology, CA, USA), IV (Abcam Co., MA, USA), and XII (kind gift from Dr. SundarRaji), and β-actin (Sigma Chem., MO, USA) in 5% skim milk solution overnight at 4 °C.