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Fluid shear stress regulates metalloproteinase-1 and 2 in human periodontal ligament cells: Involvement of extracellular signal-regulated kinase (ERK) and P38 signaling pathways

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ABSTRACT

Matrix metalloproteinase (MMP)-1, 2, with their endogenous inhibitors, tissue inhibitor of metalloproteinase (TIMP)-1, 2 are critical for extracellular matrix remodeling in human periodontal ligament (PDL) and their expression are sensitive to mechanical stresses. Shear stress as the main type of mechanical stress in tooth movement is involved in matrix turnover. However, how shear stress regulates MMPs and TIMPs system is still unclear. In this study, we investigated the effect of fluid shear stress on expression of MMP-1, 2 and TIMP-1, 2 in human PDL cells and the possible roles of mitogen-activated protein kinases in this process. Three levels of fluid shear stresses (6, 9 and 12 dyn/cm^2) were loaded on PDL cells for 2, 4, 8 and 12 h. The results indicated that fluid shear stress rearranged cytoskeleton in PDL cells. Fluid shear stress increased expression of MMP-1, 2, TIMP-1 and suppressed TIMP-2 expression. MAP kinases including extracellular signal-regulated kinase (ERK), c-Jun *N*-terminal kinase (JNK) and p38 were activated rapidly by fluid shear stress. The ERK inhibitor blocked fluid shear stress induced MMP-1 expression and P38 inhibitor reduced fluid shear stress stimulated MMP-2 expression. Our study suggested that fluid shear stress involved in PDL remodeling via regulating MMP-1, 2 and TIMP-1, 2 expression. ERK regulated fluid shear stress induced MMP-1 expression and P38 play a role in fluid shear stress induced MMP-2 upregulation.

1. Introduction

Periodontal ligament (PDL) is a band of dense connective tissue situated between the tooth-root cementum and alveolar bone. It consists of heterogeneous cell population, collagenous and noncollagenous matrix constituents (Lekic and McCulloch, 1996). PDL is supporting the teeth in the sockets. It also senses the mechanical stresses and transfers them from the tooth to the surrounding bone during mastication and tooth movement (Henneman et al., 2008; Lekic and McCulloch, 1996). PDL has high rate of turnover to suit functional changes. As the most abundant cells in PDL, PDL cells play a key role in mechanical stress induced PDL turnover (Beertsen et al., 1997; Lekic and McCulloch, 1996).

PDL cells are constantly under mechanical stresses such as shear stress, compressive stress or tensile stress during mastication and tooth movement (Wang and Thampatty, 2006). Numerous studies have shown that tensile and compressive stresses induce the expression of MMPs and TIMPs at mRNA and protein levels in PDL cells (Bolcato-Bellemin et al., 2000; Huang et al., 2008; Kook et al., 2011; Lisboa et al., 2009; Ziegler et al., 2010). The expression patterns vary under different modes of mechanical strains. Shear stress, which is produced by torsion and shear between teeth and alveolar bone, is the main type of mechanical stress loaded on PDL. PDL is also considered as a kind of porous tissue containing an extensive vascular network, criss-cross fibers and interstitial fluid (Bergomi et al.). When teeth are loaded, interstitial fluid is squeezed out of the space around the PDL cells and generates fluid shear stress on the PDL cells. van der Pauw et al. (2000) reported that fluid shear stress significantly elevated release of nitric oxide, PGE2 and decreased expression of

Abbreviations: PDL, periodontal ligament; PGE2, prostaglandin E2; IL-8, interleukin-8; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase; MIP-1α, macrophage inflammatory protein-1α; AP-1, activator protein 1; Egr-1, early growth response protein 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; BSA, albumin from bovine serum; ECM, extracellular matrix; TGF-β, transforming growth factor beta; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ADAM, a disintegrin and metalloproteinase.

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tissue non-specific alkaline phosphatase activity. Maeda et al. (2007) demonstrated that fluid shear stress increased IL-8 mRNA expression but decreased MIP-1α mRNA expression. These investigations indicated that fluid shear stress plays a role in periodontal tissues remodeling. We hypothesize that fluid shear stress can regulate MMPs and TIMPs system and is involved in PDL tissue turnover process.

Matrix metalloproteinase (MMP) constitutes a highly homologous Zn^{2+} endopeptidases family of over 25 members. MMPs can digest numerous varieties of extracellular matrix components and play a principal role in tissue remodeling (Page-McCaw et al., 2007; Varghese, 2006). The activities of MMPs are precisely regulated by their endogenous inhibitors, tissue inhibitors of metalloproteinase (TIMP) in 1:1 ratio. Regulation of the balance of MMPs and TIMPs may associate with ECM turnover, inflammation, cell growth and migration (Brew and Nagase, 2010). The MMPs and TIMPs system regulated by mechanical strain has been widely reported in endothelia cells (Magid et al., 2003; Yamane et al), smooth muscle cells (O'Callaghan and Williams, 2000; Palumbo et al., 2000) and PDL cells (Kook et al., 2011; Ziegler et al., 2010). Among MMP family, MMP-1 which digests collagen I, II, III, VII, X and gelatins serves as an initiator for major ECM proteins destruction. MMP-2 mainly cleaves collagen I, III, IV and gelatin, elastin, fibronectin. TIMP-1 and 2 inhibit the activity of most MMPs (Birkedal-Hansen et al., 1993; Sternlicht and Werb, 2001).

Mitogen-activated protein (MAP) kinases are phosphorylated at specific serines and threonines for subsequent signal transduction. It consists of extracellular signal-regulated kinase (ERK), c-Jun *N*-terminal kinase (JNK) and p38. MAP Kinases control a vast array of physiological processes, such as gene expression, cell growth and apoptosis (Johnson and Lapadat, 2002). MAP kinases are also regarded as the major cellular components involved in the mechanotransduction. Mechanical strain transiently activates MAP kinases and leads to subsequently activation of downstream transcriptional factors such as AP-1, NF-κB and Egr-1, which induce other signaling proteins expression (Hughes-Fulford, 2004). The role of MAP kinases in mechanical stresses induced MMPs/TIMPs expression has been investigated previously (Im et al., 2012; Kook et al., 2011; Ziegler et al., 2010). ERK pathway was involved in the tensile force-mediated MMP-1 and MMP-13 expression in PDL cells (Kook et al., 2011) and osteoblasts (Yang et al., 2004). P38 pathway involved in tensile stress induced MMP-2 activation in arteries (Grabellus et al., 2007).

In present study, we investigated how fluid shear stress regulated MMP-1, 2 and TIMP-1, 2 expression. Our study demonstrated that fluid shear stress regulated MMP-1, 2 and TIMP-1, 2 expression. ERK pathway was essential to fluid shear stress induced MMP-1 increase and P38 mediated MMP-2 upregulation by fluid shear stress.

2. Materials and methods

2.1. Cell culture

Human periodontal ligament cells were obtained from extracted third molars of healthy young donors (12–28 years) for orthodontic reasons. Informed consents of patients were obtained before surgery. Only the middle of the tooth roots was obtained to avoid the intermixture of gingivae and dental pulp. PDL digested with 3 mg/ml collagenase I at 37 °C for 40 min. After centrifuging for 10 min at 8000 rpm, fresh culture medium consisting of Dulbecco's Modified Eagle's Medium (DMED, Gibco, USA) with 10% fetal bovine serum and penicillin/streptomycin were added. Cell cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. PDL cells outgrew after one week. 0.25% trypsin (Sigma, USA) was used to passage the cells. Immuno-localization vimentin revealed that all cells were stained positive. Cells were negative for cytokeratin. Passage 3 to 6 of PDL cells were used in the experiments.

2.2. Mechanical strain devices

A parallel plate flow chamber was used to generate fluid shear stress as described previously (Huang et al., 2010). The entire flow chamber was placed in a humidified atmosphere of 5% CO_2 in air at 37 °C. PDL cells were cultured on glass and in DMEM with 10% fetal bovine serum and penicillin/streptomycin. Cells were exposed to a steady laminar shear flow when they were nearly 90% confluence. Cell samples and the medium were collected for further testing after 2, 4, 8 and 12 h of stimulation. PDL cells cultured at static station were considered as control.

2.3. Immunofluorescence (IIF)

PDL cells were washed with ice cold PBS, fixed with Paraformaldehyde (4%, 4 °C) for 20 min. Cells were permeabilized with 0.1% Triton X-100 in PBS and blocked by 4% BSA for 2 h at room temperature. Texas red isothiocyanate-conjugated phalloidin (Molecular Probes, Eugene, OR) at a dilution of 1:100 was added to cells for 1 h at room temperature. After wash with PBS for four times, DAPI (Sigma, USA) was used to stain the nucleolus. All fluorescent staining images were taken under a confocal microscope (Leica TCS NT) using a 63× oil immersion objective lens.

2.4. Total RNA extraction and RT-PCR

Cells were harvested after fluid shear stress stimulation and total RNA was isolated using TRIZOL reagent (Invitrogen, NY, USA). 2 μg of total RNA was used as a template to synthesize cDNA according to the manufacturer's instructions (Takara, Shiga, Japan). 1 μl of the cDNA was added to each polymerase chain reaction (PCR) amplification as template. For the MAP-kinase inhibition experiment, 10 μM of SB203580, PD98059 and SP600125 (all from Santa Cruz, CA, USA) for specific inhibition of phospho-p38, phospho-ERK and phosphor-JNK were preincubated 1 h with PDL cells before fluid shear stress loaded.

2.5. Quantitative real-time PCR

Quantification of MMP-1 mRNA was performed using an iQ^{TM}5 real-time PCR System (Bio-Rad Laboratories, Inc. USA). 1 μl of cDNA and 1 μl of primer (5 μM) were incubated with the double stranded DNA dye SYBR Green I (Takara, Shiga, Japan) in a total volume of 25 μl. MMP-1, 2, TIMP-1, 2 and GAPDH primer sequences were described in Table 1. All reactions were preincubated at 95 °C for 3 min, and then the cycles are 10 s at 95 °C, and 34 s at 60 °C (GAPDH, MMP-1, TIMP-2) or 56 °C (MMP-2, TIMP-1). Each experiment was repeated at least in triplicate. Measured MMP-1, 2 and TIMP-1, 2 mRNA levels were normalized to the mRNA level of GAPDH.

2.6. Enzyme-linked immunosorbent assays (ELISAs)

ELISA assay was established to determine quantitative expression of MMP-1 and 2 secreted to the medium. Purified human antigens of MMP-1 and MMP-2 with known concentrations were used to build standard curves and all steps according to the manufacturer's instructions (RayBiotech, Inc, USA). The MMP-1 and MMP-2 concentration in conditioned medium of control groups and fluid shear stress stimulated groups were quantified by extrapolating from the standard curves. Each sample was assayed in duplicate.

2.7. Western blot analysis

Total protein was extracted from human PDL cells and quantified with a BCA protein assay kit (Pierce, Rockford, IL). Approximately 40 μg of the extracted proteins

Table 1
Primer sequence of MMP-1, 2, TIMP-1, 2 and GAPDH for real time PCR.

Gene name	Primer sequence	
MMP-1	Forward	5′-AGCTAGCTCAGGATGACATTGATG-3′
	Reverse	5′-GCCGATGGGCTGGACAG-3′
MMP-2	Forward	5′-TGCTGGAGACAAATTCTGGAGATAC-3′
	Reverse	5′-ACTTCACGCTCTTCAGACTTTGG-3′
TIMP-1	Forward	5′-CCCAGAGAGACACCAGAGAAC-3′
	Reverse	5′-CACGAACTTGGCCCTGATGAC-3′
TIMP-2	Forward	5′-GCACATCACCCTCTGTGACTT-3′
	Reverse	5′-AGCGCGTGATCTTGCACT-3′
GAPDH	Forward	5′-TGAACGGGAAGCTCACTGGC-3′
	Reverse	5′-TCCACCACCCTGTTGCTGTA-3′