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Cyclic stretch induced gene expression of extracellular matrix and adhesion molecules in human periodontal ligament cells

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ABSTRACT

Objective: Periodontal ligament (PDL) cells play an important role in maintaining periodontal homeostasis upon force loading caused by mastication or orthodontic force. Previous studies revealed stretch-induced realignment of human PDL cells, but the mechanism for this phenomenon still remains unclear. As extracellular matrix (ECM) and adhesion molecules play critical roles in cell migration and alignment, this study aimed to identify mechanoresponsive genes related to ECM and adhesion in human PDL cells.

Design: Human PDL cells were exposed to 10% stretch strain for 6 or 24 h, and the expression of 84 genes related to ECM and adhesion were analyzed with real-time PCR array. The protein expression of integrin α5 was examined by Western blot and flow cytometric analysis.

Results: Among the genes screened, 6 were up-regulated and 3 were down-regulated after 6 h stretch. There were 12 up-regulated and 2 down-regulated genes after 24 h stretch. These differentially expressed genes included genes encoding cell-cell adhesion molecules (*CD44*, *ICAM1*), cell-matrix adhesion molecules (*ITGA5*, *ITGA6*, *ITGAL*, *ITGB2*, *SPP1*), basement membrane constituents (*SPARC*, *TNC*), collagens and ECM constituents (*COL5A1*, *COL11A1*, *FN1*), ECM proteases (*ADAMTS1*, *ADAMTS8*, *MMP8*) and inhibitors (*TIMP1*), as well as other adhesion-related molecules (*CTGF*, *CTNND2*, *TGFBI*, *CLEC3B*). Both the cytosolic and membrane integrin α5 protein levels were up-regulated in response to stretch.

Conclusion: This study identified several force-sensitive genes related to ECM and adhesion in stretched human PDL cells and should facilitate future studies on the stretch-induced cell realignment and mechanic force related periodontal remodelling by providing potential target genes.

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Abbreviations: PDL, periodontal ligament; ECM, extracellular matrix; CSU, cell strain unit; DMEM, Dulbecco's modification of Eagle's medium; FBS, fetal bovine serum; PC, personal computer; PBS, phosphate buffered saline; PCR, polymerase chain reaction; ANOVA, analysis of variance; LSD, least-significant difference; MFI, mean fluorescence intensity.

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1. Introduction

Periodontal ligament (PDL) is a specialized connective tissue, which connects cementum and alveolar bone.[1] PDL cells in this tissue are directly subject to mechanical stress during occlusal function or orthodontic tooth movement, and have been reported to have played an important role in maintaining periodontal homeostasis and in periodontal remodelling.[2] Many studies have shown that mechanical stress induced biological changes in PDL cells.[3–6] Previously, several researchers have observed the phenomenon of realignment of PDL cells under stretch conditions *in vitro*.[7,8] Our recent researches also demonstrated the stretch-induced realignment of human PDL cells: *in vitro* cultured human PDL cells inclined parallel to each other and aligned their long axes perpendicular to the stretching force vector.[9,10]

Cells attach to proteoglycans and glycoproteins in the extracellular matrix (ECM) substrate *via* adhesion molecules on their cell membrane surface to define tissue shape, structure, and function.[11] The *in vitro* cultured PDL cells attached to the matrix on the silicone membrane of the mechanical strain loading systems such as the Flexercell system and the cell strain unit (CSU),[8,10] *via* these adhesion molecules. The stretch-induced realignment of the PDL cells will inevitably involve the detachment and reattachment of the cells to the matrix and the adjacent cells. Therefore it is reasonable to suggest that ECM proteins and adhesion molecules are involved in the mechanical stress-induced biological changes in PDL cells, including the realignment of the cells. As making and breaking cellular contacts with other cells and the ECM plays important roles in both normal processes (such as cell growth, division, differentiation, and migration) and pathophysiological processes (such as wound healing and inflammation),[11] it is meaningful to investigate the role of ECM and adhesion molecules in the mechanotransduction in PDL cells subjected to mechanical stresses during occlusal function or orthodontic tooth movement.

Recently, real-time PCR array has been introduced to cytomechanical research on PDL cells to profile gene expression under stretch condition.[12–14] Most recently Saminathan et al. reported 15 adhesion-related genes which showed differential expression in cultured human PDL cells after 6–24 h cyclic stretch of 12% strain, by employing real-time PCR array analysis,[15] and it's the first and only report to date on the effect of mechanical force on the expression of adhesion-related genes by human PDL cells, to our knowledge.

When we were probing into the role of ECM and adhesion molecules in the mechanotransduction in human PDL cells, we also found some differentially expressed genes which were related to ECM and adhesion in response to cyclic stretch. Among these genes, some have been reported previously, while some have not. This article presents these new findings and we hope these findings shall contribute to the identification of mechanoresponsive adhesion-related genes and help us better understand the mechanotransduction in PDL cells by providing potential target genes for further studies.

2. Materials and methods

2.1. Preparation of human PDL cells

Human PDL cells were obtained from 2 healthy premolars (extracted for orthodontic reasons) of a 11 years old female donor, after obtaining informed consent from her parents. The protocol for harvesting human tissue from extracted teeth was approved by the Ethics Committee of Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (Reference: [2008]17). Human PDL cells were cultured as previously described.[9,10] PDL on the middle surface of the root was collected with a sterile scalpel and pieces of PDL were attached to a cell culture flask and cultured in Dulbecco's modification of Eagle's medium (DMEM, Gibco, Grand Island, NY, USA) supplemented with 20% (v/v) foetal bovine serum (FBS, Hyclone, Logan, Utah, USA) and five-fold reinforced antibiotics (500 U ml^{-1} penicillin, 500 $\mu g\ ml^{-1}$ streptomycin, Sigma, St. Louis, MO, USA) at 37 °C in a humidified atmosphere of 5% CO_2. Cells that grew out from the extracts were passaged in DMEM supplemented with 10% (v/v) FBS and antibiotics (100 U ml^{-1} penicillin, 100 $\mu g\ ml^{-1}$ streptomycin). Cells at passage 4–6 were used in the present study.

2.2. Stretch loading

Human PDL cells were stretched by a cell strain unit (CSU) which has been described previously.[9,10,14,16] The CSU includes a strainer, a controller and a personal computer (PC). Cells were seeded in a flexible-bottomed culture dish (diameter 60 mm) whose bottom is made of elastic silicon rubber (Q7-4750, Dow Corning Co., Midland, MI, USA). A spherical cap moves up and down repeatedly and stretches cells attached on the bottom of culture dish by deforming the elastic silicon bottom. All changes in stretch strain and movement of the spherical cap are controlled by the controller and PC. Cells were seeded in the flexible-bottomed culture dishes at a concentration of 1.5×10^6 cells per dish and reached confluence following 3 days' culture, and then were exposed to 10% stretch strain for 6 or 24 h at a frequency of 6 cycles min^{-1}, each cycle consisting of a 5 s stretch period followed by a 5 s relaxation period. The treatments were repeated 3 times for each of the 2 time intervals (6 and 24 h). Cells cultured in flexible-bottomed culture dishes placed in similar conditions but without stretch served as non-stretched controls. It is believed that a stretch strain no higher than 24% is reasonable for cultured PDL cells to mimic the strain which may be confronted by *in vivo* PDL cells.[17] Our recent researches demonstrated that notable realignment was induced after 6 h cyclic stretch with 10% strain in cultured human PDL cells and the realignment became more prominent after 24 h cyclic stretch.[9,10] Therefore, 10% stretch strain was chosen to load cells for 6 and 24 h in the present study. The loading frequency of 6 cycles min^{-1} (5 s stretch and 5 s relaxation) was the same to that in Matsuda et al.'s report and also our previous researches.[4,10]

2.3. RNA isolation and cDNA synthesis

Total RNA from cells in each group was isolated by using Trizol reagent (Invitrogen, Carlsbad, CA, USA) as previously