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Oligonucleotide array analysis of cyclic tension-responsive genes in human periodontal ligament fibroblasts

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Abstract

Mechanical stress results in differential gene expression that is critical to convert the stimulus into biochemical signals. Under physiological stress such as occlusal force, human periodontal ligament fibroblasts (HPLF) are associated with homeostasis of periodontal tissues however the changes in response to mechanotransduction remain uncharacterized. We hypothesized that cyclic tension-responsive (CT) genes may be used to identify a set of fundamental pathways of mechanotransduction. Our goal was to catalogue CT genes in cultured HPLF. HPLF were subjected to cyclic tension up to 16 h, and total RNA was isolated from both tension-loaded and static HPLF. The oligonucleotide arrays analysis revealed significant changes of mRNA accumulation for 122 CT genes, and their kinetics were assigned by the *K*-means clustering methods. Ingenuity Pathway Analysis was completed for HPLF mechanotransduction using 50 CT genes. This analysis revealed that cyclic tension immediately down-regulated all nuclear transcription factors except *v-fos FBJ murine osteosarcoma viral oncogene homolog* (FOS) reacting as an early responsive gene. In turn, transcription factors such as *tumor protein p53 binding protein 2* (TP53BP2), and extra-nuclear molecules such as *adrenergic receptor β2* (ADRB2) were up-regulated after 1–2 h, which may result in fundamental HPLF functions to adapt to cyclic tension. Subsequent inhibition assays using Y27632, a pharmacologic inhibitor of Rho-associated kinase (ROCK), suggested that HPLF has both ROCK-dependent and ROCK-independent CT genes. Mechanical stress was found to effect the expression of numerous genes, in particular, expression of an early responsive gene; FOS initiates alteration of HPLF behaviors to control homeostasis of the periodontal ligament.

Keywords: Cyclic tension-responsive genes; Oligonucleotide array; Human periodontal ligament fibroblasts; Pathway analysis; Rho-associated kinase

1. Introduction

Mechanical stress is thought to play important roles in regulating the function, metabolism or maintenance of connective tissues such as periodontal ligaments. Abnormal mechanical stress may cause destruction of

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tissues, and affects many pathological situations. A better understanding about cellular adaptation to changes in mechanical stress may help us design a new strategy in the treatment and prevention of mechanical stress-related disorders such as trauma, tooth/bone resorption, and atrophy or destruction of the connective tissues.

Mechanotransduction is the process by which cells transduce physiological force-induced signals into biochemical responses, and involves differential gene expression that is necessary for mediating adaptations to mechanical stress (Ingber, 2003). Evidence has accumulated that mechanical stress-induced alterations in cell shape and structure are critical for the control of cell contraction, migration, growth, differentiation, and apoptosis (Chicurel, Chen, & Ingber, 1998; Khan & Sheetz, 1997). Mechanical stresses are either transmitted to cells from the extracellular matrix, or generated within the contractile cytoskeleton of individual cells (Wang, Butler, & Ingber, 1993). Force-induced assembly of the focal adhesions and the cytoskeleton are crucial for inducing mechanotransduction in the cytoplasm (Geiger & Bershadsky, 2001; Ingber, 1997), and mediated in large by activation of the Ras-homolog of small GTPase (Rho) (Amano et al., 1997; Ridley & Hall, 1992). Rho acts as a molecular switch that cycles between an inactive GDP-bound and an active GTP-bound conformation interacting with downstream targets (effectors) to elicit cellular responses, and regulate the actin cytoskeleton (Etienne-Manneville & Hall, 2002). Rho-associated kinases (ROCK), the first and the best-characterized Rho effectors, are serine/threonine kinases that are involved in diverse cellular functions including motility, adhesion, smooth-muscle contraction, neuritic outgrowth, centrosome positioning, apoptosis, actin cytoskeleton organization and cell-size regulation. Thus, ROCK have the potential to contribute to various physiological and pathological states (Maekawa et al., 1999; Riento & Ridley, 2003). The Rho/ROCK pathway has been shown to be crucial for mediating mechanotransduction (Chiquet, Renedo, Huber, & Fluck, 2003; Numaguchi, Eguchi, Yamakawa, Motley, & Inagami, 1999) however the precise mechanisms that link mechanical stress to subsequent changes in cellular activities remains unknown.

Periodontal ligament is a connective tissue lying between alveolar bone and tooth root, and not only is a source of osteogenic cells, but also may contain multipotent stem cells that could regenerate cementum and periodontal ligament (Seo et al., 2004). Periodontal ligament fibroblasts (HPLF), in particular, can differentiate in response to a variety of extracellular stimuli to either maintain homeostasis or participate in remodeling of the periodontal ligament, and repair or regeneration of the surrounding hard tissues (McCulloch, Lekic, & McKee, 2000). These cells are subject to continuous mechanical stress under physiological condition the result of both occlusal and masticatory forces. Several studies have been published that link mechanical stress to intracellular signaling molecules in HPLF (Kletsas, Basdra, & Papavassiliou, 2002; Myokai et al., 2003) however the molecular mechanisms of HPLF mechanotransduction remain elusive. Since it is likely that the expression of many genes is altered in response to mechanical stress it will become necessary to further catalogue these genes with respect to both temporal and subcellular distribution. We hypothesized that comprehensive analysis of cyclic tension-responsive (CT) genes may provide data useful to understand the fundamental mechanisms of mechanotransduction in HPLF.

The GeneChip probe array technology allows a systematic analysis of gene expression changes, and provides significant information for a wide variety of basic biological processes, including development (Abeyta et al., 2004), tumorigenesis (Kasamatsu et al., 2005), and the immune system (Calvano et al., 2005). The array technology allows simultaneous monitoring of the activities of numerous genes because of the technique's sensitivity, specificity, and reproducibility. In the present study, HPLF were loaded with cyclic tension, and the CT genes were identified using the GeneChip probe array. The CT genes identified were further analyzed on a cellular pathway map based on Ingenuity Systems' Pathway Knowledge Base. This was done to identify genetic networks involved in mechanotransduction in HPLF. Moreover, to elucidate the effect of the Rho/ROCK pathway on CT gene expression, we analyzed the CT gene expression in the presence or absence of a pharmacologic inhibitor of ROCK activity.

2. Materials and methods

2.1. Cell culture and mechanical stress

Periodontal ligament was obtained from periodontally healthy and non-carious teeth extracted for orthodontic reasons from four donors, three Japanese female (21, 24, and 17 years old) and a Japanese male (22 years old), with informed consent. Prior to the experiment, the protocol (no. 43) was approved by the Research Ethics Committee for Human Genome/Gene Analysis Research in Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences. HPLF were maintained and expanded in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal