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Cyclic strain induces FosB and initiates osteogenic differentiation of mesenchymal cells

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Abstract

Mechanical loading is crucial for bone remodeling and osteoblast differentiation. FosB belongs to the AP-1 family of transcription factors, a group of proteins known to regulate osteoblast differentiation and bone formation. In mice, FosB is rapidly induced by mechanical stress at the transcriptional level. The aim of this study was to determine the effect of different mechanical stretch patterns on FosB gene expression and on osteogenic differentiation of human osteoblast precursor cells. Human bone-marrow-derived mesenchymal precursor cells were grown in flexible silicone dishes and stimulated by a daily application of three rounds of 2 h of cyclic stretch of either 2% or 8% elongation at 1 Hz on 3 consecutive days using a special motor-driven apparatus. By real-time PCR, we quantified FosB mRNA and the expression of genes involved in osteoblast differentiation such as Runx2 and collagen 1 to determine the osteogenic effect of mechanical stretch. Stretching induced FosB transcription and the expression of osteoblast markers in partly committed human mesenchymal precursor cells in a stretch- and time-dependent manner. We conclude that cyclic stretch-induced FosB expression and the upregulation of osteoblast genes plays a role in osteogenic differentiation of human mesenchymal precursor cells.

Keywords: Mesenchymal cells; Mechanical strain; Osteogenic differentiation; Transcriptional regulation; Real-time PCR

Introduction

Bone is a highly dynamic tissue that undergoes continuous remodeling involved in the maintenance of its architectural integrity and metabolic activity, two critical and competing functions of bone. The microstructure of bone depends on genetic determinants and on the continuous response of the skeleton to mechanical cues, adding new bone to withstand increased amounts of loading, and removing bone due to unloading or disuse during immobilization or in space (Suva et al., 2005). Mechanical stress influences bone homeostasis in post-natal remodeling processes (McNamara and Prendergast, 2007), fracture healing (Gardner et al., 2006), and in osseointegration of orthopedic implants (Leucht et al., 2007). This strain-induced adaptation and remodeling ability is frequently disturbed in low bone-mass diseases such as osteoporosis and it is therefore clinically important (Sterck et al., 1998). Thus, it is

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crucial to understand mechanisms that form a mechano-molecular interface that couples physical stimuli to intracellular molecular events, thereby modulating the genetic program and the fate of the target cells. A better understanding of the mechanisms by which mechanical strain controls skeletal remodeling and induces bone formation could be very helpful in developing novel therapeutic strategies for treating low-bone mass diseases using pharmacological approaches.

The prevailing model suggests that a key factor of bone remodeling is a strain-driven oscillatory flow of extracellular fluid in the lacunar–canalicular network in bone that is sensed by osteocytes, the terminally differentiated osteoblasts buried in extracellular matrix. Osteocytes are metabolically active and highly interconnected with each other and with other types of cells via numerous dendrites, allowing osteocytes to communicate over a distance within the bone (Bonewald, 2005, 2007). However, evidence exists that osteoblasts and even their precursors, the mesenchymal bone marrow stromal cells (BMSCs), also perceive and translate mechanical stimuli (Jagodzinski et al., 2004; Mauney et al., 2004; Inoue et al., 2004; Ignatius et al., 2005).

Different types of mechanical stimulation, such as fluid-shear stress, compression, and axial longitudinal stretch, are widely used in studies investigating the effect of mechanical stimulation on cell behavior. Although it is not clear yet what the specific differences or similarities are between different stimuli, all types of mechanostimulation have been shown to activate a divergent array of key anabolic intracellular effectors such as nitric oxide (NO) (Klein-Nulend et al., 1998), prostaglandins (Siddhivarn et al., 2006), and intracellular calcium (Chen et al., 2000), and stimulate signaling events in the cilium (Xiao et al., 2006). Most of these signaling cascades promote cell proliferation and differentiation and activate members of the mitogen-activated protein kinase (MAPK) family, including the extracellular signal-regulated kinases (ERK)-1 and 2 (Simmons et al., 2003). These signaling events activate a variety of osteoblast-related genes, including Runx2, the master regulator of osteoblast differentiation that facilitates osteoblast lineage commitment (Ducy et al., 1997; Xiao et al., 2000; Ge et al., 2007).

Activator protein-1 (AP-1) transcription factors are dimers of Fos (c-Fos, FosB, ΔFosB, Fra-1, Fra-2) and Jun (c-Jun, JunB, JunD) leucine zipper-containing proteins. Early activation of some AP-1 factors, particularly c-Fos, is known to occur in response to mechanical stimulation (Kletsas et al., 2002). Members of the AP-1 family of homo-/heterodimeric transcription factors bind a consensus sequence in the promoters of several genes that are essential for osteoblast differentiation and function, such as alkaline phosphatase, collagen type 1, osteopontin, osteocalcin, and Runx2 (Ducy and Karsenty 1995; Ducy et al., 1997).

Inoue et al. demonstrated that mechanical strain rapidly induces the transcription of the FosB gene *in vivo*, using an unloading mouse model and *in vitro*, using mouse calvarial osteoblasts (Inoue et al., 2004). The mechano-induced FosB transcription was caused by the activation of ERK1/2 by a Ca^{2+} influx. These data strongly suggest that FosB transcription participates in linking mechanical cues via transcriptional machinery into activation of an osteogenic program in bone cells of mice.

The potential physiological and pharmacological relevance of this observation led us to determine whether early immediate induction of the FosB gene expression by mechanical stimulation also occurs in mesenchymal progenitor cells of human origin. In addition, we examined the effect of different degrees of stress on induction of FosB expression and whether the rise in FosB levels was accompanied by an activation of the osteogenic program in mesenchymal precursor cells of human nature.

Materials and methods

Sample acquisition

Human bone marrow aspirates were obtained during routine orthopedic surgical procedures involving exposure of the iliac crest. The institutional ethical committee approved the procedure and written informed consent was obtained from each patient. Bone marrow aspirates (20–80 ml) were harvested from seven healthy donors using a bone marrow biopsy needle inserted through the cortical bone of the iliac crest. Four donors were female and three donors were male. The average age was 32.5 ± 6.2 years. Aspirates were cooled on ice immediately and processed within 1 h.

Primary cell culture

Human bone marrow aspirates were divided in aliquots of 25 ml, transferred into 50 ml conical tubes, and mixed with 25 ml standard tissue culture medium (DMEM/Ham's F-12 1:1 with L-glutamine, 10% human serum, 20 μg/ml amphotericin B, 100 U/ml penicillin, 100 μg/ml streptomycin) (Invitrogen, Karlsruhe, Germany). After centrifugation at $400 \times g$ for 5 min, the pellet was transferred onto 45 ml Percoll-Paque® (Pharmacia, Freiburg, Germany) density gradient solution and centrifuged for 15 min at $400 \times g$. Fourteen ml of the nucleated cell-containing supernatant were mixed with 35 ml of tissue culture medium and centrifuged at $400 \times g$ for 5 min. The supernatant was removed and the pellet was suspended in 20 ml of tissue culture medium, plated in a 75 cm^2 tissue culture flask, and incubated for 5 days in a humidified atmosphere with 5% CO_2 at