Effects of mechanical strain on proliferation and differentiation of bone marrow stromal cell line ST2

Mariko Koike · Hitoyata Shimokawa · Zuisei Kanno
Keiichi Ohya · Kunimichi Soma

Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
Tel. +81-3-5803-5529; Fax +81-3-5803-5529
e-mail: mariko.orts@tmd.ac.jp

Pharmacology, Department of Hard Tissue Engineering, Division of Bio-Matrix, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan

Received: January 19, 2004 / Accepted: September 14, 2004

Abstract

Differentiation of mesenchymal stromal cells into osteoblasts is regulated by many factors including growth factors, cytokines, and hormones. Mechanical stress has been considered to be an important factor in bone modeling and remodeling. However, biological responses of stromal cells to mechanical stimuli are still unknown. To show the correlation between magnitude of mechanical strain and differentiation of stromal cells into osteoblasts, we investigated the proliferation and the expression of osteoblast-related genes in stromal cell line ST2 that is in the process of osteoblastic differentiation by treatment with ascorbic acid and beta-glycerophosphate, under 0.8%–15% elongation using the Flexercell Strain system. The expression of osteoblast-related genes was analyzed by real-time quantitative polymerase chain reaction (PCR). Cell proliferation significantly increased at 5%, 10%, and 15% elongation compared to that of unloaded controls. Alkaline phosphatase (ALPase) activity significantly increased at 0.8% and 5% elongation but decreased at 10% and 15% elongation. At 1 h and 6 h, mRNA level of Cbfa1/Runx2 increased at lower magnitudes of strain (0.8% and 5% elongation) but decreased at higher magnitude of strain (15% elongation). At 24 and 48 h, Cbfa1/Runx2 and osteocalcin mRNAs decreased at 5%, 10%, and 15% elongation, whereas cell proliferation and expression of type I collagen mRNA increased at the same elongation. These results indicate that mechanical strain stimulates osteoblastic differentiation of stromal cells at low magnitudes of strain.

Key words

Mechanical strain · Bone marrow stromal cell · ST2 · Cbfa1/Runx2 · Real-time PCR

Introduction

Mechanical stress is known to be one of the important factors in the regulation of bone modeling and remodeling [1–3]. The effects of mechanical stress on cells are dependent on the magnitude [4,5], duration [6], and frequency [6,7] of mechanical stress. To date, several investigators have studied the responses of bone cells to mechanical stresses such as stretch [4,5,8–13], fluid flow [14], four-point bending [15], and hydrostatic pressure [6].

Bone is formed by proliferation and differentiation of mesenchymal stem cells into mature osteoblasts [16,17]. Recently, it was established that Cbfa1/Runx2, a member of the runt family of transcription factors, is a key regulator of osteoblast recruitment and differentiation from mesenchymal stem cells [18–20]. Cbfa1-binding motifs (osteoblast-specific cis-acting element, OSE2) exist in the promoter region of osteoblast phenotype-related genes encoding osteopontin [21], osteocalcin [22], and type I collagen [23] and regulate their expression. Overexpression of Cbfa1/Runx2 in C3H10T1/2 cells induces the expression of osteoblast-related genes [18,24], whereas Cbfa1 null mice completely lack bone formation. Heterozygous loss of this gene in mice and humans characterizes the cleidocranial dysplasia (CCD) syndromes [19,20,25]. Recently, it was reported that mechanical stress regulated the expression of the Cbfa1/Runx2 gene. Ahdjoudj et al. reported that skeletal unloading decreased Cbfa1/Runx2, osteocalcin, and type I collagen mRNA and reduced bone formation [2]. Ziros et al. described mechanical stress eliciting direct effects on induction of expression and DNA-binding potential of Cbfa1 [9]. Many papers have been published on the effects of mechanical stress affecting osteoblasts or osteoblastic cells; however, little is known about the effects of mechanical stress on proliferation and differentiation of bone marrow stromal cells.
To show the correlation between magnitudes of mechanical stress and differentiation of stromal cells into osteoblastic cells, we investigated the effects of various magnitudes of mechanical strain on the bone marrow stromal cells that were the initial stage in the process of osteoblastic differentiation by treatment with ascorbic acid and β-glycerophosphate. In this study, we examined cellular proliferation, ALPase activity, and mRNA level of osteoblast-related genes under different magnitudes of mechanical strain. Here we report that low magnitudes of strain increased Cbfa1/Runx2 mRNA and ALPase activity, whereas high magnitudes of strain decreased Cbfa1/Runx2 mRNA and ALPase activity and increased proliferation. Thus, we show that the bone marrow stromal cells, ST2, respond to mechanical stress in a magnitude-dependent manner.

Materials and methods

Cell culture

The bone marrow stromal cell line ST2 was obtained from the Riken Cell Bank (Tsukuba, Japan) [26]. Cells were maintained in RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS, USA), antibiotics (100 U/ml penicillin G and 100µg/ml streptomycin sulfate; Sigma, St. Louis, MO, USA) at 37°C in a humidified atmosphere of 95% air and 5% CO2. After reaching 70% confluence, the cells were detached by treatment with 10% trypsin-EDTA (Sigma), replaced on six-well, flexible-bottomed plates (type I collagen coated, Flex I; Flexcell International, Mckeesport, PA, USA) at a density of 1 x 10⁴ cells/cm². We used early-passaged cells (<9 passages) in all experiments [27]. Cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 0.2 mM ascorbic acid 2-phosphate (Wako Pure Chemical, Osaka, Japan), and 5 mM β-glycerophosphate (Sigma) for 7 days until 90% confluent. To investigate the mechanical stress on differentiation of preosteoblasts, we used ST2 that had not yet expressed osteoblastic phenotype such as alkaline phosphatase (ALPase) activity at the stage of 7-day-culture [26,28] (Fig. 1). The medium was changed every 3 days. Then, 10% FBS medium was replaced with 1% FBS and cultured overnight before strain force was applied to the cells.

Application of strain force

The plates were placed on a Loading Station (Flexcell International) with a diameter of 25 mm to produce equibiaxial strain [4]. According to the manufacturer’s instructions, there is a minimum vacuum level at which static friction is overcome on the loading stations. The membrane does not begin to stretch until this point. For the 25-mm loading stations, this level is approximately 0.70% elongation. In this experiment, cells were subjected to mechanical strain of 0.8%, 5%, 10%, and 15% elongation at 1 Hz for 2 days using the Flexcell Strain Unit (FX 3000; Flexcell International). This strain unit consisted of a computer-controlled vacuum unit and baseplates to hold the culture dishes. The computer system controlled the frequency of deformation and the negative pressure applied to the culture plates. Control cells were cultured on similar plates and kept in the same incubator without mechanical strain.

Assay of cell proliferation

Cell morphology was observed by phase-contrast microscopy. Cell proliferation was assessed by the Cell Counting Kit-8 (CCK-8; Dojin, Tokyo, Japan). This system consisted of WST-8 (2-[2-methoxy-4-nitrophenyl]-3-[4-nitrophenyl]-5-[2,4-disulphophenyl]-2H-tetrazolium, monosodium salt) that produced a water-soluble formazan dye upon bioreduction in the presence of an electron carrier [29]. WST-8 is reduced by dehydrogenase in cells to give a yellow-colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. Cultured cells were incubated with CCK-8 solution for 1 h at 37°C, and the absorbance at 450 nm was measured with a spectrophotometer.

Assay of ALPase activity

After exposure to stretch, cells were washed twice with Tris-buffered saline (TBS), scraped into extraction buffer solution [10mM Tris (pH 7.4), 0.9% NaCl, 0.1% Triton X-100, and protease inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany)], sonicated, and centrifuged at 10000g for 15 min at 4°C. The supernatants were then used for ALPase assay and protein determination. ALPase activity was measured using the ALP B-test Wako kit (Wako), and the absorbance at 405 nm was read on a

![Fig. 1. Time course of alkaline phosphatase (ALPase) activity in ST2 cells. ST2 cells in six-well plates (1 x 10⁴ cells/cm²) were cultured with RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), ascorbic acid, and β-glycerophosphate. ALPase activity of ST2 was induced after 11 days of culture indicating osteoblastic differentiation of ST2 cells. ST2 cells had low levels of ALPase activity on day 7. The values are mean ± SD. There were significant differences of *P < 0.05 and **P < 0.001](image-url)