STRETCH-INDUCED ERK PHOSPHORYLATION DEPENDS ON DIFFERENTIATION STAGE OF OSTEOBLASTS

*Jansen JHW, **Weyts FAA, ***Gussekloo-Westbroek I., Verhaar JAN, van Leeuwen JPTM, Weinans H
+Orthopaedic Research Laboratory, Erasmus MC, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

INTRODUCTION

Although mechanical loading is known to influence bone cell metabolism and bone structure, little is known about the mechanisms that enable bone cells to respond to mechanical loading. Mitogen-activated protein kinases (MAPKs) are a family of proteins that play a key role in the transfer of many extracellular signals from membrane to nucleus. Activation of extracellular signal-regulated kinase (ERK, a MAPK subgroup) has been shown to regulate differentiation of mesenchymal stem cells towards the osteogenic lineage and to be essential for growth and differentiation of human osteoblastic cells [1,2]. Recently, increased phosphorylation of ERK (ERK-P) has been reported in response to mechanical stretch in chondrocytes and periodontal ligament cells, indicating that these cells can activate this intracellular pathway upon processing mechanical signals [3,4]. These experiments were performed at high deformation levels, since both cartilage and periodontal tissue is highly deformed. In previous experiments we showed the significance of the differentiation stage of osteoblasts for the translation of mechanical signals [5]. Here, we tested 1: whether human osteoblasts respond to physiological levels of stretch with increases of ERK phosphorylation, and 2: whether this response depends on the differentiation stage of the cells.

METHODS

Cell Culture

SV40-immortalized human fetal osteoblast cells (HFO) were generously provided by Prof. M. Mori, Sapporo Medical College, Japan. HFO cells are well characterized human osteoblasts that, when cultured in medium containing 10 mM β-glycerophosphate and 1 µM dexamethasone, enter a well defined pathway of osteogenic differentiation, resulting in mineralisation of the matrix after 21 days of culture [6]. At the start of experiments (day 0), 1.105 HFO cells were transfected to full confluence, in collagen I coated 6 well plates (Flexercell, McKeessport, PA). The cells were cultured in αMEM medium without phenol red, supplemented with 2% charcoal-treated fetal calf serum.

Stretching

Stretching experiments were performed with a loading unit (Flexercell, McKeessport, PA) inside a 37°C, 5% CO2 incubator. In this setup vacuum is applied to the area under 6 well plates with a flexible, silicon culture surface. The unit produces homogeneous, biaxial stretch strains and was adapted to produce strain levels from physiological to supraphysiological (0.4%-2.5%) with minimal vertical displacement of the silicon layer. Stretching was performed for 5, 10, 15, or 60 minutes at a frequency of 0.5 Hz after 7, 14, or 21 days of culture in osteogenic medium.

Analysis

Following stretching, cells were lysed and 10 µg total protein was analysed by SDS-PAGE followed by immunoblotting as described previously [7]. Primary antibodies used were monoclonal anti-ERK-P, or anti-ERK1/2 (both 1:2000, Cell Signaling). Immuno reactive bands were quantified using Quantity One (Bio-Rad) software. Static ERK-P levels served as control.

RESULTS

DNA and protein levels increased over time during the differentiation stages of the osteoblasts (data not shown). Both at 0.4% and 2.5%, stretch resulted in a rapid increase in ERK-P levels, whereas ERK levels did not change. Increase in ERK phosphorylation was already present after 5 minutes of cyclic stretch. Maximum ERK-P levels were found between 5 and 10 minutes of stretch (fig.1). Prolonged stretching resulted in a decrease of ERK-P signal towards baseline levels. These characteristics were similar at different days of culture. Differentiation stage of the osteoblasts affected both baseline ERK-P levels and the level of ERK-P response in reaction to stretching. Baseline (static) ERK-P levels were 12 times higher at day 21 compared to day 7 (fig.2). ERK phosphorylation in response to stretching (corrected for non-stretched controls) is highest at day 7, both at 0.4% and 2.5% of stretch (fig.3).

DISCUSSION

Various downstream actions as a result of ERK activation have been described in the literature [8,9]. Therefore the activation of ERK can be an interesting reporter of mechanotransduction. This study was undertaken to examine the involvement of the ERK pathway in the response of HFO cells to physiological stretch levels at selected differentiation stages. We have demonstrated that physiological stretch levels of 4000 microstrain can induce ERK phosphorylation in osteoblastic cells. Both this response to stretch as well as baseline ERK-P levels depended on the differentiation state of the cells. Day 7 cell cultures showed low ERK-P levels relative to day 14 and 21, particularly in the control (static) group. However, day 7 cultures showed highest fold increase over static control, indicating that they may be more responsive to mechanical loading in that stage than more mature, mineralized osteoblastic cells at day 14 or 21.

REFERENCES


Listing for additional author affiliation

** Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands

Paper #0131