ACTIVATION OF EXTRACELLULAR SIGNAL-REGULATED KINASE AND P38 MAP KINASE IS REQUIRED FOR MECHANICAL STIMULATION OF CHONDROCYTE PROLIFERATION

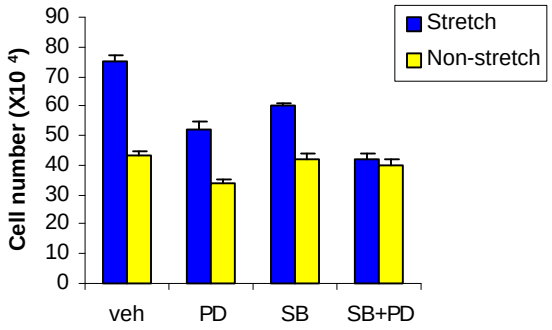
*+CHEN, Q., ZHEN, X., WU, Q.Q., and ZHANG, Y.
*+Musculoskeletal Research Laboratory Department of Orthopaedics & Rehabilitation, The Pennsylvania State University College of Medicine, Hershey, PA 17033.
Phone: 717-531-4835, Fax: 717-531-7583, email: qchen@ortho.hmc.psu.edu

Introduction:
It is well accepted that cartilage matrix deformation, as a result of mechanical loading, can regulate chondrocyte metabolism and behavior. For example, cyclic mechanical strain and hydrostatic pressure stimulate chondrocyte biosynthesis. However, very little is known about how mechanical signals are transduced within the cells, and ultimately to the nucleus, to activate biosynthesis and cellular activity. One group of the intracellular signaling molecules is the recently discovered mitogen-activated protein (MAP) kinases. MAP kinases are activated by upstream kinases induced by extracellular signals. The activated MAP kinases then translocate into the nucleus to phosphorylate transcriptional factors. As a result, biosynthesis may be stimulated and cell proliferation and differentiation altered. There are three major MAP kinase pathways. The ERK pathway (extracellular signal-regulated protein kinase) is mainly involved in transmitting signals to induce cell proliferation. The other two MAP kinase pathways, JNK (Jun-N-terminal kinase) and p38, are not activated primarily by mitogens but by cellular stress. Recent data have suggested that MAP kinases are activated by mechanical stimulation in a variety of cell types including cardiac myocytes and endothelial cells. The aim of this study is to examine whether MAP kinases in chondrocytes are activated by mechanical signals, and if so, whether this activation is required for transmitting mechanical signals intracellularly to stimulate chondrocyte activities.

Method:
Chondrocytes were isolated from embryonic avian sterna, and cultured in a new three dimensional culture system. This system consists of a sponge of collagen networks for seeding the cells, and a computer controlled "Bio-stretch" system to apply precise mechanical loading to the sponge. The sponge was stretched to a 5% elongation at 60 cycles/min, 15 min/hr. After different periods of mechanical stretching, cells were collected by digesting the sponge with 0.05% collagenase. The total number of cells was counted for quantifying the proliferation rates. Quantifying MAP kinase activities was performed as follows. Cells were washed before lysed in a lysis buffer. Lysates were centrifuged and aliquots of supernatant were used in immunoprecipitation. For immunoprecipitation, antibodies against ERK, JNK, and p38 were used in an overnight incubation, respectively. Immunoprecipitates were collected and washed before being used for kinase assays. The ERK assay was performed with substrate PKI, the JNK assay with c-jun, and p38 assay with MBP, respectively. The reaction was initiated by the addition of 10 mM (g-^{32}P) ATP followed by incubation for 20 min at 30^{o}C. The reactions were stopped by the addition of 2 X Laemmli sample buffer and 20 ml of each reaction was subjected to 12 % SDS-PAGE. The gels were dried for autoradiography or quantifying by phosphoimager. The inhibitor of ERK, PD98059, and the inhibitor of p38, SB203580 were from CalBiochem (la Jolla, CA). They were added to medium of some cultures containing at a final concentration of 10 μM.

Result:
A cyclic deformation of collagen sponge containing cultured chondrocytes was performed with an intermittent stretch pattern (5% elongation, 60 stretches/min, 15 min/h). This extent of matrix deformation was comparable to that experienced in vivo. Under this stretch pattern, two of the major intracellular MAP kinases, ERK and p38, were activated rapidly and significantly, while the third major kinase JNK was barely activated. The activities of these kinases were quantified by the extent of phosphorylation of their respective substrates. The activation of ERK and p38 by matrix deformation had specific time courses and amplitudes. The activation of ERK was more than two fold, and reached its peak after only 15 minutes of stimulation. The activation of p38 was more than 5 fold, but it took 60 minutes of stretch to reach that extent. In contrast, JNK was not greatly activated (less than 50%). This demonstrates that mechanical stretch of extracellular matrix activates ERK and p38 MAPK specifically.

The long term effect of the mechanical stimulation was a dramatic increase of chondrocyte proliferation. Cell number relative to nonstretched cells increased 85% after 2 days, and 101% after 3 days. Cell doubling time was reduced from 72 hours to 43 hours. We then determined whether the activation of ERK and p38 MAPK was required for the increase of cell proliferation by mechanical stimulation. Chondrocytes were incubated in the presence of 10 uM PD98059, which specifically inhibited ERK activity, or in the presence of 10 uM SB203580, which specifically inhibited p38 activity, or both. In addition, chondrocytes were incubated in the presence of 0.5uM



DMSO, which served as a vehicle for both inhibitors. In the presence of 0.5uM DMSO, cell number increased more than 80% under stretched conditions after 2 days (Fig, Veh). This demonstrates that DMSO at such concentration does not have toxicity for mechanically stimulated cell proliferation. The presence of either inhibitor alone partially reduced the stimulatory effects of mechanical stretch on chondrocyte proliferation (Fig). Combination of both inhibitors completely demolished the stimulatory effects of mechanical signals (Fig, SB+PD). This indicates that both ERK and p38 are responsible for transducing mechanical signals to stimulate chondrocyte proliferation.

Discussion:
We have shown that two intracellular MAP kinase pathways, ERK and p38 MAPK are rapidly activated in cultured chondrocytes that receive mechanical signals from a 3D matrix environment. Furthermore, these two MAP kinases are responsible for transmitting mechanical signals to stimulate chondrocyte proliferation. Although a blockage of one of these pathways is not sufficient to abolish the stimulatory effect of cyclic deformation on chondrocyte proliferation, combination of the two inhibitors abolishes the stimulatory effects completely. It is interesting to note that ERK has been associated with transmitting signals to stimulate cell proliferation, whereas p38 has been identified to transmit stress signals. We have shown for the first time that ERK and p38 act in concert to transmit mechanical stress signals to stimulate proliferation in chondrocytes. Thus, extracellular mechanical signals stimulate chondrocyte proliferation via ERK and p38 MAPK pathways.

Acknowledgement: This study is supported by grants AG14399 and AG00811 from NIH, and by a grant from the Arthritis Foundation

☐ One or more of the authors have received something of value from a commercial or other party related directly or indirectly to the subject of my presentation.

☒ The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.