Mechanical stress for activation of p44/42 MAP kinase and NF-κB in frozen shoulder

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Introduction: Pathology of frozen shoulder is classified into three categories: idiopathic, traumatic and diabetic; however, the mechanism of its pathogenesis remains unclear. One of the pathogenic factors could be abnormal mechanical loading, because it is known that mechanical stress results in deformity of matrix in skeletal tissues (1). Arthroscopic analysis has recognized synovial proliferation at the site of the long head of biceps (LHB) and rotator interval (RI) in frozen shoulders. Our hypothesis is that mechanical stress of LHB may activate a series of intracellular signaling molecules including MAP kinases and NF-κB in the synovium, which in turn induce the expression of cellular adhesion molecules thereby altering tissue mechanics in the shoulder. As the first step to test this hypothesis, we performed immunohistochemical analysis of synovial tissues taken from frozen shoulder patients to examine whether these intracellular kinases are activated under frozen shoulder conditions.

Materials and Methods: Synovial tissues were taken from four patients with frozen shoulders with mean age of 55.5 (46-62) and mean blood sugar of 126 (94-200), and embedded and cut in serial paraffin sections (2 μm). Sections were stained with hematoxylin and eosin (H&E) for histology analysis. For immunohistochemistry, sections were deparaffinized and ethanol dehydrated before autoclaved at 121o in 10mM sodium citrate buffer (pH6.4) for 20 minutes. Sections were allowed to cool to room temperature, and then rinsed in detergent solution (0.05M PBS, pH7.6) for 3 times of 3 minutes. Tissue sections were blocked for 10 minutes in PBS containing 20% rabbit serum, followed by incubation at 4oC overnight with the following antibodies: Anti-human phospho-p38 MAPK (Tyr180/Tyr182) mouse monoclonal antibody (1:500 dilution; cellsign #3216S), anti-human phospho-p44/42 MAPK (Tyr202/Tyr204, ERK1/ERK2) mouse monoclonal antibody (1:500 dilution; cellsignal #9106S), anti-human phospho-p44/42 MAPK (Tyr180/Tyr182) mouse monoclonal antibody (1:500 dilution; cellsignal #3216S), anti-human NF-κB p50 rabbit polyclonal antibody (1:200; Santa Cruz Biotechnology, #sc-7178), anti-TNF-α mouse monoclonal antibody (1:1000; Biogenesis, Pool, UK), anti-human CD29/β1 integrin) mouse monoclonal antibody (1:350 dilution; Novocastra #NCL-CD29), MMP-3(1:100; Biogenesis #5980-0311), IL-6(1-6:1000, Rockland Inc. #109-401-310), CD56(1:1000; Novoceastra # NCL-CD56), CD68(1:1000; DAKO #M0814), S-100(1:500; DAKO #20311) and VEGF(1:800, upstate #05-443). After rinsing (0.05M PBS, pH7.6), endogenous peroxidase was blocked for 10 minutes with 0.3% hydrogen peroxide in Tris buffered saline (10 mm Tris HCL, 140 mM NaCl, pH 7.4), followed by 30 minutes’ incubation with a biotinylated species-specific anti-IgG secondary antibody (Vector, Burlingame, CA). Sections were then incubated for another 30 minutes with the appropriate Vectastain ABC reagent (Vector), using 3, 3’-diaminobenzidine-4HCl (DAB) (Sigma) for the color reaction, which resulted in brown staining of antigen expressing cells.

Results: H&E histological analysis showed vascular proliferation with fibrin and fibrous tissues. This indicates there is active angiogenesis in the synovium from frozen shoulder patients. NF-κB expression was detected in blood vessels, while TNF-α was expressed weakly around the vascular tissue. Interestingly, p42/44 (ERK1/ERK2) was highly activated in the epithelial cell layer of the vascular tissue, as indicated by the strong staining of phosphorylated ERK. In contrast, p38 MAPK was not activated. This indicates that activation of MAP kinase pathways in frozen shoulder was specific. In parallel to the activation of ERK kinase, CD29/β integrin expression was detected in vascular tissue and the superficial cells of synovial tissues. MMP-3 and VEGF expressed on surface layer and vascular, CD68 expressed on the surface layer. IL-6 expressed interstitial layer but CD56 and S-100 did not expressed. Therefore the synovial tissue in mechanical stress environment at LHB and RI, MMP-3, IL-6, VEGF and CD68 expressed without peripheral nerve adhesion protein. Consistent with the previous studies that have shown that altered mechanical loading activates ERK MAP kinase and NF-κB in various tissues, we propose that mechanical stress environment at LHB and RI may activate intracellular signaling molecules including ERK1/ERK2 and NF-κB, which induces the expression of adhesion molecule CD29 in the synovial tissues. The increase of the cellular adhesion molecules may result in capsule adhesion, thereby contributing to the frozen shoulder syndrome.

Discussion: We have reported mechanical stress induced pericellular matrix protein of chondrocytes (1). In this study, we found the activation of mechanical stress related signal molecule, ERK1/ERK2 with expression of CD29/β integrin active in LHB and RI, in frozen shoulder conditions. Mechanical stress around LHB and RI shoulder may induce activation of MAP Kinase and NF-κB.