GENE EXPRESSION PROFILE ANALYSIS BY OLIGO-ARRAY IN THE CAPSULE TISSUE OF PATIENTS WITH FROZEN SHOULDER

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Introduction

Frozen shoulder is a common condition characterized by gradual loss of active and passive shoulder motion. The prevalence of frozen shoulder is more than 30% in the shoulder pain population and is slightly greater than 2% in the general population. Although it's great prevalence, the etiology of frozen shoulder remains enigmatic. A few studies have recently been tried to elucidate the pathophysiology of the adheisve capsulitis, but these reports demonstrated very limited information about the process of this condition, including some cytokines and histologic changes in the capule tissue of the patient with frozen shoulder. Therefore, a study that screen and review overally all kinds of adhesion molecules and the related cytokines with adhesive capsulitis is required for better understanding the basic pathology.

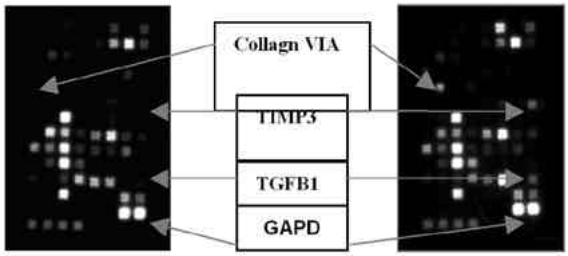
The purpose of our study is to examine the gene expression profile of adhesion molecules of the capsule samples derived from the patients with adhesive capsulitis and compare it with that of normal capsule. Understanding the basic mechanisms behind the pathogenesis of adhesive capsulitis may lead to the development of new treatment regimens for this common and painful condition.

Materials and Methods

Capsular tissues in the rotator interval were obtained intraoperatively from patients during shoulder surgery and analyzed using Oligo-Array technique. 21 patients (mean age 53.2, range 43~65) were candidates for this study. They were divided into two groups: Group I; 17 patients with frozen shoulder, Group II; 4 patients with shoulder instability or fracture. Samples were placed in RNAlater® solution (Ambion, Austin, TX) immediately after surgery and frozen at -70℃ within 2 hours of suspension. RNA was extracted from the samples using the RNeasy® Fibrous Tissue Mini Kit (Qiagen, Valencia, CA) and stored at -70℃. Complementary DNA-array hybridization was done using Oligo-GEArrayTM Series Kits (SuperArray, Frederick, MD) for human extracellular matrix and adhesion molecule. The arrays were hybridized with Biotin-dUTP(Roche Applied Science, IN) labeled cDNA probes, which were prepared from the extracted

RNA according to the manufacturer's protocol. Images of the bursal specimens were obtained by chemiluminescent detection on X-ray films. Data analysis and normalization was accomplished using ScanAlyze and GEArrayTM Analyzer software. Mann-Whitney U test was used for statistical analysis. The P values less than 0.05 were considered significant. Human tissue were obtained with the approval of the Catholic University Institutional Review Board.

Results



Control Frozen shoulder

Fig. 1. One sample of x-ray films of chemiluminescent detection membrane for Human Extracellular Matrix & Adhesion Molecule Gene Array. Collagen VIA = Collagen 6 alpha, TIMP 3 = Tissue Inhibitor of Metalloproteinase 3, TGF B1 = Transforming Growth Factor betha 1 GAPD=Glyceraldehyde-3-phosphate dehydrogenase.

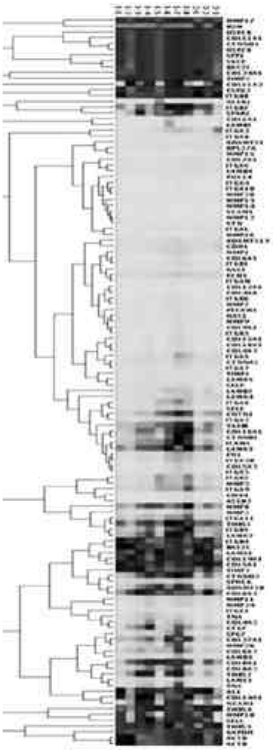


Fig 2. Cluster analysis of expression of extracellular matrix and adhesion molecule genes in capsular specimen. The all molecules list at the right side of column. The patients list on the top of column; the control lie on the right three patients and the patients with frozen shoulder lie on the left eight. This figure demonstrated that the over all expression of extracellular matrix and adhesion molecules was same in both group, but some molecules showed a different pattern of gene expression between two groups.

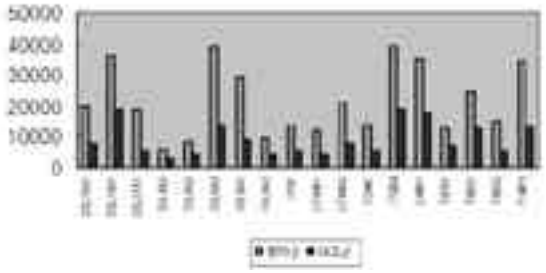


Fig 3. Average relative intensity of expression of extracellular matrix and adhesion molecule genes in capsular specimen.

The gene expression of collagen molecules (COL4A2, COL6A2, COL6A3, COL8A1, COL8A2, COL16A1, COL19A1, COL27A1) and the other adhesion molecules (Connective tissue growth factor; CTGF, Catenin; CTNNB1,D2, Intercellular adhesion molecule; ICAM1, Integrin; ITGB4, Laminin; LAMA1, Transforming growth factor; TGFB1, Thrombospondin; THBS1,S2, Tissue inhibitor of metalloproteinase; TIMP3) were increased in Group I (frozen shoulder) compared to Group II (normal capsule) ($p<0.05$).

Discussion & Conclusion

We demonstrate that many extracellular matrix/adhesion molecules are increased significantly in the rotator interval capsule tissue of patients with frozen shoulder. These specific molecules will be the key to elucidate the basic pathophysiology of the inflammatory and fibrotic processes in adhesive capsulitis and the result of this study will serve as the basis for the future investigations of the adhesive capsulitis. Understanding the basic mechanisms behind the pathogenesis of frozen shoulder is an important step in the development of clinically useful antifibrotic agent that can serve as novel treatments for patients with this common and painful condition.