Gene Expression Profiling of Human Knee Osteoarthritic Subchondral Bone

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Introduction: Osteoarthritis of the knee joint (KOA) is generally considered as a disease of articular cartilage (AC) accompanied by secondary bone changes. The degradation of the AC has been sought as a main part of the disease but subchondral bone (SB) has also been thought as another important part of this disease because the integrity of AC depends much on the mechanical properties of its bony bed. In our previous report, TNF-α, Cox-2, and substance-P positive cells were detected in osteoarthritic SB of affected compartment and relationship between pain evocation and pathological changes of SB was indicated(1). To further analyze characteristic changes of SB, we examined specific gene expression seen in osteoarthritic SB with microarrays (MA).

Materials and Methods: Medial-type KOA receiving total knee arthroplasty (TKA) in our institution from April 2006 were involved in this study. In the including patients, there was eburnation of AC on their weight-bearing (WB) area of the medial femoral condyle (MFC) and normal or superficial fibrillating AC on their WB area of lateral femoral condyle (LFC). According to that criterion, in amount of the 15 patients, 7 were chosen for the study. WB area of SB of MFC and that of LFC were immediately frozen in liquid nitrogen after the thorough removal of remaining AC and stored at –80°C until RNA extraction. RNA extraction was performed using the RNeasy Mini kit (Qiagen). To analyze the differential gene expression in medial (MSB) and lateral (LSB) SB of the femoral condyles, the GeneChip Human Genome U133 Plus2.0 (Affymetrix) was employed. It is equipped with 54675 probe sets. Total of 7 KOA were involved; specimens from initial 3 knees were used for MA analysis and all the 7 samples were used to confirm the MA results were used for real-time PCR analysis using the ABI 7500 PCR system (Applied Biosystems). Primer and probe sets were purchased from TaqMan Gene Expression Assays for the set of genes to be studied: asporin (ASPN), periostin (POSTN) and transient receptor potential (TRP) cation channel, V1–4, M8, A1 with β-actin, as an internal control.

Results: 1. Microarray

The expression analysis revealed that 27259±1021 (Mean±SD) of all probe sets were present in MSB and 28128±560 in LSB. We found a pattern of unanimously higher (1705) or lower (1630) expressed genes in the MSB vs LSB. One hundred sixty six genes of all probe sets indicates a 2-fold higher expression than in LSB in all 3 samples in common. Furthermore, 16 genes indicated a 4-fold difference (MSB > LSB) (Figure 1). We focused on and picked up some genes that are known to have relation with OA in previous reports or suggested to be involved in OA. These were classified into 5 groups. Proteases: MMP2, 3, 9, 13, 14, TIMP1, ADAM10 was significantly higher in MSB. Growth factor: There was no differences in expression of genes including TGF-β and IGF between MSB and LSB. Extracellular matrix protein: ASPN and POSTN was significantly higher in MSB. Cytokine: No differences in expression of genes including IL1, IL6, IL8, and TNF-α. Inflammation: No differences in expression of genes including Cox-1, 2, Substance, Bradykinin and Serotonin. Nociceptor: TRPV2 was significantly higher in MSB.

2. Realtime PCR

The expression of mRNA for ASPN, POSTN and TRPV2 in MSB was higher in all 7 specimens. (Figure 2-3)

Discussion: We conducted a comprehensive analysis of the gene expression in human SB of affected compartment in medial-type KOA with MA. ASPN, POSTN, TRPV2 in SB of affected side was higher than that of non-affected side with MA and PCR. It was suggested that protease and extracellular matrix protein such as MMPs, ASPN, POSTN and so,relation to TGF-β network, implicate in the pathogenesis of KOA. TRPV2 appeared to association with pain of KOA, in particular, with mechanical stress including WB.

References: (1) Origin of Osteoarthritic Knee Pain: Immunohistochemical Analysis of Subchondral Bone: S.Ogino et al.; ORS transactions 2006; 31: 246