Exosome derived from osteoarthritis cartilage induces catabolic factor gene expressions in synovium

INTRODUCTION:
Osteoarthritis (OA) is the most widespread connective tissue disorder which is characterized as cartilage degeneration. Despite its high prevalence, the etiology of OA has not been elucidated. Several studies focused on articular cartilage and synovial tissues were conducted, however, the pathogenesis has been still unclear, especially the relationship articular cartilage and synovium during the progression of OA. Recently, it became clear that microRNA (miRNA), small non-coding RNA, play a role in several human diseases. As for OA, Miyaki et al demonstrated that miRNA-140 play a crucial role in maintain the homeostasis of articular cartilage and synovial tissues. Therefore, there is the possibility that exosome play a role in tissue-to-tissue communication, such as articular cartilage-synovial tissue, during OA progression. The purpose of this study is to investigate the role of exosome from OA cartilage to OA synovial tissue on the expression of catabolic factor in OA synovial tissues.

METHODS:
Articular cartilage and synovial tissues were obtained during total knee arthroplasty from 4 OA patients with written permission. All patients were classified as grade 4 according to Kellgren-Lawrence classification. The articular cartilage and synovial tissues were immediately cut and sliced at the size of 1cm x 1cm, and then cultured in DMEM containing 10%FBS and antibiotics in 24 well culture plate. Cartilage tissues were treated with or without IL-1β for 24 hours, then we isolated exosomes by ExoQuick™ (SBI) from IL-1β stimulated medium and control medium at a volume of 500µl. Isolated exosomes fraction was measured for its protein content using BCA protein assay kit (Thermo Science), we also verified isolated exosomes by Westren blot using antibody against commonly found exosomes biomarker protein, CD63. 5µl exosome was applied in each 24 well containing OA cartilage, while these previous studies focused on the intracellular role of miRNAs in regulating gene expression, recently, miRNAs were found in the extracellular space including blood and other body fluid. The extracellular RNAs are packaged in secretory microparticles such as exosomes, representing genetic material that is transferable from tissue to tissue and from human to human. Therefore, miRNA may be not only a regulatory molecule within the cell, but also, like cytokines, a paracrine regulatory molecules for cell-cell, tissue-tissue communication.

RESULTS:
Real time PCR analysis revealed that the expression of MMP13 in synovial tissue treated with IL-1β stimulated exosome was higher than that from control medium (Figure 1).

Figure 1. The expression of MMP13 by real time PCR analysis. Real time PCR analysis revealed that the expression of MMP13 in synovial tissue treated with IL-1β stimulated exosome was higher than that from control medium. Exo (+); exosome from OA cartilage stimulated without IL-1β. Exo (-); exosome from OA cartilage stimulated with IL-1β.

Figure 2. Immunohistochemistry of IL-1β, TNFα, and COX2. IL-1β, TNFα, and COX2 positive cells were observed in synovial membrane treated with IL-1β stimulated exosome, while they were not observed in synovial membrane treated with exosome from control medium. Bar; 50µm.

Discussion:
Exosomes are one type of microvesicles (MV’s), which are 100-1000nm diameter circular fragments of membrane released from the endosomal compartment as exosomes or shed from the surface membranes of most cell types of endocytic origin released by many cells. The exosomes are small particles (30nm-100nm in diameter) that are released into the extracellular environment on fusion of multivesicular bodies with plasma membrane. Secreted exosomes have surface receptors/ligands of the originating cells and have the potential to selectively interact with specific target cells. Previous studies reported that exosomes play a role in cell-to-cell communication. Indeed, the MVs, including exosomes, may directly stimulate target cells through receptor-mediated interactions or may transfer from the cell of origin to the recipient cell various bioactive molecules such as proteins, miRNAs and miRNAs. Recently, exosomes were shown to carry RNA for information transfer between cells. Importantly, miRNAs exist in exosomes and are protected from RNases. Our results suggested that exosome from OA cartilage stimulated with IL-1β might induced the expression of catabolic factor in synovial tissues in OA joint. Exosome might be released into synovial fluid from chondrocytes by IL-1β, and up taken in synovial fibroblast. Subsequently synovial fibroblast might produce the catabolic factors, especially MMP13, which lead progression of OA. Further examination should be needed to elucidate the mechanism via cell-cell communication by cartilage derived exosome.