INTRODUCTION:
Proliferation of synoviocytes and infiltration of inflammatory cells into the synovium is one of the major characteristics of rheumatoid arthritis (RA). The synovial tissue in patients with osteoarthritis (OA) also shows proliferation of synoviocytes and infiltration of inflammatory cells, which however is much milder than that in RA. In fact, systemic inflammatory signs of CRP and ESR are elevated in RA but not in OA generally. One of the factors that contribute to this difference is considered to be abnormality of immune systems in RA. In addition to the immunological factors, nature of synoviocytes in RA would be different from that of OA. One of the differences is hyperplasia of synovial fibroblasts in synovium of patients with RA (1). Further more, isolated and serially cultured RA synovial fibroblasts maintain their invasive and destructive behavior (2). This supported the concept that synovial fibroblasts in RA are persistently activated. Although the nature of synovocytes is different, few studies have been available on biochemical differences of synoviocytes between RA and OA so far. It would be important to survey the difference of the nature of the synoviocytes between these two diseases.

In recent years, comprehensive analyses have started to be used to survey disease-specific changes at each level of genome, mRNA, and proteins. Nevertheless, from the viewpoint that proteins have various functions of proteins. Therefore we suggested that clarification of biochemical differences of synoviocytes between RA and OA would be important.

In this study, we clarified the difference of protein profiles of synoviocytes between RA and OA comprehensively, using proteomics at phosphorylation levels. We further investigated annexin VII which was highly phosphorylated in OA than in RA.

METHODS:
Synoviocytes, obtained from 5 patients with RA and 5 with OA, were cultured separately. This experiment was approved by the institutional review board and informed consent. Total proteins were extracted from the cultured synoviocytes and were then separated by 2-dimensional electrophoresis. Specifically, the proteins were separated by isoelectric focusing (IEF) on the liner strips of pH4 to 7 as a first electrophoresis. Then the separated proteins were further separated by their molecular weights by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were then detected by a fluorescent dye of Sypro-Ruby Protein Gel stain and phosphorylated proteins were detected by a fluorescent dye of Pro-Q Diamond Phosphoprotein Gel Stain. The gels were analyzed to obtain information on the position and phosphorylation levels of each phosphoprotein. The phosphorylation levels were compared between 5 samples from RA and 5 from OA by the Ettan Progenesis program. Detected proteins, which were over- or under- phosphorylated in the samples from patients with RA, were further subjected to protein identification by the combination of peptide mass-fingerprinting and the MS/MS analysis. For this analyses, proteins in the spots of interest were digested with trypsin in-gel, extracted from the gel spot, and then were measured for their masses by matrix associated laser-dissociated ionization time-of-flight mass spectroscopy (MALDI-TOF-MS). The obtained mass data was used for searching of SWISS-PROT protein data base by the Mascot program. The conditions of phosphorylation were investigated in each identified proteins.

RESULTS:
By the two-dimensional electrophoresis of the synovial proteins, more than 1300 proteins were detected in each sample. By the staining of phosphoproteins with Pro-Q staining, about 800 proteins were detected in each sample. Comparison between 5 result from patients with RA and 5 from patients with OA revealed that proximally 100 proteins were different more than two-fold at phosphorylation levels. Thus, phosphoproteome profiles of the synoviocytes were found to be considerably different between the two diseases.

A part of these proteins were next identified by the peptide mass fingerprinting and MS/MS analysis. One of the proteins whose phosphorylation levels were higher in RA than in OA was identified as annexin VII (synexin), of which phosphorylation was 3.3- fold stronger in RA than in OA. First we focused on this annexin VII, because of its membrane fusion activity in the process of exocytosis. It is strongly suggested that phosphorylation of annexin VII induces exocytosis of some proteins, for example catecholamine from chromaffin cells, surfactant from alveolar type II cells, and insulin from islet of Langerhans. The expression of total annexin VII in synoviocytes was significantly up-regulated in RA compared with OA by immunoprecipitation and western blotting.

Further, the expression and localization of annexin VII in the section of synovium were confirmed using immunohistological staining in 5 patients with RA and 5 with OA. Annexin VII was strongly stained in the lining layer cells and infiltrated inflammatory cells in RA, on the other hand, annexin VII was slightly stained a part of lining layer cells in OA. This supports a hypothesis that annexin VII-induced exocytosis is much stronger in the synovium of RA patients than in that of OA patients.

In the next step, we are focusing on identified 4 proteins which were strongly phosphorylated in OA than in RA.

DISCUSSION:
We evidenced for the first time that phosphoproteome of the synoviocytes were considerably different between RA and OA. This may reflect different nature of synoviocytes like strong proliferative potential in RA. From the results, the proteomic approaches would be useful methods to characterize synoviocytes both in RA and OA.

One of the over-phosphorylated and over-produced proteins in RA was annexin VII as described above, further investigation of the differently phosphorylated and over-produced proteins in RA was annexin VII. Phosphorylation of annexin VII is as essential process for exocytosis, its over-phosphorylation and over-production would be reasonable, since various cytokines and proteases including matrix metalloproteases (MMPs) were reported to be secreted by synoviocytes in RA. Consequent investigation of molecules differently phosphorylated between in RA and OA would help us to understand the pathogenesis of both RA and OA.

We here demonstrated that profiles of phosphoproteins in synoviocytes of patients with RA were distinct from those in OA. Including annexin VII as described above, further investigation of the differently phosphorylated proteins in RA and OA would elucidate characteristics of synoviocytes in both disease conditions.

REFERENCES

AFFILIATED INSTITUTION
**Department of Orthopaedic Surgery, Yokohama City University Kanagawa, Japan