INTRODUCTION: Osteoarthritis (OA) is characterized by degradation of articular cartilage that frequently leads to disability in performing daily activities among the elderly. A better understanding of the degradation mechanism should elucidate OA pathogenesis. Although a large number of biomechanical and biochemical studies have been attempted to clarify the mechanism of cartilage degradation, it remains unclear [1]. For this clarification, a new analytical approach must be considered. Glycobiology has been recently applied to molecular-based works in the biomedical field. The majority of glycans attached to proteins are classified into N-glycan and O-glycan [2]. Among both types of glycans, several N-glycan functions related to diseases have been made clear. Our recent study, using a rabbit OA model, first demonstrated that the alterations in cartilage N-glycans occur from an early phase of OA aggravation [3]. Although this indicates the crucial role of N-glycans in the mediation and progression of OA, the biological correlations between the alterations in N-glycans and chondrocyte metabolism related to cartilage degradation are still unclear. The hypothesis of this study was that the alterations in N-glycans with cartilage degradation would occur by the changes in chondrocyte metabolism responding to stimulation inducing the degradation. The objectives of this study were to identify alterations in cartilage N-glycans and chondrocyte mRNA expression of genes of enzymes on the altered N-glycan biosynthesis (N-glycanes) related to the biosynthetic pathway of the altered N-glycan in chondrocytes and to determine the localization of the altered N-glycan in living articular cartilage.

METHODS: Culture of Cartilage Explants. According to the established ethical guidelines approved by the local animal care committee, in vitro cartilage catabolism was analyzed by culturing femoral head cartilage of 4-week-old C57BL6 female mice with interleukin-1α (IL-1α). The explants were classified into the following two groups: degradation group, cultured with IL-1α; control group, without IL-1α. To determine the degenerative changes in the cultured cartilage, histological analysis and quantification of proteoglycan release from cartilage explants were performed in both groups.

Structural Analysis of Cartilage N-glycans. In the structural analysis of N-glycans, the N-glycan structures were first pyridylaminated (PA-N-glycans). Then, PA-N-glycans were analyzed using high-performance liquid chromatography (HPLC) and mass spectrometry (MS). Pyridylaminated-N-glycans were separated with HPLC on an ODS column, and each peak was applied individually to an amide column. Elution positions of PA-N-glycans on the ODS and amide columns were converted to glucose units (GU). PA-N-glycan structures were estimated with corresponding elution positions of a database. To identify the PA-N-glycan structure, some PA-N-glycans separated by HPLC were further analyzed by MS. Identification of N-glycanes Related to the Altered N-glycans. The mRNA expression of N-glycanes related to the biosynthetic pathway of the altered N-glycan was measured by real-time RT-PCR analysis. Lectin Staining. To identify localization of the altered N-glycan, lectin staining was performed on the samples of both groups. Surgical Induction of OA. The right knee joint of 8-week-old mice was destabilized by transection of the medial collateral ligament and the meniscus (OA side). A sham operation was performed on the left knee joint using the same approach without ligament transection and menisectomy (Sham side). At 2, 4 and 8 weeks after surgery, each section of knee joint was stained with HE, Safranin-O, and lectin. Statistical Analysis. Significant differences between the groups were assessed by unpaired t-tests. P values of less than 0.05 were considered significant.

RESULTS: Cartilage Degradation. After cultivation, an apparent reduction in Safranin-O staining was found in the articular cartilage and growth plate in samples of the degradation group (Figure 1). The application of IL-1α stimulated the release of proteoglycan from cartilage explant by ECM degradation. (P<0.01).

Alterations in N-glycan Structures with Cartilage Degradation. In comparison with the control group, a significant alteration in the N-glycan peak pattern was detected in cartilage of the degradation group (Figure 2). This peak suggested that the structure is M5.1, a high-mannose type N-glycan. MALDI-TOF spectra of this peak with DHB as a matrix corresponded with a protonated PA-N-glycan which consists of HexHexNAc2, as M5.1 component. Alterations in N-glycanes Related to the Altered N-glycan Biosynthesis. The biosynthetic pathway of M5.1 highlights the importance of enzymes α-1,2-mannosidase IA (ManIA), IB (ManIB), IC (ManIC), and β-1,2-N-acetylglucosaminyltransferase 1 (Gnt-I). The obtained results showed that the expression of ManIA, ManIB and Gnt-I significantly increased in the samples of the degradation group (Figure 3). Localization of the Altered N-glycan in the Cartilage (in vitro). In the lectin staining, samples of the control group had a reactivity to concanavalin A (ConA) on the cell surface and in the cytoplasm of the articular cartilage chondrocytes and hypertrophic chondrocytes. In contrast, such a reactivity to ConA apparently decreased in the samples of the degradation group (Figure 4). Alterations in Chondrocyte N-glycans with OA Progression (in vivo). Concanavalin A staining suggested that the chondrocyte high-mannose type N-glycans decreased with OA progression. The knee joints showed no apparent OA changes during the first 2 weeks after surgery. However, reactivity to ConA in OA lesions apparently decreased from 2 weeks after surgery (Figure 5). At 8 weeks postoperatively, no reactivity to ConA was found in any OA lesions (Figure 5).

DISCUSSION: This study showed the significant alteration in the peak pattern of M5.1, which belongs to high-mannose type N-glycans, with cartilage degradation and significant upregulation of mRNA expression related to the biosynthetic pathway of this N-glycan in chondrocytes responding to IL-1α stimulation. These results indicate that the alteration in cartilage N-glycans with cartilage degradation occurs by upregulation of the chondrocyte N-glycanes related to the identified N-glycan. In our in vivo study, the weak reactivity to ConA in OA lesions from 2 weeks postoperatively indicates that the cartilage high-mannose type N-glycans decrease from an early stage of OA. Based on these in vivo and in vitro results, we conclude that the alteration in cartilage high-mannose type N-glycans is involved in OA pathogenesis at an early stage and /or initiation of OA. The results obtained here provide the possibility of high-mannose type N-glycans as a predictive molecule of OA progression and a target for the treatment of this disease.

REFERENCES: