INTRODUCTION: Osteoarthritis (OA) is characterized by degradation of the cartilage extracellular matrix. Therefore, elucidation of the pathogenesis of OA requires a better understanding of the cartilage degradation mechanism. Despite the large number of gene-based or protein-based studies performed to clarify the mechanism of cartilage degradation, the pathogenesis of OA is not fully understood. Glycosphingolipids (GSLs) are ubiquitous membrane components that modulate transmembrane signaling and maintain cell-to-cell interactions [1]. GSL expression is decreased in the articular cartilage of humans with OA [2]. Although decreased GSL expression leads to alterations in the biochemical composition of the chondrocyte membrane, the role of these changes in cartilage metabolism and in the pathogenesis of OA is unknown. Our working hypothesis was that alterations in chondrocyte GSLs could be responsible for disturbances in cartilage homeostasis, contributing to the development of OA. The objective of this study was to determine the functional role of GSLs in the development of age-associated and instability-induced OA.

METHODS: Targeting and mice. To determine the functional significance of GSLs in cartilage, we generated mice with knockout of the chondrocyte-specific Ugcg, which encodes an initial enzyme of major GSLs synthesis, (Col2-Ugcg−/−), using the Cre/loxP system. Col2-Ugcg−/− mice were generated by breeding Col2a1-Cre transgenic mice with UgcgloxP/loxP mice [3]. Wild-type littermates (UgcgloxP/loxP) were used as controls. The present experiments were approved by the institutional animal care committee. Histological evaluation. Samples were fixed in 10% buffered formalin and decalcified in 10% EDTA (pH 7.5). Each tissue was dehydrated and embedded in paraffin, and sectioned into 5-μm thick slices. A single observer quantified the osteoarthritis severity using the Mankin scoring system [4]. Age-associated OA model. Mice were followed for the spontaneous development of OA up to 15 months of age. Instability-induced OA model. To create an instability-induced OA model, the right knee joint of 8-week-old mice was destabilized by transecting the medial collateral ligament and removing the cranial half of the medial meniscus. To evaluate the histological findings, the mice were euthanized and entire knee joints were dissected 8 weeks after surgery. Culture of cartilage explants. The femoral head cartilage was harvested from 4-week-old mice and pre-cultured for 48 h, and then cultured for an additional 72 h in serum-free Dulbecco's Modified Eagle’s Medium plus 10 ng/ml mouse IL-1α. The proteoglycan content in the medium and digested cartilage was measured using a dimethylmethylene blue assay. The amount of proteoglycan released from a cartilage explant into the medium was quantitatively expressed as the percentage of proteoglycan (%PG). Mass spectrometry (MS). GSLs were extracted from mouse chondrocyte pellets. Glycans of GSLs were digested by EGCase II. The solutions treated by EGCase were subjected to glycoblotting using a protocol similar to that used for N-glycome analyses reported previously [5]. Samples were applied to MALDI TOF MS analysis on an Ultraflex II TOF/TOF mass spectrometer equipped with a reflector, and controlled by the FlexControl 3.0 software package. Statistical analysis. Data are expressed as mean ± s.e.m. Means of groups were compared by two-tailed unpaired t-tests. P values less than 0.05 were considered significant.

RESULTS: Normal growth and histology. Col2-Ugcg−/− mice developed and grew normally, indicating no apparent effect of chondrocyte-specific deletion of Ugcg on the development and organization of cartilage tissue in young mice. Enhanced development of age-associated OA in Col2-Ugcg−/− mice. Although there were no apparent OA changes in the knee joints in either genotype at 4 months of age, the OA was more progressed in Col2-Ugcg−/− mice at 15 months of age, through the overexpression of MMP-13 and chondrocyte apoptosis, compared to those of wild-type littersmates (n = 10, Mankin score: 6.9 ± 0.4 vs 4.9 ± 0.3, P < 0.05, Fig. 1). Enhanced development of instability-induced OA in Col2-Ugcg−/− mice. Histological findings of instability-induced OA were more progressed in the knees of Col2-Ugcg−/− mice compared to those of wild-type littersmates (n = 10, Mankin score: 10.8 ± 0.3 vs 5.8 ± 0.3, P < 0.05, Fig. 2).

DISCUSSION: The data presented here indicate that GSLs maintain cartilage molecular metabolism and prevent OA progression, although GSLs are not essential for chondrogenesis of progenitor and stem cells and cartilage development in young mice. Our mechanism-of-action studies suggest that GSLs have a chondroprotective role against IL-1α stimulation in the cartilage degradation process by reducing MMP-13 secretion and apoptosis of chondrocytes. GSLs are present in the plasma membranes of eukaryotic cells, including chondrocytes. Therefore, we speculate that GSLs participate in modulating the signaling pathways of some nearby receptors related to chondrocyte metabolism. A limitation of this study was that our speculation was not proven here.

SIGNIFICANCE: This is the first study to show the functional role of GSLs in the development of OA. Although further studies are required to confirm our speculation, GSLs may be target molecules for a novel and effective strategy for the treatment of OA.