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Depletion of gangliosides enhances cartilage degradation in mice

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SUMMARY

Objective: Glycosphingolipids (GSLs) are ubiquitous membrane components that play a functional role in maintaining chondrocyte homeostasis. We investigated the potential role of gangliosides, one of the major components of GSLs, in osteoarthritis (OA) pathogenesis.
Design: Both age-associated and instability-induced OA models were generated using GM3 synthase knockout (*GM3S*$^{-/-}$) mice. A cartilage degradation model and transiently *GM3S*-transfected chondrocytes were analyzed to evaluate the function of gangliosides in OA development. The amount of each series of GSLs in chondrocytes after IL-1α stimulation was profiled using mass spectrometry (MS).
Results: OA changes in *GM3S*$^{-/-}$ mice were dramatically enhanced with aging compared to those in wild-type (WT) mice. *GM3S*$^{-/-}$ mice showed more severe instability-induced pathologic OA *in vivo*. Ganglioside deficiency also led to the induction of matrix metalloproteinase (MMP)-13 and ADAMTS-5 secretion and chondrocyte apoptosis *in vitro*. In contrast, transient *GM3S* transfection of chondrocytes suppressed MMP-13 and ADAMTS-5 expression after interleukin (IL)-1α stimulation. GSL profiling revealed the presence of abundant gangliosides in chondrocytes after IL-1α stimulation.
Conclusion: Gangliosides play a critical role in OA pathogenesis by regulating the expression of MMP-13 and ADAMTS-5 and chondrocyte apoptosis. Based on the obtained results, we propose that gangliosides are potential target molecules for the development of novel OA treatments.

Introduction

The pathology of osteoarthritis (OA) is characterized by a progressive degradation of articular cartilage. In healthy individuals, the homeostasis of articular cartilage is maintained primarily by chondrocytes that are responsible for the synthesis and degradation of the extracellular matrix (ECM). However, the actual mechanism(s) of cartilage degradation remain unclear, despite a large number of gene- and protein-based studies designed to address the process[1–3]. The development of OA therapies will require the identification of new molecular targets involved in the degradation mechanism.

Glycosphingolipids (GSLs) are a group of glycolipids that are widely distributed on vertebrate plasma membranes. These molecules form clusters on cell membranes, where the GSLs modulate transmembrane signaling and mediate cell-to-cell and cell-to-matrix interactions[4,5]. GSLs are known to be critical for the maintenance of chondrocyte homeostasis[6]. However, mice that are homozygous null for *Ugcg* (the gene encoding UDP-glucose ceramide glucosyltransferase (GlcCer synthase), the first committed step in GSL synthesis) also exhibit an embryonic lethal phenotype[7,8]. Nonetheless, specific subclasses of GSLs that influence individual systems and conditions are considered ideal targets for clinical applications.

GSLs comprise diverse types of glycolipids, and are classified into several groups depending on their structural features, including lacto-series, neo lacto-series, globo-series, isoglobo-series, and ganglio-series (gangliosides) [Fig. 1][9]. Gangliosides constitute one of the most abundant molecules in mammalian cells. In the context of OA, previous studies have shown that the total ganglioside content of OA cartilage is decreased by 40%[10,11].

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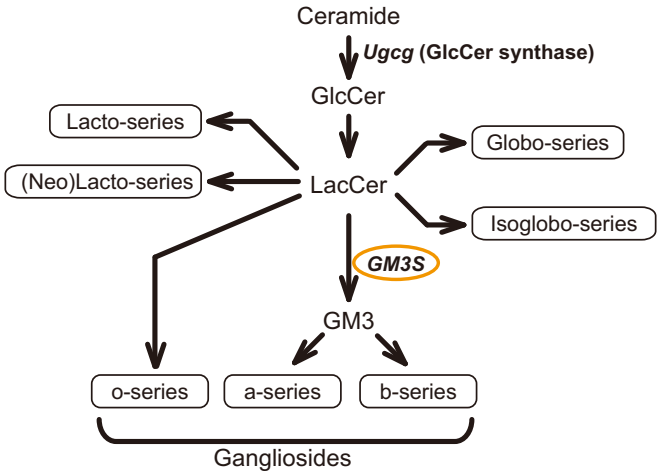

Fig. 1. Schematic of the GSL synthetic pathway in mammalian cells. GSLs from ceramide through glucosylceramide (GlcCer) are synthesized by GlcCer synthase, encoded by the *Ugcg* locus. Lactosylceramide (LacCer) is the key branching point of GSL biosynthesis, with four alternate pathways (lacto-series, neo lacto-series, globo-series, and isoglobo-series) branching off from LacCer. Virtually all the ganglio-series GSLs (gangliosides) are detected as a-series and b-series gangliosides, both of which are synthesized from the shared precursor molecule GM3, itself the product of GM3 synthase (GM3S, encoded by the *GM3S* locus). The o-series gangliosides typically are detected only in trace amounts.

Additional research revealed that gangliosides suppress the secretion of interleukin (IL)-17 and prevent the progression of collagen-induced arthritis in mice[12].

These results suggest that gangliosides may play a crucial role in OA pathogenesis. To test this hypothesis, we employed a strain of mice genetically engineered to lack GM3 synthase (GM3S). GM3 serves as a precursor molecule for most of the more complex ganglioside species; mice lacking GM3S are deficient in almost all of the gangliosides synthesized from GM3[13]. The aim of the present study was to analyze the functional roles of gangliosides in OA pathogenesis, thereby validating the GM3S as a potential clinical target molecule in the treatment of OA.

Method

Animals and generation of gene deletions

GM3 synthase knockout ($GM3S^{-/-}$) mice were generated as described previously[13]. Male adult (4-week-old and 8-week-old) and 6-day-old C57BL/6 mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Mice were utilized after 7-day-acclimatization following transportation[14]. All the purchased mice recovered normal behavior within 24 h after transportation. Mice were housed in a temperature- and humidity-controlled environment under 12-h light/12-h dark conditions and fed a standard rodent diet. All experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee of the Hokkaido University Graduate School of Medicine (Sapporo, Japan).

Age-associated OA model

As a model of age-associated OA, joints were compared between wild-type (WT; $GM3S^{+/+}$) and mutant ($GM3S^{-/-}$) mice sacrificed at 4 or 15 months of age. For each animal, the entire knee joint was dissected and evaluated for the spontaneous development of OA[15,16].

Instability-induced OA model

An instability-induced OA model was created in 8-week-old WT or mutant mice as previously described[15,17,18]. Animals were anesthetized and the right knee joint was destabilized by transection of the medial collateral ligament and removal of the cranial half of the medial meniscus using a microsurgical technique (OA side). A sham operation was performed on the left knee joint using the same approach without ligament transection and meniscectomy (sham side). Mice were recovered for 8 weeks and then sacrificed for histologic assessment.

Histologic analysis

Samples were fixed in 10% buffered formalin, decalcified in 10% ethylenediaminetetraacetate (pH 7.5), dehydrated, embedded in paraffin, and sectioned at 5-μm thicknesses. Sections then were subjected to staining with hematoxylin and eosin (H&E) and Safranin O. OA severity in each mouse was quantified using the Mankin scoring system[19,20]. In addition, we evaluated the changes in synovium and subchondral bones using a scoring system with low power images. We used the same scoring system previously described[12]. Each sample was evaluated independently by three observers who were blinded with regard to experimental group, and the resulting scores were averaged to provide a mean score.

Culture of femoral head cartilage explant

In vitro cartilage catabolism was analyzed by culturing mouse femoral head cartilage with IL-1α (Sigma, St. Louis, MO)[15,21,22]. The femoral head cartilage was harvested from 4-week-old mice and precultured for 48 h at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, using Dulbecco's modified Eagle's medium (DMEM; Sigma) containing 1% antibiotic solution (penicillin–streptomycin, Gibco-BRL, Grand Island, NY), 2 mM glutamine (Wako, Osaka, Japan), 10 mM 4-(2-HydroxyEthyl)-1-PiperazineEthaneSulfonic acid (HEPES) (Gibco-BRL), 50 μg/ml ascorbate (Sigma), and 10% fetal bovine serum (FBS; Nichirei Biosciences Inc., Tokyo, Japan). The explants then were washed three times with serum-free DMEM and cultured for an additional 72 h in serum-free DMEM containing 10 ng/ml mouse IL-1α.

Immunohistochemistry

Processed but unstained sections of mouse femoral head cartilage were deparaffinized, and endogenous peroxidase activity was quenched. After treatment with chondroitinase ABC (0.25 units/ml, Sigma–Aldrich, Tokyo, Japan), the sections were incubated overnight at 4°C with polyclonal antibody against the carboxyl-terminus of matrix metalloproteinase (MMP)-13 (1:200 dilution; Chemicon, Temecula, CA). Samples then were washed three times with phosphate-buffered saline (PBS) and incubated with a biotinylated secondary antibody, the primary antibody was omitted for the negative control. For semi-quantitative data, at 400× magnification, the positive cells were counted from three different fields of observation; the number of counted cells was then averaged. Positive cell ratio was calculated with the total chondrocyte number of each field. Each sample was evaluated independently by three observers who were blinded with regard to the experimental groups they were observing.

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay

To investigate chondrocyte apoptosis, the TUNEL assay was performed using an *in situ* Apoptosis Detection Kit according to the