Depletion Of Gangliosides Accelerated The Articular Cartilage Repair In Mice

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Introduction: Articular cartilage injuries precede to cartilage degradation, osteoarthritis (OA) and lower the quality of life. Although there have been many attempts to enhance cartilage repair and reasonable clinical outcomes can be achieved, the existing attempts are insufficient to restore a native cartilage tissue. The mechanisms of articular cartilage repair should be clarified in order to achieve better clinical outcomes. To clarify the mechanism of the articular cartilage repair, we previously established a novel mouse model that can analyze the molecular biological process of the cartilage repair (1). We are focusing on the Glycosphingolipids (GSLs) which are known to be critical for the maintenance of chondrocyte homeostasis (2). The depletion of GSLs accelerated the cartilage degradation and the depletion of gangliosides showed almost same phenotypic changes (2, 3). Our previous reports showed GSLs involved cartilage metabolism especially in OA. Therefore, we hypothesized that GSLs also might involve the articular cartilage repair. To test this hypothesis, we employed genetically modified mice lacking GM3 synthase (GM3-/− mice) (4). These mice are deficient in almost all of the gangliosides synthesized from GM3. The purpose of the current study was to analyze the GSLs functions in the articular cartilage repair.

Methods: We adopted GM3-/− mice and wild-type C57Bl/6 mice as controls (4). Articular cartilage full-thickness injuries were generated on 4-week old mice as we previously reported (1). Briefly, medial para-patella approach was performed, and then longitudinal full thickness injuries were made in the patellar groove using a 27-G needle covered with 21-G needle. The histological score for joint surface repair was evaluated as previously reported (1, 5). For immunostaining of GM3, frozen sections were prepared from mice knee obtained at each period of time. Compact bone-derived mesenchymal stem cells (MSCs) were harvested as previously reported (6), and then used for the chondrogenic differentiation using pellet culture. The pellets were analyzed by quantitative real-time reverse transcriptase-PCR (qPCR) at day 21. Total RNA was extracted from the pellets and relative messenger RNA (mRNA) expressions of chondrocyte specific genes were measured by ΔΔCt method. Mean histological scores were statistically compared by non-parametric Wilcoxon tests between pairs of groups. The relative mRNA expressions were statistically compared using unpaired t tests to evaluate differences between pairs of groups. Significance was accepted with a p value < 0.05.

Results: We analyzed the articular cartilage repair in WT mice and GM3-/− mice 8 weeks postoperatively.

GM3-/− mice showed superior cartilage repair compared with WT mice (Fig.1A-D). The score for the articular joint surface was significantly higher in GM3-/− mice than WT mice (mean ± SEM; 7.67 ± 0.53 in WT mice versus 9.88 ± 0.49 in GM3-/− mice [P = 0.0039]) (Fig. 1E). In addition, GM3 was transiently enhanced surrounding the repair tissue peaking 6 weeks postoperatively (Fig. 2). In pellet culture, the type II collagen mRNA wasn’t significantly different between WT and GM3-/− mice (type II collagen, mean ± SEM; 2.72 ± 0.48 in WT mice versus 4.00 ± 0.73 in GM3-/− mice [P = 0.18] Fig.3A), whereas Sox9
and Aggrecan mRNA were significantly increased in GM3-/- mice (Sox9, 0.30 ± 0.033 in WT mice versus 0.47 ± 0.080 in GM3-/- mice [P = 0.049] Fig.3B) (0.61 ± 0.11 versus 1.72 ± 0.32 [P = 0.016] Fig.3C) and type X collagen mRNA was significantly increased in WT mice (0.0015 ± 0.00032 versus 0.00039 ± 0.00019 [P = 0.012] Fig.3D).

**Discussion:** Based on our previously report that the depletion of the gangliosides accelerated the OA progression (3), we have speculated that gangliosides deficiency might decelerate cartilage repair. Surprisingly, the depletion of the gangliosides enhanced the articular cartilage repair as compared to wild mice. Moreover, the depletion of gangliosides inhibited the hypertrophic differentiation in vitro (Fig.3D). In the articular cartilage repair, the repair tissues are often entering the hypertrophic pathway (7). In the current study, the inhibition of the hypertrophic differentiation might lead to the superior morphology. When paired with our previous study, GM3-/- mice showed both the inhibition of the hypertrophic differentiation and the progression of OA (3). In previous reports, the inhibition of hypertrophic differentiation frequently decreased cartilage degradation (8), suggesting that the inhibition of hypertrophic differentiation may be a future direction for the acceleration of cartilage repair. In conclusion, the depletion of gangliosides enhanced articular cartilage repair possibly because the inhibition of hypertrophic differentiation.

**Significance:** To the best of our knowledge, this was the first study that reported GSLs regulated hypertrophic differentiation. Although further studies are required, our speculations will give a new insight to the tissue engineering of cartilage metabolism.

![Figure 1. Representative histology of the articular cartilage repair. WT mice (A, B) and GM3-/- mice (C, D) were stained with Hematoxylin & eosin and safranin-O staining. (E) The articular cartilage repair score at 8 weeks postoperatively (n=20, ** P < 0.01). The scale bar shows 100 μm. All values are expressed as mean ± SEM.](image)
Figure 2. Representative histology of immunostaining of GM3. (A-C) 4 weeks postoperatively, (D-F) 6 weeks postoperatively, (G-I) 8 weeks postoperatively. White dot lines show the margin of the patella groove.

Figure 3. Chondrogenic differentiation at day 21 (n=5, * P < 0.05). (A) Type II collagen mRNA, (B) Sox9 mRNA, (C) Aggrecan mRNA, (D) type X collagen mRNA.

All values are expressed as mean ± SEM.