News Release

Title

Conditional knockdown of hyaluronidase 2 in articular cartilage stimulates osteoarthritic progression in a mice model

Key Points

- ○The absence of Hyal2 in articular cartilage was associated with accumulation of HA and stimulation of OA development and progression
- ○Progression of osteoarthritic change was observed in natural course model and destabilization of the medial meniscus model of mice, in addition to the explant culture experiment using IL-1α.
- ○Knockdown of Hyal2 in articular cartilage induced increased expression of MMP-13 and ADAMTS-5.

Summary

Assoc. prof. Yoshihiro Nishida, Dr. Yoshitoshi Higuchi at Department of Orthopaedic Surgery, Nagoya University Graduate School of Medicine (Dean: Kenji Kadomatsu, MD, PhD), revealed knockdown of Hyaluronidase 2 (Hyal2) in articular cartilage induced osteoarthritis development and progression

Hyaluronan (HA) is abundant in extracellular matrix of connective tissues including articular cartilage. Among several hyaluronidases, two hyaluronidases (Hyal1 and Hyal2) have been considered to play key roles in HA degradation, particularly of articular cartilage.

The details of HA catabolism in articular cartilage remains unclear. A previous report analyzed the effects of HYAL1 deficiency on articular cartilage degeneration, however, effects of HYAL2 deficiency has not been elucidated on the the development of osteoarthritis The aims of this study were to investigate the effects of conditional Hyal2 knockdown in articular cartilage on the development of osteoarthritis (OA) using genetic manipulated mice. Destabilization of the medial meniscus (DMM) model of Hyal2 knockout ($Hyal2^{-/-}$) mice was established and examined in addition to the natural course model of mice. Age related and DMM induced alterations of articular cartilage of knee joint were evaluated with modified Mankin score, immunohistochemical staining of MMP-13, ADAMTS-5 and biotinylated- hyaluronan binding protein staining, and histomorphometrical analyses. Effects of Hyal2 suppression were also analyzed using explant cultures of an IL-1α induced articular cartilage degradation model. The amount and size of hyaluronan in articular cartilage were higher in $Hyal2^{-/-}$ mice. $Hyal2^{-/-}$ mice exhibited aggravated cartilage degradation in age-related and DMM induced mice. MMP-13 and ADAMTS-5 positive chondrocytes were significantly higher in $Hyal2^{-/-}$ mice. Articular cartilage was more degraded in explant cultures obtained from $Hyal2^{-/-}$ mice. Knockdown of Hyal2 in articular cartilage induced OA development and progression possibly mediated by an imbalance of HA metabolism.

Research Background

Hyaluronan (HA) is a major constituent of extracellular matrices (ECMs) of various tissues, particularly in connective tissues including articular cartilage in addition to synovial fluid of the joint and the vitreous humor of the eye. The balance of anabolism and catabolism in HA is crucial for maintenance of extracellular matrix quality in connective tissue. The details of HA catabolism in articular cartilage has not been clarified yet.

Two hyaluronidases (Hyal1 and Hyal2) and cell surface HA receptor (CD44) have been surmised to play key roles in HA degradation (Fig.1).

Deficiency of Hyal1 has been demonstrated to exhibit mucopolysaccharidosis (MPS) IX, a lysosomal storage disorder characterized by joint abnormalities due to HA accumulation and osteoarthritis. Hyal2$^{-/-}$ mice has been reported to display craniofacial and cervical vertebral abnormalities, however, the effects of Hyal2 deficiency on articular cartilage and development of osteoarthritis has not been reported.

The aims of the current study were to analyze the roles of Hyal2 in the development and progression of osteoarthritis (OA) using non-treated aged mice (natural course) and joint instability OA (destabilization of the medial meniscus; DMM) models of mice, in addition to an explant culture inflammation model of articular cartilage.

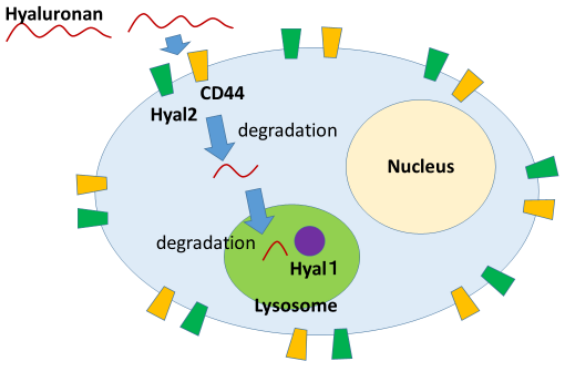

Fig.1 Hyaluronan catabolism

Research Results

Natural course of conditional knockout mice

Results of Safranin-O staining demonstrated there was a marked reduction in Hyal2$^{-/-}$ mice compared with wild mice at 9 months of age (Fig.2A, E, I). Biotinylated- hyaluronan binding protein (B-HABP) staining revealed a difference in HA accumulation in articular cartilage between wild and Hyal2 knockout mice (Fig.2B, F). The amount and size of HA in articular cartilage were higher in Hyal2$^{-/-}$ mice compared to wild mice (Fig.3). More ADAMTS-5 and MMP-13-positive chondrocytes were observed in Hyal2 knockout articular cartilage compared with those in wild cartilage (Fig.2C, D, G, H, J).

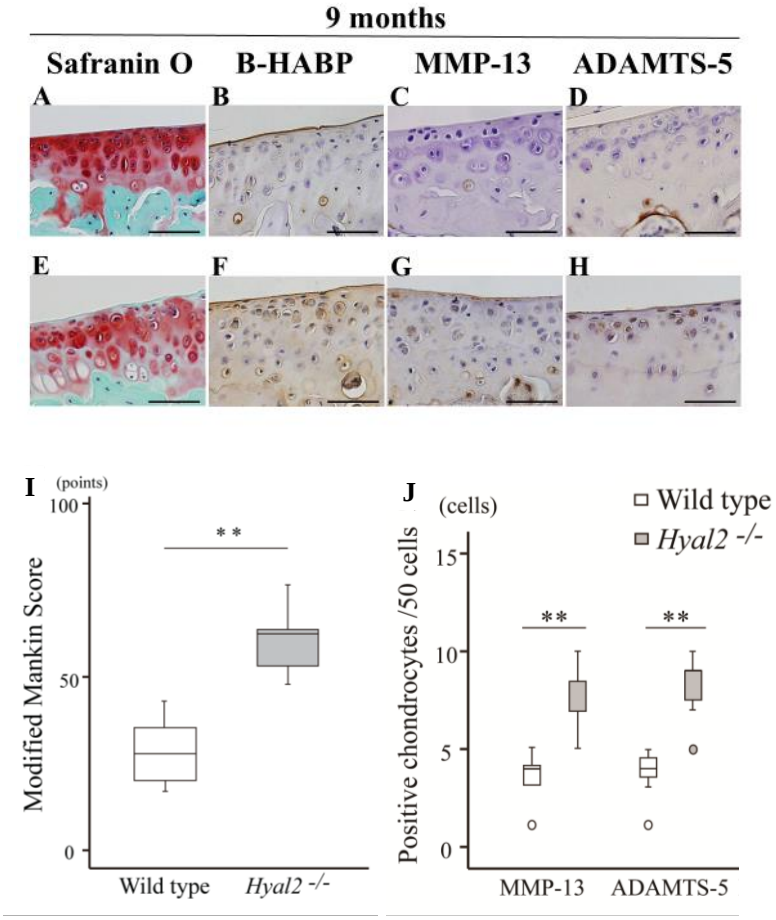

Fig.2 Histologic analysis of structural damage of articular cartilage in natural course of wild mice (A-D) and Hyal2$^{-/-}$ mice (E-H) at 9 months of age. B-HABP staining: wild and Hyal2$^{-/-}$ mice (B and F, respectively). Immunohistochemical staining for MMP-13: wild and Hyal2$^{-/-}$ mice (C and G, respectively). Immunohistochemical staining for ADAMTS-5: WT and Hyal2$^{-/-}$ mice (D and H, respectively).

Modified Mankin score of medial tibia in wild and Hyal2$^{-/-}$ mice at 9 months (**P <0.01) (I). The numbers of immunostaining positive chondrocytes for MMP-13, and ADAMTS-5 in Hyal2$^{-/-}$ mice were significantly elevated compared to that in wild mice (**P <0.01, respectively) (J)

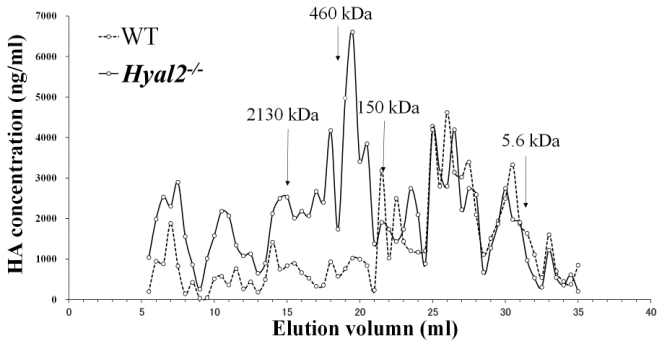

Fig.3 Hyal2 deficiency in cartilage causes the accumulation of high molecular weight hyaluronan.

Hyaluronan extracted from knee joints and femoral heads of 9-month-old male mice and hyaluronan references with known molecular sizes were subjected to Sephacryl S-1000 gel filtration chromatography (0.7×40cm). Hyaluronan contents in each fraction were determined using a competitive ELISA . The arrows indicate the positions of peaks of hyaluronan references.

OA development in mice subjected to DMM surgery

The results of Safranin O staining at 10 weeks revealed that stainable proteoglycan was reduced and osteoarthritic structural change in Hyal2$^{-/-}$ mice compared to wild mice (Fig.4A, E, I). HA deposition detected by B-HABP staining was increased in the pericellular area and in and around the chondrocytes in articular cartilage of Hyal2$^{-/-}$ mice compared with wild mice (Fig.4B, F). More ADAMTS-5 and MMP-13-positive chondrocytes were observed in Hyal2$^{-/-}$ articular cartilage compared with those in wild cartilage (Fig.4C, D, G, H, J, K).

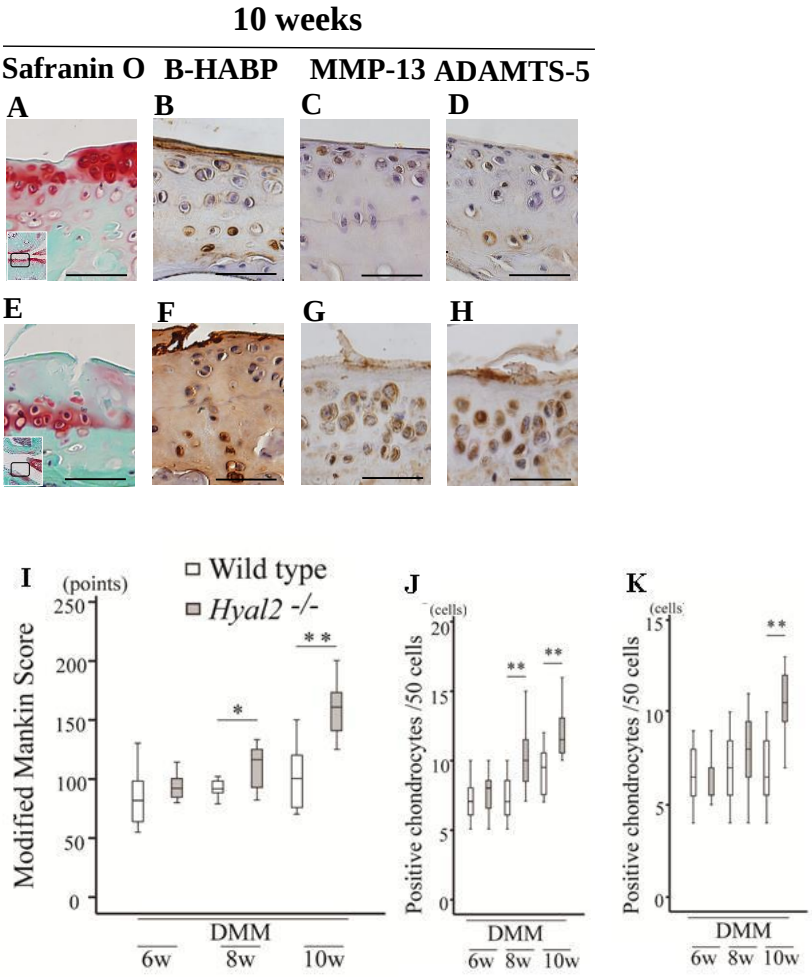

Fig.4 Histological, B-HABP and immunohistochemical staining findings of articular cartilage in DMM treated wild mice (A-D) and Hyal2$^{-/-}$ mice (E-H).
Modified Mankin score (I) of mice articular cartilage (n=8 per group). * P <0.05, ** P <0.01
The numbers of positive chondrocytes for MMP-13 (J), ADAMTS-5 (K) were graphed (** P <0.01).

Effects of IL-1α on Hyal2 deficient cartilage explant

Three days' culture with IL-1α resulted in a marked reduction of Safranin O positive staining in Hyal2$^{-/-}$ mice cartilage compared to that in wild mice cartilage (Fig.5A, E). Results of immunohistochemistry showed that HA was abundantly deposited in and around the chondrocytes in Hyal2$^{-/-}$ mice compared with wild mice (Fig.5B, F). More ADAMTS-5 and MMP-13-positive chondrocytes were observed in chondrocytes of Hyal2$^{-/-}$ cartilage, where stainable proteoglycan was reduced with safranin O staining, compared with that in wild cartilage (Fig.5C, D, G, H, I).

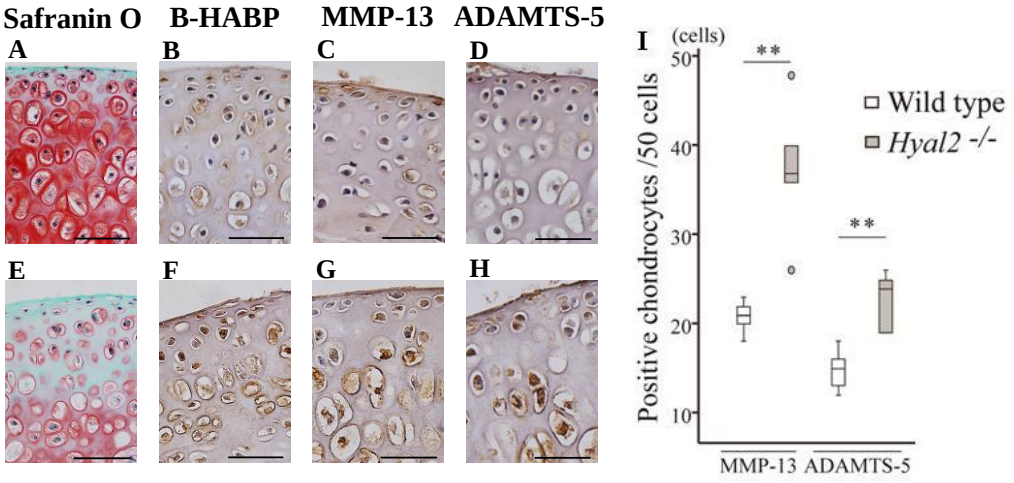

Fig.5 Articular cartilage explant culture with IL-1α of wild and Hyal2$^{-/-}$ mice.
Pieces of articular cartilage were subjected to Safranin O staining (A; wild, E; Hyal2$^{-/-}$).
B-HABP staining (B; wild, F; Hyal2$^{-/-}$), MMP-13 staining (C; wild, G; Hyal2$^{-/-}$), ADAMTS-5 staining (D; wild, H; Hyal2$^{-/-}$).
The numbers of positive stainable cells were graphed (** = P <0.01) (I) **.** Small circle represented outlier.

Research Summary and Future Perspective

The current study demonstrated that the absence of Hyal2 in articular cartilage was associated with accumulation of HA and stimulation of OA development and progression. It was previously reported that Hyal1 deficiency in a mouse model of MPS IX exhibited OA. Hyal2 deficiency showed similar OA features, suggesting a novel MPS-like disorder of HA storage. Generally, HA content is reduced in articular cartilage of osteoarthritis. This study revealed that abnormal accumulation of HA also induces osteoarthritic change in articular cartilage. Clinically, there are many types of osteoarthritis patients. Among them, some patients may revealed abnormal accumulation of HA in articular cartilage. Elucidation of osteoarthritis patients with increased HA accumulation may establish the novel patient cohort of osteoarthritis, and contribute to the novel treatment modality such as inhibition of HA synthesis.

Publication

Yoshitoshi. Higuchi, Yoshihiro Nishida, Eiji Kozawa, Lisheng Zhuo, Eisuke Arai, Shunsuke Hamada, Daigo Morita, Kunihiro Ikuta, Koji Kimata, Takahiro Ushida, Naoki Ishiguro. Conditional knockdown of hyaluronidase 2 in articular cartilage stimulates osteoarthritic progression in a mice model. Scientific Reports, published online on August 1st, 2017.
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Japanese ver.

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