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COMMENTARY

Pathophysiology of Hyaluronan Accumulation/Depolymerization in Osteoarthritic Joints

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In healthy articular cartilage, the extracellular matrix (ECM) is properly maintained by the overall anabolic and catabolic balance of slow synthesis and degradation, a so-called homeostatic state, under the influence of synovial metabolism. Though the ECM of articular cartilage tissue contains low amounts of hyaluronan (HA), its main components are collagen, type II, IX, and XI, and proteoglycans, such as aggrecan. Nevertheless, HA plays crucial roles in cartilage, especially in retaining aggrecan in tissues.[1] Previous studies on ECM degradation in osteoarthritis (OA) focused on the degradation of the proteoglycan, aggrecan,[2] but not that of HA. The mechanism of HA synthesis in articular cartilage and synovium has been elucidated by the expression and functional analysis of hyaluronan synthase 1 to 3. However, research on the HA degradation system has not progressed because the main enzymes involved are not well understood. The research article by Momoeda et al,[3] in this issue of *The American Journal of Pathology*, provides new findings on the involvement of a novel hyaluronidase in OA development using genetically manipulated mice.

Among the molecules in the hyaluronan-degrading enzyme (HYAL) family, HYAL1 and HYAL2 are widely expressed and have been hypothesized to be the major hyaluronidases involved in HA catabolism in somatic tissues. However, research data on HYAL1 and HYAL2 do not support them being the central hyaluronidases that cleave high-molecular-weight (HMW) HA on the cell surface or extracellular space. This is because both HYAL1 and HYAL2 have enzymatic activity in the acidic condition,[4,5] whereas HYAL2, which has enzymatic activity on the cell membrane, has 50 times lower enzymatic activity than HYAL1.[6]

Recently, two new molecules with strong hyaluronan degrading activity have been identified. First, *KIAA1199*, a deafness gene of unknown function, was reported to play a central role in HA degradation in the dermis of healthy skin and the synovium of arthritis patients.[7] The authors proposed to name KIAA1199 as HYBID (a hyaluronan-binding protein involved in hyaluronan depolymerization).[8] This molecule is also named CEMIP (cell migration−inducing protein) because it also has an important role in oncology.[9] Second, transmembrane protein 2 (TMEM2) was identified as a cell surface hyaluronidase. Unlike HYBID/CEMIP/KIAA1199, TMEM2 does not require the participation of live cells for its hyaluronidase activity.[10] The culture supernatant of HYBID-transfected cells has almost no HA-degrading activity.[7] In contrast, TMEM2 has been shown to act in the extracellular environment.[10]

When and how these old and new hyaluronidases (HYAL1, HYAL2, HYBID/KIAA1199/CEMIP, and TMEM2) are involved in the onset and progression of OA is unknown.

Involvement of Hyaluronidase in the Onset and Progression of OA

HA degradation by HYAL1 occurs intracellularly, and deficient mutation of *Hyal1* causes a lysosomal storage disorder, mucopolysaccharidosis IX.[11] Histologic analysis of the knee joint of Hyal1 null mice showed that proteoglycan loss occurred at 3 months and progressed with age. An increased number of chondrocytes, showing strong pericellular and/or cytoplasmic HA staining, was detected in the epiphysis and articular cartilage of null mice, indicating HA accumulation. These results indicate that osteoarthritis is an important disease feature of Hyal1 deficiency, but it is not known whether high expression of Hyal1 is involved in the development of

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OA. Cartilage-specific conditional *Hyal2*$^{-/-}$ mice, Col2a1-dependent conditional knockout mice using the Cre-loxP system, revealed accelerated OA development not only in the natural course of aged mice, but also in a destabilization of the medial meniscus (DMM) surgery-induced OA model.[12] HA deposition was increased in the pericellular area and in and around the chondrocytes in *Hyal2*$^{-/-}$ mice compared with wild-type (WT) mice with DMM surgery at 10 weeks. Proteoglycan was reduced in *Hyal2*$^{-/-}$ (DMM) mice compared with WT (DMM) mice with Safranin-O staining. The authors concluded that Hyal2 deficiency showed similar OA features to those of Hyal1 deficiency, suggesting that defects of Hyal2 cause a novel mucopolysaccharidosis-like disorder of HA storage. *Hyal3*$^{-/-}$ mice were viable, fertile, and showed no gross phenotypic abnormalities. In addition, a study of 12- to 14-month−old mice showed no difference in knee joint tissue between *Hyal3*$^{-/-}$ mice and *Hyal3*$^{+/+}$ mice.[13] These findings suggest that Hyal3 does not play crucial roles in the development of OA or ECM degradation of articular cartilage. As described above, HYAL1 and HYAL2 seem to achieve homeostasis of the ECM of articular cartilage by regulating the metabolism of HA, thereby avoiding the development of OA.

In contrast to these studies, Momoeda et al,[3] in this issue of *The American Journal of Pathology*, reported that in nonintervention mice, observed up to 60 weeks of age, the results showed only minimal structural changes and proteoglycan depletion in both Hybid null and WT mice. HA staining with HA-binding protein also showed no difference between WT and Hybid null mice. Unlike the Hyal1 and Hyal2 null mice, the Hybid null mice did not cause a spontaneous OA change, suggesting that Hybid has less constitutive activity in articular cartilage. They provided the results using two types of OA-induced models (medical collateral ligament transection with meniscus removal model and DMM model) with *Hybid*$^{-/-}$ mice. Destruction of articular cartilage is suppressed in Hybid null mice in parallel with the accumulation of high-molecular-weight HA in joint tissue. In contrast, WT mice show high expression of Hybid in synovial cells and chondrocytes and a lower ratio of HA species >1000 kDa compared with Hybid null mice. Hybid expression is elevated in an OA-inducing environment, suggesting that it is deeply involved in OA onset and progression. The OA-induced model of Momoeda et al[3] had elevated expression of Tmem2, although not as much as Hybid, suggesting that Tmem2 may play some roles in OA onset and development.

Hyaluronidase in Developed OA, Potential Therapeutic Intervention

A previous study indicated that synovial tissues obtained from knee OA patients had a 5.5-fold expression of HYBID compared with normal control synovium, and TMEM2 expression was similar between OA and normal tissues.[14] Of the 12 factors examined, IL-6 significantly up-regulated HYBID expression and HA degradation activity in osteoarthritis synovial fibroblasts. For articular cartilage, Shimizu et al[15] demonstrated that HYBID is overexpressed by chondrocytes in the HA depleted area of OA cartilage, correlating directly with the Mankin score, and HYBID expression in the OA cartilage cells is up-regulated by tumor necrosis factor-α. They also indicated that HYAL1 and HYAL2 expression was up-regulated in OA chondrocytes; however, suppression of HYBID with siRNA eliminated HA depolymerization activity, and siRNA against HYAL1 and HYAL2 did not suppress HA depolymerization. Ohtsuki et al[16] reported that CEMIP/HYBID expression was transiently increased by IL-1β stimulation in chondrocytic cells *in vitro*. These results suggest that HYBID is highly expressed in OA cartilage and synovial tissues and might play a central role in HA depolymerization under inflammatory conditions.

Can HYBID, which is highly expressed in OA, be a therapeutic target? If highly expressed HYBID is suppressed in a state of OA, can reversible changes in the pathophysiology of OA be expected and, if possible, reversal of symptoms? The stage of OA (Kellgren-Lawrence classification) HYBID that can be a therapeutic target has not been studied yet. Because OA is multifactorial, if it progresses to some extent, it may progress if only HYBID is targeted, and therefore may require some combination therapy. These will be important research topics for the future.

Does Exogenously Administered HMW-HA Compensate for HA Degradation of OA?

Momoeda et al,[3] in this issue of *The American Journal of Pathology*, reported that injections of HMW-HA inhibited the cartilage destruction in a WT mouse OA group. HA injected into joints has been reported to have a half-life of <24 hours,[17] and it is unlikely that HA in the joint fluid can act as a long-acting viscosupplement having a sustained inhibitory effect on OA development. HYBID, a strong hyaluronidase, may catabolize exogenously added HA in joint fluid much faster under inflammatory conditions. Momoeda et al[3] suggested that HMW-HA itself may have a biological activity to attenuate OA development. Their data showed that HYBID null mice accumulated HMW-HA in the OA model, and injection of HMW-HA suppressed cartilage degradation in the OA model using WT mice. Another possibility is that exogenous HMW-HA can act as a competitive inhibitor of endogenous HA breakdown. Because the mechanism of action of HMW-HA on suppression of cartilage degradation was not clear, the findings of Momoeda et al[3] have provided new mechanistic insights into the role of HMW-HA in preventing cartilage degradation.

In articular cartilage, which contains abundant high-quality ECM, is it possible for exogenously added HMW-HA to penetrate and/or accumulate in cartilage tissue? Fukuda et al[18] show that exogenous HA does not penetrate cartilage, and remains on the surface. However, if treated with IL-1, exogenous HA deposits pericellular matrix of

chondrocytes in articular cartilage.[18] This suggests that, in inflammatory conditions, exogenous HA could affect the deep-seated chondrocytes.

HA injection in human OA has some effect. The American Medical Society for Sports Medicine issued a scientific statement in 2016 regarding intra-articular HA injections for knee OA.[19] There is evidence to support intra-articular HA treatment for Kellgren-Lawrence classification grade 2 or 3 knee OA in patients aged ≥60 years. However, intra-articular HA treatment might not be effective in patients with Kellgren-Lawrence classification grade 4. Taken together, the effect of exogenous HA may differ depending on the stage of OA. It seems highly improbable to compensate for the effects of overexpressed HYBID in any state of OA.

Perspectives

HYBID/CEMIP/KIAA1199 is highly expressed in OA, is clearly associated with its onset and progression, and appears to play a greater role than Hyal1 or Hyal2. A drug that suppresses the expression and function of HYBID/CEMIP/KIAA1199 during OA onset is likely to suppress or delay the onset of OA. There is evidence for the efficacy of exercise and weight loss as treatments for OA. It would be interesting to examine the effects of these interventions on HYBID expression and function.

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