主論文の要約

Hyaluronan in articular cartilage: Analysis of hip osteoarthritis and osteonecrosis of femoral head

（関節軟骨中のヒアルロン酸分析：変形性股関節症と大腿骨頭壊死症の比較）

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【Introduction】

Osteoarthritis（OA）is a degenerative disease causing joint pain, stiffness, and swelling as the main symptoms. The most common pathological changes in OA joints include loss and destruction of articular cartilage, formation of osteophytes, synovial inflammation, and degeneration of ligaments.

In human articular cartilage, hyaluronan (HA) plays important roles as one of the main components of extracellular matrix (ECM) molecules. Many aggrecans bound to HA maintain the required compressive resilience.

HA is synthesized by hyaluronan synthase (HAS), of which there are three isoforms. HAS2 is considered to be most important enzyme among the three of them in articular cartilage. HAS2 is known to produce high molecular weight (MW) HA. Previous reports indicated that HA degradation mainly relied on hyaluronidase (HYAL) 1,2; however, research results on HYAL1 and HYAL2 do not support their being central HYALs.

Recently, KIAA1199 has been reported to be an enzyme that powerfully degrades HA independently of CD44 and HYAL1,2. The discovery of this new HYAL, KIAA1199, has led to several studies on the involvement of KIAA1199 in OA cartilage and synovium, and the results have been reported. KIAA1199 is upregulated in OA chondrocytes and leads to the degradation of high MW HA in OA cartilage. KIAA1199 expression is significantly higher in OA synovium than in normal control synovium. However, no studies have reported KIAA1199 involvement in osteonecrosis of femoral head (ONFH) in comparison to hip OA.

ONFH is a disease characterized by the death of osteocytes and bone marrow mostly caused by ischemia of the femoral head. Bone necrosis eventually causes collapse of the femoral head. Due to lack of cartilage nutrition, the collapsed area will become degraded and softened. Several studies performed gene expression analysis on ONFH, and reported high expression of some chondrogenesis- or ECM-related genes. However, these studies could not identify any HA-related genes including KIAA1199. In the present study, HA status and metabolism in articular cartilage of human ONFH were investigated focusing on KIAA1199 in comparison to OA and control cartilage.

【Material and Methods】

Human articular cartilage tissues of ONFH, OA, and control were obtained from femoral head (n = 27, mean ± SD age: 59.4 ± 14.03) (Table 1). Tissues of ONFH and OA were retrieved during total hip arthroplasty. Control cartilage tissues were obtained during artificial head replacement for femoral neck fracture or hip disarticulation surgery for malignant bone and soft tissue tumors at our institution. OA cartilage specimens were taken from the macroscopically damaged part of the femoral head. ONFH cartilage specimens were taken from the cartilage in collapsed areas of the femoral head. Control group cartilage specimens were taken from femoral head cartilage was macroscopically normal and without any

osteoarthritic changes on preoperative X-ray.

During the process of extracting HA from cartilage, we use deferoxamine to inhibit the depolymerization of high molecular weight (MW) HA. The amount of HA and the MW in the cartilage tissue were analyzed by competing HA ELISA and chromatography, respectively. The mRNA expression of HAS1, HAS2, HAS3, HYAL1, HYAL2, and KIAA1199 was evaluated using quantitative RT-PCR, and the tissue distribution of KIAA1199 and HA was analyzed by immunohistochemical staining for KIAA1199 and staining with HA-binding protein (HABP).

All data are expressed as mean ± SD. The Mann–Whitney U-test followed by Bonferroni corrections was used to assess two independent groups, with values of <0.05 considered significant.

【Results】

According to the immunostaining results. Overexpression of KIAA1199 was observed in the region of fibrillation and cracks in OA cartilage and positivity of HABP staining was reduced. There was remarkably decreased expression of KIAA1199 in the cartilage of the ONFH collapsed part, and a slight decrease in HABP staining was observed.

The MW distribution results show that MW of HA in OA cartilage was increased, while in ONFH it was decreased.

The HA content in OA cartilage (0.33 ± 0.23 μg/mg) was lower than control cartilage (0.80 ± 0.40 μg/mg); however, it was not significant ($p = 0.154$). The HA content in the ONFH cartilage varied widely, and no particular tendency was observed.

Results of RT-PCR showed that mRNA expression of HAS2 and KIAA1199 was increased in OA cartilage, and the mRNA expression of genes related to HA catabolism was decreased in ONFH cartilage.

【Discussion】

HAS2 is considered to be the key HA synthase in cartilage and produces high MW HA. Overexpression of HAS2 in OA cartilage in the present study might be the main cause of the increase of HA MW, whereas, inactivated HA metabolism may result in the lowered HA MW distribution of ONFH.

Regarding the pathogenesis of ONFH, ischemia is considered the main cause of femoral head necrosis, cartilage in ONFH was considered to be in a nutrient deficiency environment. Meanwhile, the synthetic process of HA requires sufficient energy supply. We speculate the nutrient deficient environment cause the inactivation of molecules which related to the HA metabolism, resulting in the reduction of HA molecular weight in ONFH cartilage.

The amount of HA in OA cartilage showed a downward trend but was not statistically significant, and it is speculated that it might be caused by the imbalance of HA catabolism and anabolism. Although the lytic enzyme of HA, KIAA1199, shows very active, overexpression

of HAS2 may compensate for part of the HA loss in OA cartilage. Considering that KIAA1199 was found to play a key role in HA catabolism in arthritic synovium, we guess that excess HA in OA synovial fluid may partially originate from degenerated cartilage and be depolymerized into small molecules by KIAA1199 in synovium.

According to the previous report, cartilage above necrotic zone in ONFH may not have progressed to identical pathological state, it might be responsible for the large variance of HA content and size in ONFH cartilage.

KIAA1199 was found to be overexpressed in OA knee cartilage and synovial tissue and play a key role in the process of HA degradation. The expression of KIAA1199 was also increased in hip OA cartilage, but not in ONFH. These results help to make a clear distinction between the pathophysiology of articular cartilage in OA and ONFH.

【Conclusions】

HA metabolism in ONFH cartilage was suggested to be slow and was activated in OA with high expression of KIAA1199. The fact that the MW of HA in OA cartilage did not decrease may be related to the increase in the synthetic system, HAS expression.

Table 1

	Age mean± SD	Classification	Case
Control	59.6±11.95	7	
OA	63.8±11.64	K-L Grade	Case
		2	1
		3	4
		4	5
ONFH	54.8±17.12	Ficat stage	Case
		III A	5
		III B	2
		IV	3