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Original Article

Mild degenerative changes of hip cartilage in elderly patients: an available sample representative of early osteoarthritis

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Abstract: This study investigated the cellular and molecular changes which occur in cartilage from adults with femoral neck fracture (FNF) and osteoarthritis (OA), and explored the similarities in hip cartilage obtained from elderly patients and patients with early OA. Femoral heads were retrieved from 23 female patients undergoing total hip arthroplasty (THA). This group included 7 healthy patients with FNF (hFNF), 8 elderly adults with FNF (eFNF), and 8 elderly patients with hip OA (OA). After high-field MRI T2 mapping, osteochondral plugs were harvested from the weight-bearing area of femoral heads for subsequent macroscopic, histologic, and immunochemical evaluation. Additionally, the contents of cartilage matrix were analyzed, and gene expression was detected. The surface of cartilage from hFNF and eFNF patients appeared smooth, regular, and elastic, whereas it showed irregularities, thinning, and defects in OA patients. Elevated T2 values and decreased accumulation of glycosaminoglycans (GAGs) were detected in cartilage from eFNF patients. Furthermore, type I collagen accumulation was slightly increased and type X collagen concentration was obviously elevated in eFNF patients; however, type II collagen distribution and the contents and anisotropy of collagen fibrils in eFNF patients showed no significant changes. Consistent with histology and immunohistochemical results, aggrecan was downregulated and type X collagen was upregulated, while collagens types I and II showed no significant changes in eFNF patients. The cellular and molecular characteristics of hip cartilage in eFNF patients who showed no symptoms of OA were similar to those in patients with mild OA. Thus, eFNF cartilage can serve as a comparative specimen for use in studies investigating early OA.

Keywords: Femoral neck fracture, osteoarthritis, elderly patients, articular cartilage degeneration, available sample

Introduction

Osteoarthritis (OA) is a global health problem, and its clinical progression often results in severe pain and limited mobility. Moreover, due to its avascular nature, cartilage has a very limited inherent capacity for self-renewal. The obvious radiographic signs and symptoms of OA mostly appear in a relatively advanced stage of the disease, and other than prosthetic arthroplasty, the therapeutic options for late OA are very limited [1]. Thus, early diagnosis of OA plays a critical role in the treatment and outcome of the disease. The recent development of high field strength imaging and functional MRI T2 mapping allows for quantitative assessment of early degenerative alterations in articular cartilage, such as changes in water contents, GAG amounts, collagen contents, and collagen anisotropy [2-5].

Nevertheless, cartilage samples from patients with early or mild OA are not easily acquired, because the clinical signs and imaging features of OA are usually not yet evident. Therefore, various animal models of OA have been developed to study the cellular and molecular events associated with early stage OA [6-8]. Matyas et al. demonstrated that aggrecan levels and type II collagen degradation increased in a canine model of early OA induced by unilateral transection of the anterior cruciate ligament. Their

results suggested that changes in the composition joint fluid, serum, and urine were sensitive and specific biomarkers for changes in the cartilage matrix [9]. Little and coworkers reported the effect of abnormal weight-bearing on the metabolism of aggrecan, biglycan, and decorin in a meniscectomy induced ovine model of early OA. Their data indicated that unnatural mechanical loading caused changes in the phenotypic expression of proteoglycans by chondrocytes [10]. Moreover, Kyrkos et al. showed that intramuscular administration of calcitonin delayed development of early OA caused by knee joint instability in a rabbit model [11].

However, there are limitations associated with using animal models. First, the species differences between human and animals are reflected in the OA development process [12]. Moreover, animals generally have smaller joint sizes and thinner cartilage than humans, and these factors limit the amounts of cartilage that can be obtained for analysis [13, 14]. Additionally, animals usually display different joint loading patterns when compared to humans [15, 16]. Thus, it is of great importance to acquire a homologous sample of human cartilage when investigating the early events in development of OA.

In this study, homogenous specimens of human cartilage were obtained by punching cartilage-bone plugs from the femoral head of elderly patients with a femoral neck fracture (FNF) after total hip arthroplasty (THA). We hypothesized that the cellular and molecular characteristics of hip cartilage from elderly adults who had no symptoms of OA would be consistent with the characteristics of cartilage obtained from patients with mild OA. The goal of this study was to identify and investigate a sample of human cartilage that would be representative of early OA.

Materials and methods

Study population and cartilage source

Femoral heads were harvested from 23 female patients (mean age, 66.5 years; range, 43-84 years) during unilateral total hip arthroplasty (THA). The harvested femoral heads were then divided into 3 groups: healthy patients with femoral neck fracture (hFNF) (mean age, 49.1 years; range, 43-53 years; n = 7); elderly adults with femoral neck fracture (eFNF) (mean age 73.6 years; range, 62-81 years; n = 8); elderly adults with hip OA (OA) (mean age 74.5 years; range 64-84 years; n = 8). The protocol for this study was approved by the Research Ethics Committee of Nanjing Medical University of China, and a signed written informed consent was obtained from all participants.

T2 relaxation time at 7.0 T

An MRI examination of each femoral head was performed using a 7.0-T MRI system (Bruker PharmaScan; Bruker BioSpin, Karlsruhe, Germany) equipped with a 60 mm coil. Sagittal T2 mapping was performed with the following parameters: repetition time (TR) 7953.6 ms; echo time (TE) 15.3 ms; flip angle (FA) 180.0 deg; field of view (FOV) 60 × 60 mm; pixel matrix 384 × 384; section thickness 1.2 mm; scan time 38 min 10 s 634 ms. T2 maps were then generated using a Bruker ParaVision 5.0 system. The weight-bearing area of each femoral head was selected as the region of interest (ROI), and totals of 21, 24, and 24 ROIs (3 ROIs for each sample) were identified from the hFNF, eFNF, and OA patients, respectively. The average T2 relaxation times were calculated using a Bruker ParaVision 5.0 system, and ROIs were analyzed by a senior musculoskeletal radiologist with 6 years of experience in that field of medicine.

Gross observations and histology

Following examination by MRI, osteochondral plugs (8 mm in diameter) were obtained from the weight-bearing area of each femoral head and divided into two halves using a scalpel. The specimens were photographed, fixed in 10% neutral formalin for 24 h, and decalcified in 5% nitric acid for 72 h. The samples were then dehydrated through a series of graded ethanol solutions, cleared in xylene, and embedded in paraffin. Paraffin sections (5 μm in thickness) were stained with hematoxylin-eosin (H&E) and Safranin-O/Fast-Green (SO) for examinations of cell morphology and proteoglycan accumulation, respectively. Staining results were observed using a light microscope (Ci-L; Nikon, Tokyo, Japan). Additionally, separate sections of tissue were stained with Sirius Red for examination of collagen anisotropy, and visualized by polarized light microscopy (PLM) (Ci-L; Nikon, Tokyo, Japan).

Table 1. Primer sequences used for RT-PCR

Gene	Primer nucleotide sequence
Type I collagen	Forward: 5-CAGCCGCTTCACCTACAGC-3
	Reverse: 3-TTTTGTATTCAATCACTGTCTTGCC-5
Type II collagen	Forward: 5-GGCAATAGCAGGTTCACGTACA-3
	Reverse: 3-CGATAACAGTCTTGCCCCACTT-5
Type X collagen	Forward: 5-CAAGGCACCATCTCCAGGAA-3
	Reverse: 3-AAAGGGTATTTGTGGCAGCATATT-5
Aggrecan	Forward: 50-TCGAGGACAGCGAGGCC-3
	Reverse: 3-TCGAGGGTGTAGCGTGTAGAGA-5
GAPDH	Forward: 5-ATGGGGAAGGTGAAGGTCG-3
	Reverse: 3-TAAAAGCAGCCCTGGTGACC-5

Immunohistochemistry

Separate tissue slices (5 μm in thickness) were incubated with proteinase K enzyme for 30 min for antigen retrieval, and then immersed in 3% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity. After blocking nonspecific protein binding with 10% goat serum, the sections were incubated for 1 h at 37°C with mouse anti-type II collagen monoclonal antibody (Novus Biologicals, Littleton, CO, USA), mouse anti-type I collagen antibody (Sigma, St. Louis, MO, USA), and rabbit anti-type X collagen antibody (Abcam, Shatin, N.T., Hong Kong), respectively. The sections were then incubated with goat anti-mouse/rabbit secondary antibody (Nanjing KeyGen Biotech. Co., Ltd., Jiangsu, China) for 30 min at 37°C; after which, they were incubated in 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution for 3-5 min at room temperature and counterstained with hematoxylin for 8 min. Following counterstaining, the sections were mounted with neutral balsam for microscopic observation.

Biochemical assays

Cartilage samples for biochemical assays were harvested from the weight-bearing portion of each femoral head, and the wet weights were determined. The samples were then dried for 2 days at 65°C to remove water and re-weighed. The water content of the cartilage was calculated as wet-dry weight/wet weight.

The total DNA contents of cartilage samples were quantified using a Quit-iT dsDNA kit (Invitrogen, Eugene, OR, USA). In brief, a solution of papain-digested cartilage was reacted with Hoechst dye 33258 for 30 min in the dark. Fluorescence intensity was determined with a microplate reader (Bio-Rad Laboratories, Richmond, CA, USA) using excitation at 360 nm and emission at 460 nm. DNA from salmon testes (Sigma) was used to establish the standard curve.

The GAG contents of cartilage samples were measured using a Blyscan™ sulfated GAG assay kit (Biocolor, Newtonabbey, UK) and a standardized protocol. Briefly, a papain-digested solution of cartilage was mixed with Blyscan dye reagent and centrifuged at 12,000 rpm for 10 min. The non-bound GAG dye was then removed and dissociation reagent was added to the remaining pelleted material. A microplate reader (Bio-Rad Laboratories, Richmond, CA, USA) was used to measure absorbance at 656 nm.

The collagen components of cartilage samples were quantified using a Sircol Collagen Assay Kit (Biocolor, Newtonabbey, UK) according to the manufacturer's instructions. In brief, a pepsin-digested cartilage solution was mixed with Sircol dye reagent and centrifuged at 12,000 rpm for 10 min. After removing the supernatant, the pelleted material was washed with acid-salt wash reagent and dissolved in alkali reagent. A microplate reader (Bio-Rad Laboratories, Richmond, CA, USA) was used to measure the absorbance at 555 nm.

Gene expression

The weight-bearing cartilage of each femoral head was collected and analyzed for gene expression. Briefly, the cartilage specimens were lysed in Trizol reagent (Invitrogen, California, USA) at room temperature for 10 min, and the total RNA was reverse transcribed to cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). RT-PCR and an ABI Prism 7500 sequence detection system (Applied Biosystems, CA, USA) were used to assess the expression of genes for type I collagen (COL1), type II collagen (COL2), type X collagen (COL10), aggrecan (ACAN), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH was used as the housekeeping gene for normalization of expression levels. The

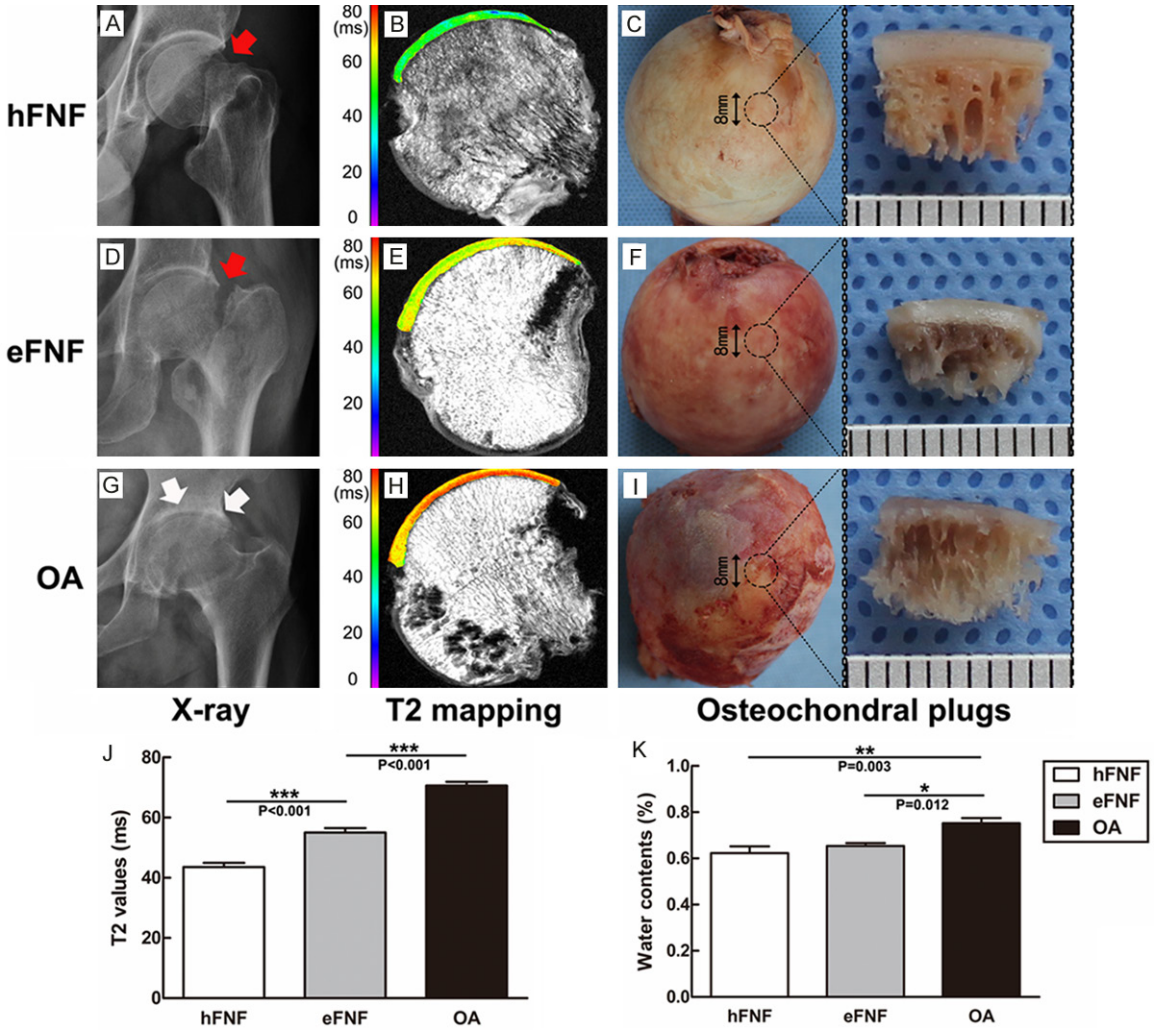

Figure 1. Results of high-field MRI T2 mapping, macroscopy, and determination of water contents. A, D, G: Anterior-posterior X-rays of hip joints. Red arrows denote the region of femoral neck fracture (FNF) and white arrows denote hip osteoarthritis. B, E, H: Cartilage T2 mapping demonstrated higher signal intensity in elderly adults with FNF (eFNF) compared to healthy patients with FNF (hFNF), but lower signal intensity than in elderly adults with hip OA (OA). C, F, I: Cartilage-bone plugs (8 mm diameter) punched from the femoral heads of hFNF and eFNF patients were smooth and regular in appearance, while cartilage-bone plugs punched from femoral heads of OA patients appeared irregular, thin, and defective. J: The average T2 value for eFNF cartilage was significantly higher than that for hFNF cartilage ($P < 0.001$), but lower than that for OA cartilage ($P < 0.001$). K: Water content in eFNF cartilage was insignificantly higher than that in hFNF cartilage ($P = 0.351$), but significantly lower than that in OA cartilage ($P = 0.012$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

PCR system was run for 40 cycles. The primer sequences were designed using Primer 5 software and are listed in **Table 1**.

Statistical analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA). The Fisher's least significant difference (LSD) *post-hoc* test was used to compare differences in T2 values, water, DNA, GAG, and collagen contents, and gene expression in hFNF, eFNF, and OA cartilage samples. *P* values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS for Windows, Version 13.0 Chicago, IL: SPSS Inc.

Results

MRI T2 mapping, macroscopy and water contents

The study patients diagnosed as FNF (**Figure 1A**, **1D**) or OA (**Figure 1G**) underwent THA. T2

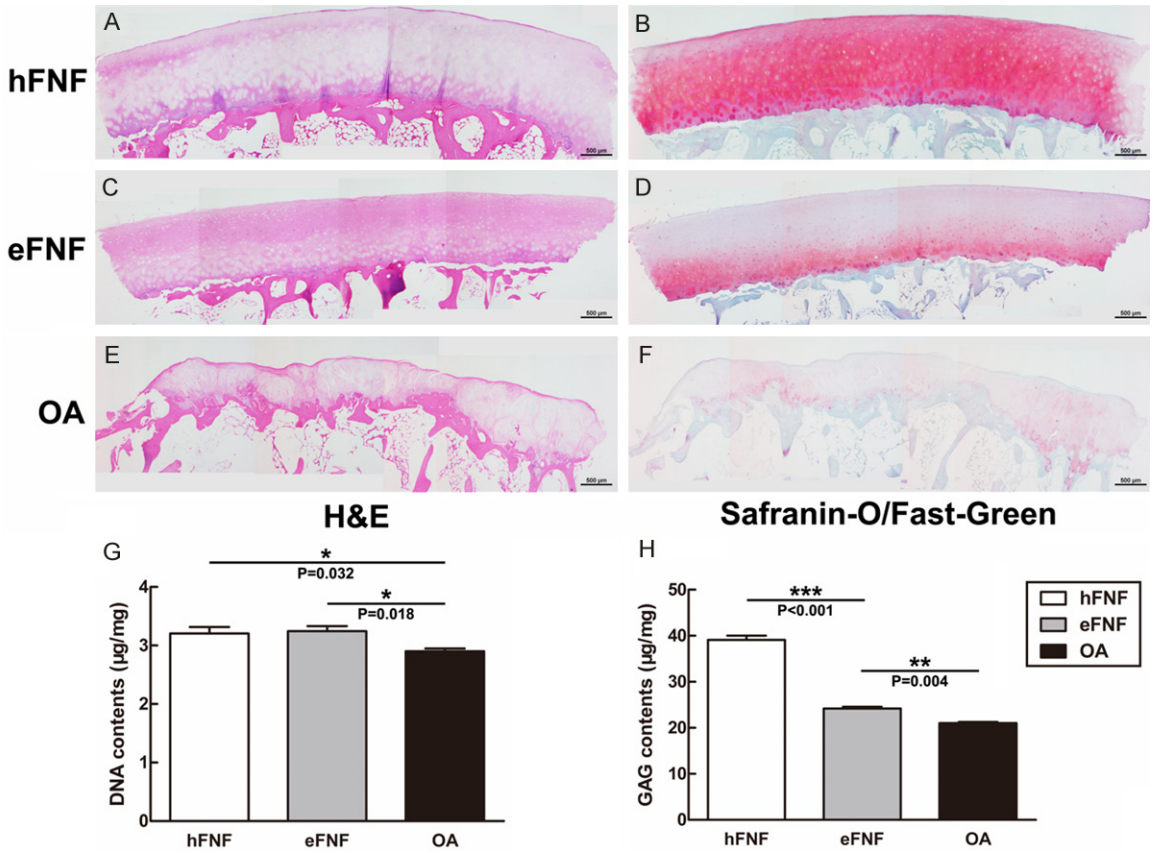

Figure 2. Cell morphology and proteoglycan accumulation. (A, B) Chondrocytes and proteoglycans were regularly distributed in hFNF cartilage, (C, D) whereas slightly hypertrophic chondrocytes were showed in the superficial layer of eFNF cartilage and reduced SO staining were detected in the upper and intermediate zone of cartilage. (E, F) An uneven distribution of chondrocyte clusters and marked loss of SO staining in all layers were shown in OA cartilage. (G) The total DNA contents of hFNF and eFNF were significantly higher than that of OA cartilage, while hFNF and eFNF cartilage showed no significant differences in DNA contents. (H) Amounts of GAG were higher in hFNF cartilage compared to eFNF cartilage, and much higher than amounts in OA cartilage. Magnification ×40. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

mapping of eFNF cartilage (**Figure 1E**) demonstrated higher signal intensity than obtained from hFNF cartilage (**Figure 1B**), but lower signal intensity than obtained from OA cartilage (**Figure 1H**). Furthermore, the average T2 value of eFNF cartilage (55 ± 3.3 ms) was significantly higher than that of hFNF cartilage (43.5 ± 3.2 ms) ($P < 0.001$), but lower than that of OA cartilage (70.6 ± 2.8 ms) ($P < 0.001$) (**Figure 1J**).

The surfaces of hFNF and eFNF cartilage appeared smooth and regular (**Figure 1C**, **1F**), whereas OA cartilage showed surface irregularities, thinning, and defects (**Figure 1I**). Moreover, the water content of eFNF cartilage was significantly lower than that of OA cartilage ($P = 0.012$). There was no significant difference in water content between hFNF cartilage and eFNF cartilage ($P = 0.351$) (**Figure 1K**).

Cell morphology and proteoglycan accumulation

Microscopic examinations showed that chondrocytes were regularly distributed in hFNF cartilage (**Figure 2A**), slightly hypertrophic in the superficial layer of eFNF cartilage (**Figure 2C**), and unevenly distributed and arranged in clusters in OA cartilage (**Figure 2E**). The surface of OA cartilage was rough and broken by fissures which extended downwards into the deep layers of cartilage tissue (**Figure 2E**). Additionally, the total DNA contents of hFNF and eFNF were significantly higher than that of OA cartilage ($P = 0.032$ and $P = 0.018$, respectively), while hFNF and eFNF cartilage showed no significant differences in DNA contents ($P = 0.738$) (**Figure 2G**).

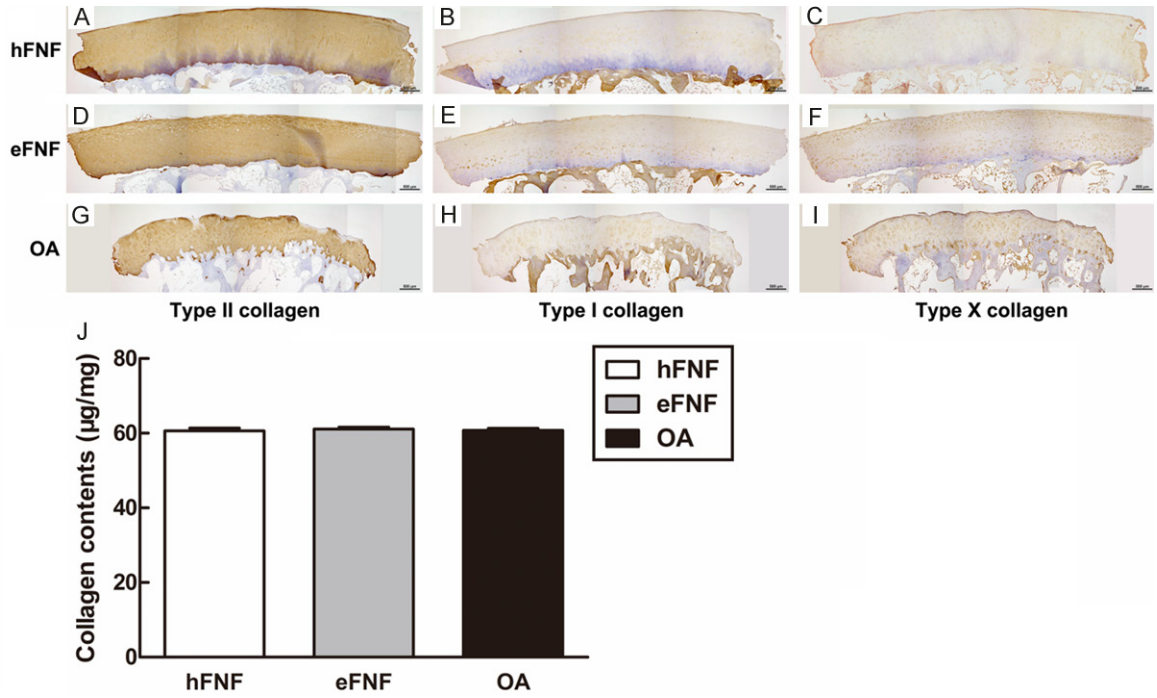

Figure 3. Collagen distribution. A-C: Type II collagen was uniformly distributed throughout the cartilage zones, whereas types I and type X collagen were not detected in the hFNF cartilage. D: Distribution of type II collagen showed no obvious changes in all layers of eFNF cartilage. E, F: However, a slightly increased accumulation of type I collagen and an obviously enhanced concentration of type X collagen were found in the superficial and deep regions of eFNF cartilage. G: Furthermore, type II collagen concentrations were reduced throughout the upper region of cartilage, H, I: whereas deposition of collagens type I and X were increased mainly around chondrocyte clusters in OA cartilage. J: However, there were no significant differences in the total collagen contents of the different groups. Magnification ×40.

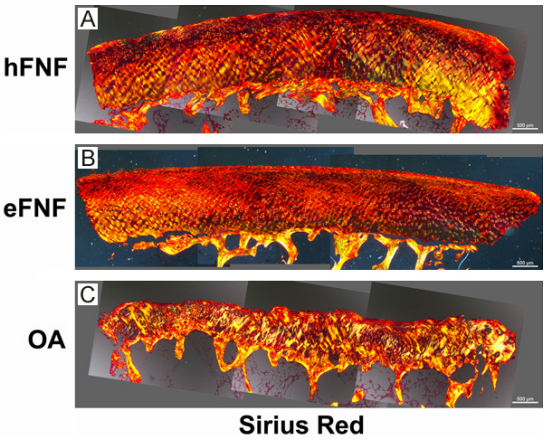

Figure 4. Anisotropy of collagen fibrils. A, B: PLM revealed normal organization of collagen fibrils in cartilage from both hFNF and eFNF patients. C: However, disruption of birefringence and disordered collagen fibers were shown in cartilage from OA patients. Magnification ×40.

Samples of hFNF cartilage displayed a uniform concentration of proteoglycans throughout the layers of tissue (**Figure 2B**), while reduced SO staining was observed in the upper and intermediate layers of eFNF cartilage (**Figure 2D**). Moreover, a marked loss of SO staining was noticeable in full-thickness samples of OA cartilage (**Figure 2F**). The mean GAG content of eFNF cartilage (24.2 ± 0.7 μg/mg) was significantly lower than that of hFNF cartilage (39.1 ± 1.8 μg/mg) ($P < 0.001$), but higher than that of OA cartilage (21 ± 0.6 μg/mg) ($P = 0.004$) (**Figure 2H**). These results were in agreement with the results of SO staining.

Collagen distribution and anisotropy

Immunohistochemical staining of hFNF cartilage showed that type II collagen was uniformly distributed throughout the cartilage layers (**Figure 3A**), while collagens type I and X were not detected in the hFNF cartilage (**Figure 3B**, **3C**). Concentrations of type II collagen showed no significant changes throughout the regions of eFNF cartilage (**Figure 3D**), whereas type I collagen was slightly distributed and type X col-

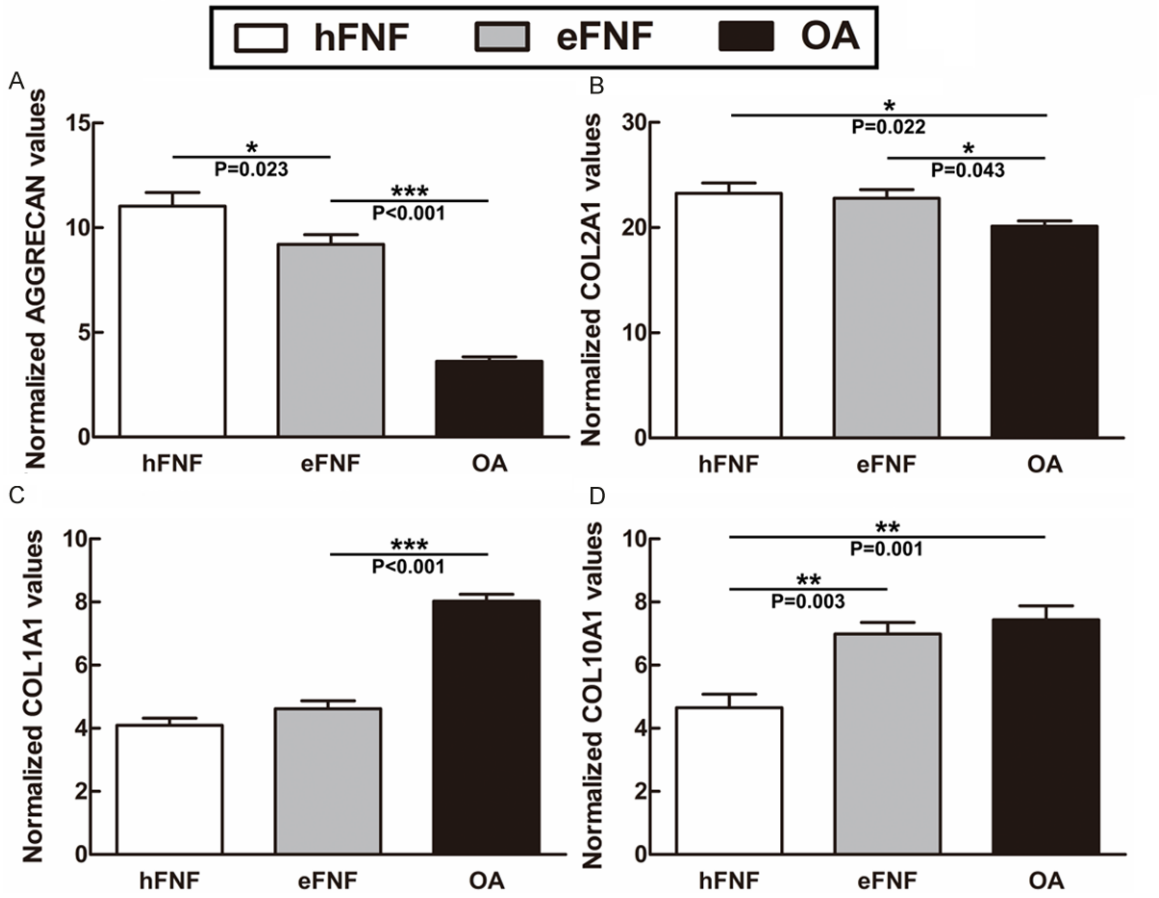

Figure 5. Gene expression. A: ACAN levels in hFNF cartilage were somewhat higher than those in eFNF cartilage (P = 0.023), and much higher than those in OA cartilage (P < 0.001). B: Levels of COL2 in eFNF cartilage was higher than that in OA cartilage (P = 0.043), whereas no significant difference was detected between eFNF and hFNF cartilage (P = 0.684). C: Levels of COL1 in eFNF cartilage was significantly lower than those in OA cartilage (P < 0.001), there was no obvious difference found in eFNF and hFNF cartilage (P = 0.141). D: Levels of COL10 in hFNF cartilage were lower than those in eFNF cartilage (P = 0.003) and OA cartilage (P = 0.001). *P < 0.05, **P < 0.01, ***P < 0.001.

lagen was obviously detected in both the superficial and deep layers of eFNF cartilage (**Figure 3E**, **3F**), suggesting a chondrocyte phenotypic shift towards hypertrophy. OA cartilage showed reduced and uneven deposition of type II collagen throughout the upper region of cartilage (**Figure 3G**), whereas collagens type I and X were accumulated mainly around chondrocyte clusters in the OA cartilage layers (**Figure 3H**, **3I**), indicating cartilage hypertrophy and calcification. However, hFNF, eFNF, and OA cartilage samples showed non-significant differences in their total content of collagen (P > 0.05) (**Figure 3J**).

Examination of hFNF and eFNF cartilage samples by PLM revealed that the collagen fibers were distributed perpendicular from the superficial layer to the deep zone of cartilage, and oriented parallel to the cartilage surface in the superficial layer (**Figure 4A**, **4B**). However, investigations of anisotropy in OA collagen indicated disruption of birefringence and disorganization of collagen fibers (**Figure 4C**).

Gene expression analysis

RT-PCR showed that ACAN expression in eFNF cartilage was significantly lower than that in hFNF cartilage (P = 0.023), but much higher than that in OA cartilage (P < 0.001) (**Figure 5A**). While COL2 levels in OA cartilage were significantly lower than those in eFNF (P = 0.043) and hFNF cartilage (P = 0.022), there was no significant difference in COL2 expression in eFNF and hFNF cartilage (P = 0.684) (**Figure 5B**). Moreover, while higher levels of COL1 were found in OA cartilage compared to either eFNF

or hFNF cartilage ($P < 0.001$), there was no obvious difference in the COL1 levels found in eFNF and hFNF cartilage ($P = 0.141$) (**Figure 5C**). Consistent with results from immunohistochemical and biochemical assays, COL10 levels in hFNF cartilage were lower than those in eFNF cartilage ($P = 0.003$) and OA cartilage ($P = 0.001$), indicating a shift towards a hypertrophic chondrocytes. However, there was no significant difference in COL10 expression in eFNF and OA cartilage ($P = 0.463$) (**Figure 5D**).

Discussion

Samples of human OA cartilage are in great demand for use in studies exploring the mechanism of OA; however, such specimens are not easily acquired. In this study, we investigated the cellular and molecular changes which occur in femoral head cartilage from eFNF patients, and found they resembled the changes which occur in early OA. Several previous studies have investigated the differences between normal and OA cartilage [17-21]. Lippiello et al. examined collagen metabolism in OA and normal cartilage, and further compared the differences in cartilage between OA and normal subjects. Their results revealed higher rates of collagen synthesis in normal cartilage compared to OA cartilage, and showed a correlation between collagen synthesis and OA severity [19]. Martin and associates examined levels of cartilage matrix gene expression in normal and OA cartilage, and showed higher ratios of type II collagen/type I collagen and agreecan/versican in normal vs. OA cartilage, suggesting that expression of genes related to chondrocyte differentiation was distinguished between OA and normal cartlilage [20]. Moreover, Hosseininia et al. investigated the differences in water, GAG, and collagen contents between OA and normal hip cartilage, and further suggested that changes in biochemical components precede changes of OA [21]. However, those OA cartilage samples were obtained from patients with severe OA, when the radiographic and clinical signs of disease are obvious, and few therapeutic targets are available for intervention. These previous findings support the importance of using specimens of early OA cartilage when investigating the mechanism and treatment of OA.

However, due to their unremarkable imaging and clinical characteristics, human cartilage samples from early stage OA patients are difficult to acquire. Additionally, there are obvious differences between humans and animals (joint size, cartilage thickness, and joint loading, etc) that can affect the validity of using animal cartilage to study human arthritic disease [13-16]. We hypothesized that the cellular and molecular changes which occur in weight-bearing area cartilage obtained from elderly subjects who had no signs or history of OA might be similar to cartilage changes which occur in early OA.

In the present study, we first examined the cartilage changes which occur in hFNF, eFNF, and OA patients using high-field MRI T2 mapping. This technique was selected due to its potential for investigating the early degenerative changes of cartilage [2, 3]. Our results showed that higher signal intensities and T2 values were found in eFNF cartilage compared to hFNF cartilage. Additionally, the surface of eFNF cartilage appeared regular and smooth, reflecting the changes which occur in early OA. We next investigated the concentrations and structure of various cartilage matrix components, including water, GAG, and collagen. In normal cartilage, chondrocytes usually lie parallel to the cartilage surface in the superficial layer and perpendicular to the surface in the intermediate and deep layers. Moreover, proteoglycans and type II collagen are distributed uniformly throughout the cartilage matrix, while type I collagen accumulates in the uppermost layer and type X collagen is absent in the cartilage zones. In contrast to results from previous studies [2, 22, 23], we found no significant differences in the water contents of hFNF and eFNF cartilage; however, the water content of OA cartilage was somewhat higher than that of eFNF cartilage ($P = 0.012$), and much higher than that of hFNF cartilage ($P = 0.003$). Progression of early stage OA is characterized by the presence of fibrillations and decreased Safranin O staining in the superficial zone of cartilage [24, 25]. These changes are followed by the complete loss of proteoglycans in the upper, middle, and deep layers of cartilage in advanced OA [25]. In the present study, eFNF cartilage showed changes which were similar to those found in early OA cartilage, including insignificant changes in total DNA contents, a loss of SO staining in the upper and intermediate cartilage layer, and a reduced concentration of GAG. Early OA cartilage is also characterized by a regular distribution of type II collagen, whereas distribution of

type I collagen is increased in the superficial layer of early OA cartilage. While severe OA cartilage usually shows accumulations of type I and type X collagen [24, 26], total collagen content remains unchanged during development of OA [27]. In our study, eFNF cartilage showed no obvious changes in the accumulation of type II collagen in the cartilage layer, whereas a slightly increased distribution of type I collagen and an obviously enhanced concentration of type X collagen were detected in the superficial and deep layers of cartilage. However, eFNF cartilage showed no significant changes in total collagen content, suggesting that collagen metabolism was in balance. We next investigated the collagen architecture of cartilage using PLM, because of its potential for detecting the orientation and birefringence of collagen fibrils [22, 28-30]. Consistent with our findings for early OA cartilage, our results also showed normal birefringence and organization of collagen fibers in eFNF cartilage. Numerous previous studies have reported abnormal gene expression in various models of OA [31-33]. In this study, we demonstrated differential gene expression of aggrecan, type I collagen, type II collagen, and type X collagen in eFNF cartilage, and these results were in line with results shown by immunohistochemical and biochemical assays used to characterize changes in early OA.

A major limitation of this study is that the metabolic, biochemical, and biomechanical properties of cartilage differ among joints, and these differences may be linking to the differential progression of OA in different joints [34]. Additionally, samples of OA cartilage obtained from the same species often show various degrees of heterogeneity [35]. Thus, future investigations need to focus on the diversity of cartilage found in different joints from the same individual and at different sites in the same joint.

In the present study, we demonstrated that the cellular and molecular changes found in hip cartilage from eFNF patients largely resemble the changes which occur in early OA. To our knowledge, this study is the first to explore the changes which occur in hip cartilage in elderly individuals with early OA, and then suggest that cartilage specimens from eFNF patients are suitable for investigating and identifying early changes which lead to OA. In the future, preventative treatments can be implemented in elderly patients who show no signs of OA, but demonstrate early degenerative changes detected by high field and functional MRI.

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Disclosure of conflict of interest

None.

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