Cellular and Epigenetic Features of a Young Healthy and a Young Osteoarthritic Cartilage Compared with Aged Control and OA Cartilage

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ABSTRACT: Osteoarthritis (OA) is generally a disease of the elderly population, but can occur in young patients in exceptional cases. This study compares the cellular and epigenetic features of primary old-age OA with those of secondary OA in a 23-year-old patient with developmental dysplasia of the hip. In addition, control cartilage from a 14-year-old was compared with that from patients with a fracture of the neck of femur (#NOF) to establish to what extent the latter is a useful control for OA. Articular cartilage was obtained from discarded femoral heads after hip arthroplasty. MMP-3, MMP-9, MMP-13, and ADAMTS-4 were immunolocalized and the methylation status of specific promoter CpG sites was determined. Both primary and secondary OA were characterized by loss of aggrecan, formation of clones, and abnormal expression of the proteases that correlated with epigenetic DNA demethylation. The latter indicated that the abnormal expression of the cartilage-degrading proteases was not due to a short-term up-regulation, but a heritable, permanent alteration in gene expression. Comparing cell densities in young and old control cartilage estimated an age-related cell loss of ~1% per year. In aged #NOF cartilage, some superficial-zone chondrocytes expressed the proteases, but the majority of cells were immunonegative and their promoters were hypermethylated. The cellular and epigenetic features of the intermediate and deep zones of #NOF cartilage are thus similar to those of young healthy cartilage, justifying the use of #NOF cartilage as control cartilage for OA, providing the superficial zone is removed. © 2008 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 27:593–601, 2009

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Osteoarthritis (OA) is characterized by defective integrity of the articular cartilage and adjacent tissues at diarthrodial joints.[1,2] Primary OA has no obvious predisposing cause, whereas for secondary OA factors such as trauma or abnormal loading favor development of the disease. Because primary OA occurs predominantly in older people, age-related factors may be involved, such as "wear and tear", oxidative stress,[3] or cellular senescence.[4,5] However, OA is not a necessary outcome of aging, as the disease also occurs in young people. An example is developmental dysplasia of the hip (DDH), a condition where the femoral head does not develop correctly within the acetabulum. New-born babies are generally screened for DDH so that problems can be corrected by reduction using a Pavlik harness.[6] In some patients, however, the condition is not diagnosed correctly during childhood or treatment fails to correct the dysplasia. The resulting abnormal anatomy often results in secondary OA at an early age, which requires surgical treatment.[7,8] We obtained OA cartilage from the femoral head of a 23-year-old female DDH patient. This provided a unique and rare opportunity to compare the features of secondary OA in a young patient with those of primary old-age OA and thereby delineate how age per se influences OA. In addition, we obtained articular cartilage from a 14-year-old male with spastic quadriplegia. This normal cartilage was compared with the articular cartilage from osteoporotic patients, aged 80–90 years, who had sustained a fracture (#) of the neck of femur (#NOF). Because patients with osteoporosis rarely have OA, and vice versa,[9] the cartilage from #NOF patients is frequently used as control cartilage for OA, but to what extent is this justified?

The present study concentrated on the cellular and epigenetic features of the various types of articular cartilage, because these are central to the patho-etiology of the disease. The cellular features are well established.[1,10,11] Briefly, chondrocytes change from anabolic to catabolic cells that produce large quantities of matrix-degrading enzymes, which include aggrecanases (ADAMTS [a disintegrin and metalloproteinase with thrombospondin motifs]-1, -4, and -5) and matrix metalloproteinases (MMP-1, -2, -3, -9, -13). We have previously also established the epigenetic features of primary OA by showing that the abnormal expression of MMP-3, -9, -13, and ADAMTS-4 was associated with loss of DNA methylation at specific cytosine-phosphate-guanine (CpG) sites in the promoters of the respective enzymes.[12,13] DNA methylation, together with histone modifications, are epigenetic mechanisms that silence those genes that are normally not expressed, thus assuring genomic stability.[14]

The aims of the present study were: (i) to compare and contrast the cellular and epigenetic features of the young normal cartilage with those of aged #NOF cartilage; (ii) to characterize the cellular features of OA in a young DDH patient by histological comparison with primary OA; and (iii) to determine whether the epigenetic features that had previously been observed in primary OA[12] were also present in the young DDH patient.

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METHODS

Selection of Material

All samples were obtained with patients' consent and the approval of the local ethics committee.

Fourteen-Year-Old Male

This patient suffered from spastic quadriplegia and both hips were neurologically dislocated. The patient was in great pain, but surgical reconstruction was not indicated. Instead, the proximal femur was excised, enabling us to obtain extremely rare young cartilage for histology and DNA extraction.

DDH Patient

A Charnley total hip arthroplasty was performed on a 23-year-old female for secondary OA following the sequelae of DDH. Although she had had multiple surgical interventions, her hip had failed, due to degenerative changes and subluxation. The femoral head was severely deformed and osteophytes had formed on both acetabulum and femoral head. Most of the bone of the femoral head was used for bone grafting (Fig. 1), but cartilage present in the circumference of a ~1 cm thick slice of the femoral head was available for histology and DNA extraction. This is the first time that cartilage from a young OA patient has been available for such studies.

Aged Patients

Femoral heads were obtained after arthroplasty for OA or hemi-arthroplasty following a #NOF. This is an ongoing study, with more than 30 patients in each group. Representative examples were chosen for comparisons with the cartilage from the 14 or 23 year old. For histology, cartilage was sampled from both weight-bearing and non-weight bearing regions. For DNA analyses, cartilage was sampled from the deep zones of #NOF patients, but from the surface zones of OA patients, because, as confirmed by histology, the former contained normal, healthy chondrocytes, whereas the latter contained typical OA chondrocytes.

Histological Analyses

Full cartilage depth samples (~4 × 10 mm) were dissected. To label live/dead cells, the tissue pieces were incubated for 2–3 h with the fluorescent marker CellTracker Green to label viable, metabolically active cells, and with Ethidium homodimer-1 to label dead or dying cells.[15] Samples were fixed in 4% paraformaldehyde, dehydrated with ethanols, cleared in chloroform, and embedded in paraffin wax. Twenty-five to forty sequential sections (7 μm) were cut and stored at 4°C. The distribution of fluorescent labels was viewed with a LEICA TCS SP2 confocal laser-scanning microscope in unstained sections without deparaffinization.

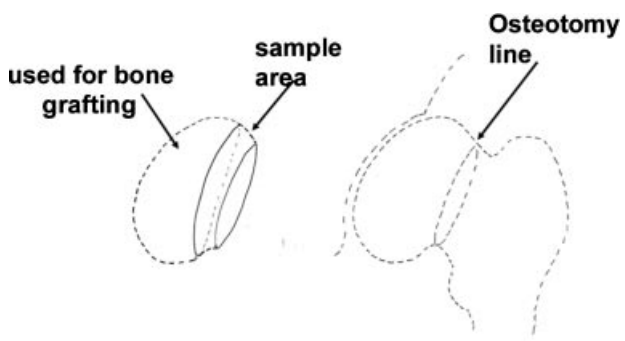

Figure 1. Sketch to illustrate position of the sampled 1 cm slice of the femoral head obtained from the DDH patient.

For other staining procedures, paraffin wax was removed. Sections were stained with Safranin-O to identify regions of proteoglycans, and with Weigert's Haematoxylin/Alcian blue/Sirius red to demonstrate better the regions of proteoglycan loss, because Sirius red stains the cartilage collagen only in those areas where proteoglycans are absent.[16] Immunohistochemistry was carried out with the following primary antibodies: monoclonal antihuman MMP-3 (MAB3312; Chemicon) and MMP-9 (MAB3309; Chemicon); rabbit antihuman MMP-13 (AHP751; Serotec, Oxford, UK) and ADAMTS-4 raised to the C-terminal region (AHP821; Serotec) or to the disintegrin-like domain (AB19165; Chemicon). Some sections were pretreated with hyaluronidase (2 mg/mL in acetate buffer, pH 5, for 60 min at 37°C) to demonstrate matrix staining of the enzymes. Sections were incubated with the primary antibodies overnight, followed by biotinylated secondary antibodies (60 min) and avidin-peroxidase (30 min) with 3-amino-9-ethyl-carbazole (AEC) as the final chromogen. The slides were counterstained briefly with 1% Alcian blue, mounted in glycerol jelly, and viewed with a Zeiss Universal light microscope (Zeiss, Welwyn Garden City, UK). Images were captured with a digital camera.

Cell Counting

To obtain an approximate estimate of the age-related loss of chondrocytes, four to six representative sections of the cartilage from the 14 year old and from aged #NOF patients were photographed at a magnification of X100. The images were transferred to a PowerPoint slide and enlarged so that each image covered the slide fully and could be viewed with a final magnification of X400. A grid consisting of 7 × 10 squares, corresponding to an area of 280 × 400 μm was overlaid and the cells were counted in each subsection grid. The numbers were normalized to an area of 100 × 100 μm, the mean values and standard deviations were calculated, and the two groups were compared using the Student's *t*-test.

Cell diameters were determined in images of representative sections, transferred to a PowerPoint slide as above. The shortest diameter of 40 small chondrocytes, located in the superficial and intermediate zones, and 40 large chondrocytes, located near the osteophyte, was measured in the same section, using an image analysis program (Image J; NHI, Wayne Husband, Bethesda, MD). The mean values and standard deviations were calculated and the two groups were compared using the Student's *t*-test.

DNA Methylation Status

Genomic DNA was extracted directly from the human cartilage samples after they were ground in a liquid nitrogen-cooled freezer mill and the methylation status of individual CpG sites in the promoters of MMP-3, -9, -13, and ADAMTS-4 was determined with the aid of methylation-sensitive restriction enzymes, followed by semi-quantitative polymerase chain reaction (PCR), as previously described.[12]

RESULTS

Comparison of Young Versus Old Non-OA Cartilage

The femoral head obtained from the 14-year-old male was deformed, but was covered evenly with smooth, visually healthy cartilage. The femoral heads from

#NOF patients were also covered with healthy cartilage, although some wear was sometimes seen in the weight-bearing region and the histological features varied in different #NOF patients. To illustrate the range, two sets of images are shown that show the two extremes of the observed features: (i) from non-weight bearing regions that showed little or no degradation; and (ii) from weight-bearing regions that showed early signs of degradation. Because the staining characteristics of intermediate and deep zones did *not* differ between the non-weight bearing and weight bearing samples, the images in Figures 2 and 3 are from the superficial zone only.

As expected, the cartilage from the 14-year-old patient had a smooth surface and good Safranin-O staining, except for some loss at the very surface (seen at higher magnification) (Fig. 2A). The surface of cartilage from the non-weight bearing regions of #NOF patients was smooth and its chondrocytes were flattened and aligned with the surface (Fig. 2B,E, and insert in E), as would be expected for normal cartilage. However, the surface of the cartilage from the weight-bearing regions was irregular (Fig. 2C,F), and the cells were rounded (insert in Fig. 2F), suggesting that the zone of flattened cells had been eroded. It is noteworthy that these cells were not directly at the surface, because that region no longer contained cells (Fig. 2F). Loss of proteoglycan near the surface was more pronounced (Fig. 2B,C), as also seen in Figure 2E,F.

Specimens from non-weight bearing regions had very few dying cells and many cells near the surface were metabolically active (Fig. 3A), whereas in weight-bearing regions many chondrocytes near the surface were dying or dead (Fig. 3B). Of the matrix-degrading enzymes, only MMP-3 showed positive staining in the 14 year old (Fig. 2G). All specimens from #NOF patients contained some cells that were immunopositive for all matrix-degrading enzymes. In the non-weight bearing regions, immunopositive cells were encountered rarely (arrows), but their numbers increased in cartilage from weight-bearing regions (compare Fig. 2H,L with Fig. 2J,M and Fig. 3D,G with 3E,H). Immunopositive chondrocytes were, however, present only at the surface of the superficial zone, never in intermediate and deep zones (not shown). The location of immunopositive cells always closely matched the region of proteoglycan loss.

As expected, cell density was higher in the cartilage of the 14-year-old, whereas the cartilage of #NOF patients contained large acellular regions, presumably due to chondrocyte death. In the grid of 280×400 μm^2, 345 ± 9.5 cells were present in the 14-year-old cartilage, while only 132 ± 22 cells were counted in the #NOF

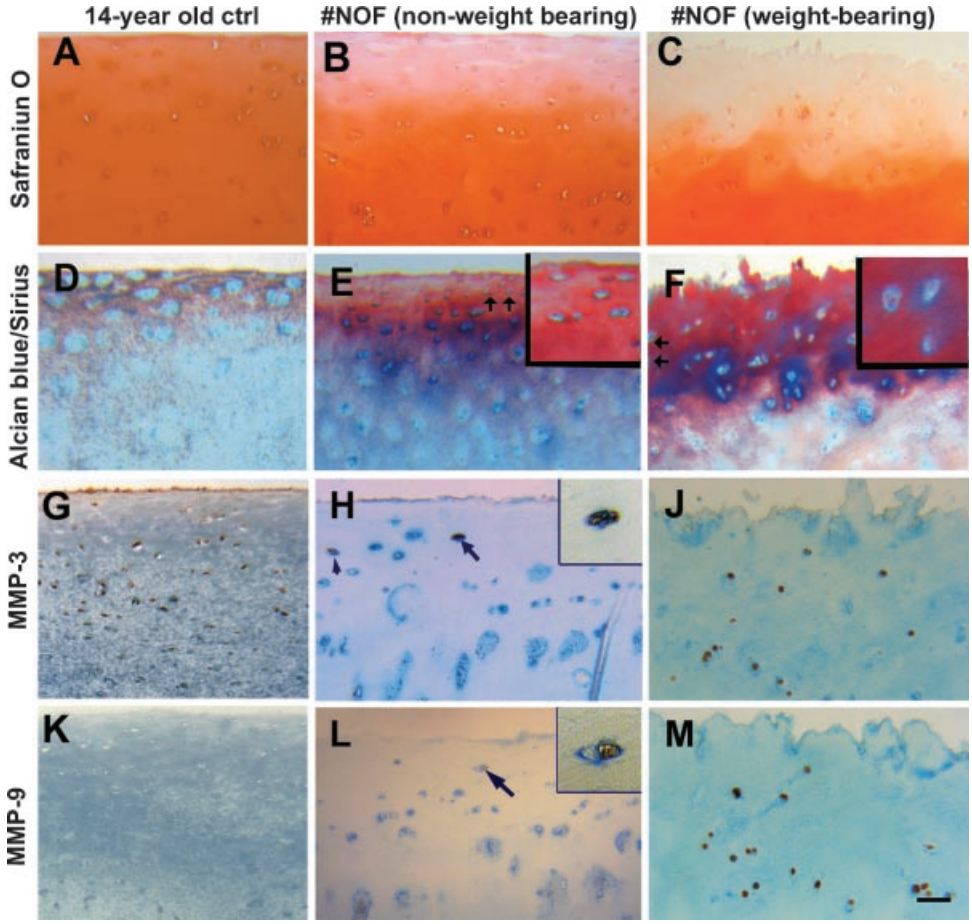

Figure 2. Comparison of the superficial zones of articular cartilage from a 14-year-old male (A,D,G,K) with that obtained from osteoporotic patients following a fracture of the neck of femur (#NOF). Panels (B,E,H,L) show examples of the smooth cartilage from non-weight bearing regions, while (C,F,J,M) were taken from weight-bearing regions. (A–C) Loss of Safranin O staining at the surface demonstrates progressive loss of proteoglycans illustrating the effect of age and weight-bearing at the joint surface. (D–F) Alcian blue/Sirius red staining. The loss of proteoglycan makes the cartilage collagen accessible for Sirius red staining so that the intense red staining at the surface also demonstrates proteoglycan loss. (G–J) The thick arrows point to the regions that are enlarged 2.4X in the insets of (E) and (F). Immunolocalization of MMP-3 and (K–M) of MMP-9. MMP-3 was expressed by chondrocytes in the 14 year old (G), whereas MMP-9 (K) was absent. For the patients with a #NOF, the numbers of immunopositive cells were always higher in the cartilage from weight-bearing regions, but a few chondrocytes were always immunopositive even in the non-weight bearing regions. The thin arrows point to single immunopositive cells that are seen in higher magnification in the insets (2.8X enlargement in H and 3.1X in L relative to the main image). Bar = 25 μm. All main images are of the same magnification.

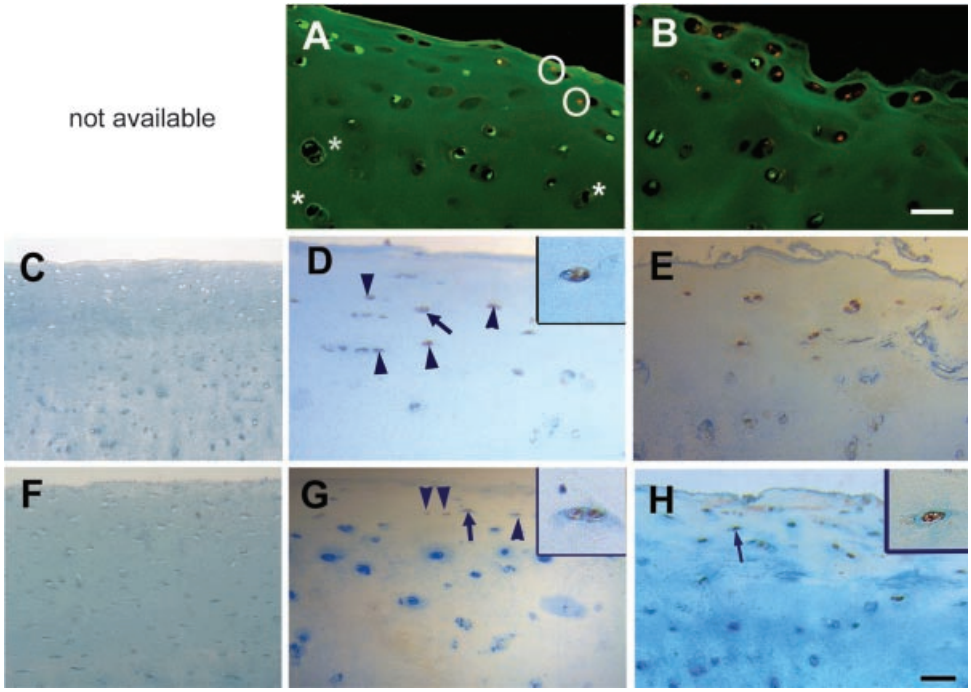

Figure 3. Live/dead staining in the superficial zone of the non-weight-bearing region (A) shows mostly viable cells, but the incidence of cell death is higher in the weight-bearing regions (B). Cells below the surface do not show fluorescence (*), indicating that they have low metabolic activity. No expression of the proteases MMP-13 or ADAMTS-4 was found in the 14 year old (C,F). For the patients with a #NOF, the number of immunopositive cells was always higher in the cartilage from weight-bearing regions, but a few chondrocytes were always immunopositive even in the non-weight bearing regions. Compare (D,E) for MMP-13 and (G,H) for ADAMTS-4. The arrows point to those cells that are seen in higher magnification in the inset. Arrowheads point to other immunopositive cells. Bar = 25 μm. All images are of the same magnification.

specimens ($p < 0.001$). Normalization to a surface of $100 \times 100\ \mu m^2$ gives an estimate of 31 cells/0.01 m^2 for the 14 year old, compared with 12 cells/0.01 m^2 in the aged controls.

Early Stages of OA in DDH Patient

In primary OA, the various stages of cartilage degradation (as evaluated by Osteoarthritis Research Society International (OARSI) grading[17]) can be identified in different regions of the femoral head. Cartilage sampled near the weight-bearing regions always has a higher score than cartilage from non-weight bearing regions. Similarly, by sampling from different regions, it was possible to identify different histological stages of OA in the DDH patient. However, the total specimen consisted only of a femur slice that was 1 cm thick, sampled near the femoral neck. This region is non-weight bearing and would show little evidence of erosion in most patients with primary OA. However, the cartilage from the DDH patient showed various degrees of erosion. In the area seen in Figure 4A, an early osteophyte is seen on the left and full-thickness cartilage on the right. The surface of the full-thickness cartilage was mostly smooth and not much proteoglycan had been lost. The density of the cells was high (Fig. 4B–E), similar to that seen in the 14 year old. This cartilage may therefore be at an early stage of erosion (or at least at an earlier stage than the cartilage shown in Fig. 5). However, unlike the cartilage from low-grade primary OA, the incidence of cell death was high, particularly in the superficial region (Figs. 4B,C). However, not all cells were dying. In some regions, the chondrocytes were highly active, as shown by the intense CellTracker green fluorescence (Fig. 4E). Moreover, close proximity of two or more cells (arrows in Fig. 4E) was consistent with the notion that the surviving chondrocytes proliferated.

Immunostaining of parallel sections for MMP-3 (Fig. 4D), MMP-9 (Fig. 4F), and the other proteases (not shown) showed that almost all chondrocytes expressed proteases. Enzyme activity was also detectable in the matrix near the surface of the cartilage (Fig. 4D), suggesting secretion of the enzymes. It is noteworthy that the chondrocytes located near the osteophyte were considerably larger than the chondrocytes in the superficial and intermediate zones (compare Fig. 4G and 4H). The average diameter of chondrocytes in the superficial and intermediate zones was 4.2 ± 1.1 μm and that of the large chondrocytes was 16.5 ± 2.5 μm ($p < 0.001$). This three- to fivefold enlargement is consistent with the interpretation that these cells had further differentiated to hypertrophy prior to osteophyte formation via endochondral ossification. No typical clusters/clones of primary OA were seen in this section.

Late Stages of OA in the DDH Patient

Cartilage specimen from a different region resembled specimens from high-grade OA. Proteoglycans had been lost from the upper third of the tissue (Fig. 5A,B). The surface was highly fibrillated and had a fibrous appearance. The latter may have resulted from fibrous overgrowth that can sometimes be observed in primary OA specimens. However, the latter tends to be demarcated by a layer of dense cells in the overlying fibrous tissue (see Fig. 5C), which was not seen in the DDH sample, whose cartilage matrix was continuous with the fiberlike superficial zone, with only few cells in this upper region (Fig. 5D,E). These remaining few

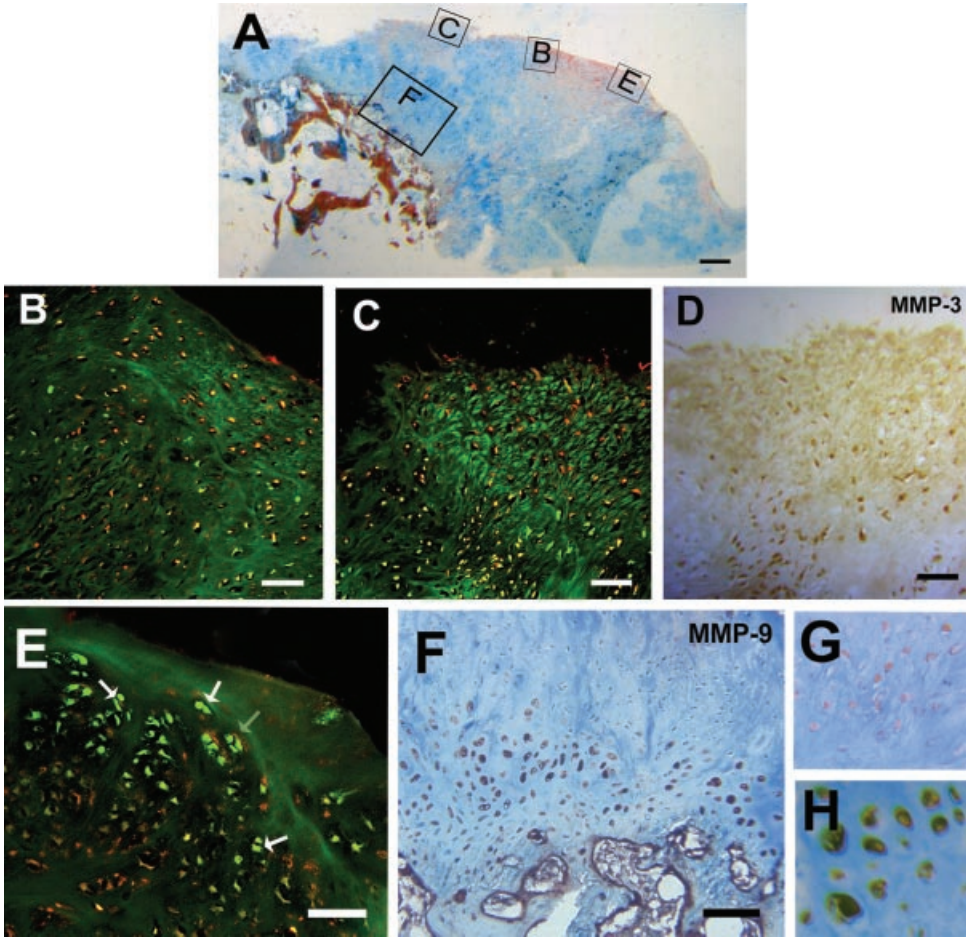

Figure 4. Example of an osteophyte and lower grade OA in the DDH patient (OARSI grade ~1–2). (A) Alcian blue/Sirius red-stained overview of the section, showing thick cartilage (blue) and a bony osteophyte (red) on the left. (B,C) Live-dead staining with CellTracker green and Ethidium homodimer-1 demonstrates a high incidence of cell death (red) as well as some metabolically active cells (green). (D) MMP-3 immunostaining in the same region as that shown in (C) illustrates that most chondrocytes produced the protease (brown stain). In a different area (E), viable (green) cells were closely approximated, suggestive of cell division (arrows). Near the osteophyte (F), the size of the chondrocytes had increased three- to fivefold (G,H) and nearly all cells were immunopositive for MMP-9. Bars = 500 μm in (A), 50 μm in (B–E), 150 μm in (F), and 25 μm in (G,H).

cells were metabolically active and immunopositive for MMP-3 (Fig. 5F), ADAMTS-4 (Fig. 5G), MMP-9 (Fig. 5H), and MMP-13 (not shown). Significant enzyme activity was also present in the matrix throughout the upper fibrous regions. Ongoing cell death occurred predominantly in chondrocytes in the upper cartilage/lower fibrous region (Fig. 5D,E).

This sample of DDH cartilage also contained the typical clones of OA cartilage. The cells of the clones were viable (Fig. 6A) and expressed MMP-3, MMP-9, MMP-13, and ADAMTS-4 (Fig. 6B–E). These characteristics matched those in primary OA (Fig. 6F–K).

Evaluation of DNA Methylation Status

As virtually all chondrocytes of the DDH patient expressed proteolytic enzymes, it was of interest to investigate whether this abnormal expression was associated with an "unsilencing" via DNA demethylation, as had been shown for primary OA.[12] Figure 7 illustrates the DNA methylation status of specific CpG sites in the promoters of the four proteases for each of the four cartilage specimens. Consistent with absence of MMP-13 expression, bands were present at all examined CpG sites in the MMP-13 promoter in the two control cartilage specimens, although the band intensity was reduced at −134 in the #NOF patient. In our previous study we also found a reduced or absent band at this site in 3/10 #NOF patients. Not all CpG sites are equally important for silencing and we had previously shown that it was loss of DNA methylation at the CpG site at −110 bp that was the most critical for "unsilencing" of MMP-13.[12] Indeed, in both aged OA and the DDH patient, loss of DNA methylation had occurred at both −134 bp and −110 bp with an additional loss at −14 bp in the DDH patient. For MMP-9, the CpG sites at −712 bp and at least one of the two sites at −624 and −562 bp were hypo-methylated in all specimens in agreement with previous findings, suggesting that these sites play a minor role in transcriptional silencing. By contrast, the CpG sites close to the transcription start were demethylated in both primary OA and DDH samples, but not in the controls. These sites, therefore, were most likely involved in transcriptional silencing in healthy cartilage. For MMP-3, there was a marked difference between the #NOF control samples, whose CpG sites were methylated throughout, corresponding to absence of MMP-3 expression, and those of the 14-year-old patient, where there was no methylation at −1923 bp and considerable loss of methylation at −635 bp. In both OA and the DDH samples it was the same CpG sites that showed absence or considerable

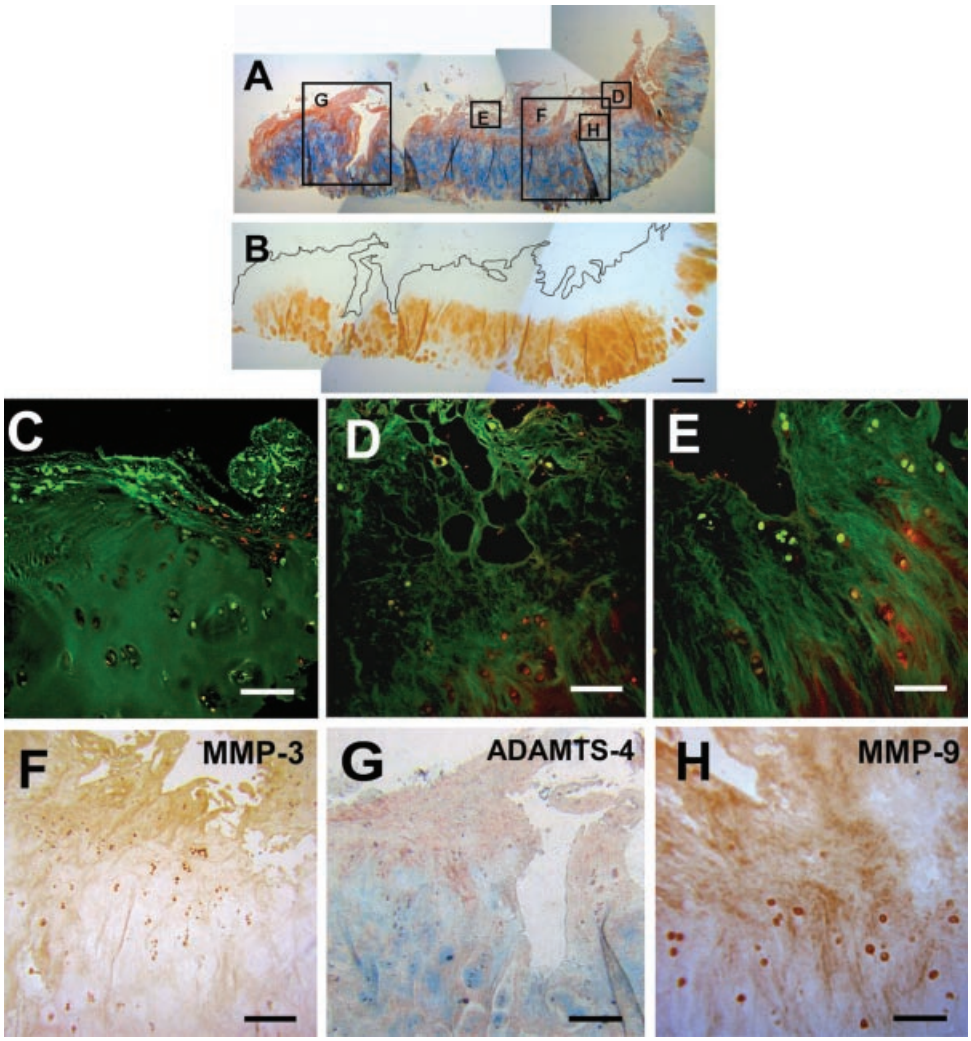

Figure 5. Features of high-grade OA in the DDH patient (OARSI grade 4). (A,B) Composite images to show an overview of the whole section. Comparison of the Alcian blue/Sirius red stained section (A) with the Safranin O stained section (B) clearly illustrates the loss of proteoglycan. (C) The typical appearance of fibrous overgrowth in idiopathic OA. The demarcation between cartilage and fibrous overgrowth is clearly seen. (D,E) The fiberlike region at the surface of the DDH patient. The few cells that remain in the fiberlike region are viable (green), but dead cells (red) are present in the upper cartilage zone. (F–H) Extensive protease activity in almost all chondrocytes and in the matrix. This is illustrated for MMP-3 (F), ADAMTS-4 (G), and MMP-9 (H). The sections had been pretreated with hyaluronidase, which uncovers binding sites in the matrix (brown stain). Bars = 500 μm in (A,B); 50 μm in (C–E) and (H); and 200 μm in (F,G).

reduction in methylation. For ADAMTS-4, both OA samples showed loss of DNA methylation at the CpG sites located between −232 and −829 bp. Data on the methylation status of the ADAMTS-4 sites were not available for the 14-year-old patient.

Overall, there was a good correlation between induction of expression of the typical OA proteases and loss of DNA methylation in the relevant promoter regions. The DNA methylation status of the samples from the 14-year-old patient was identical to that of the aged #NOF

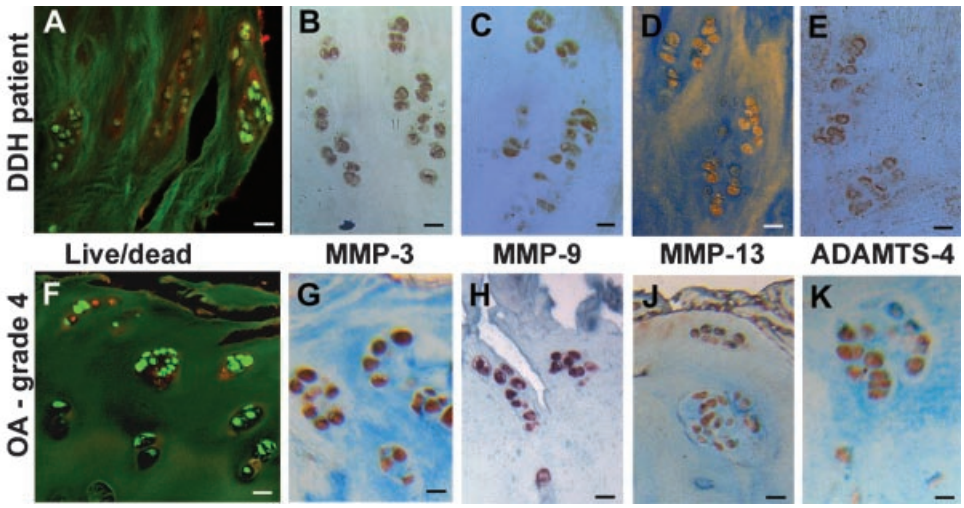

Figure 6. The typical cell clusters of severe OA cartilage in the DDH patient (A–E) and in late-stage primary OA (F–K). (A,F) Live-dead staining demonstrates that most cells are viable and metabolically active (bright green fluorescence), although some cell death is also apparent (red cells in F). All the cells within a clone are immunopositive for MMP-3 (B,G), MMP-9 (C,H), MMP-13 (D,J), and ADAMTS-4 (E,K), illustrating the similarity between OA in the young DDH patient with that in old-age idiopathic OA.

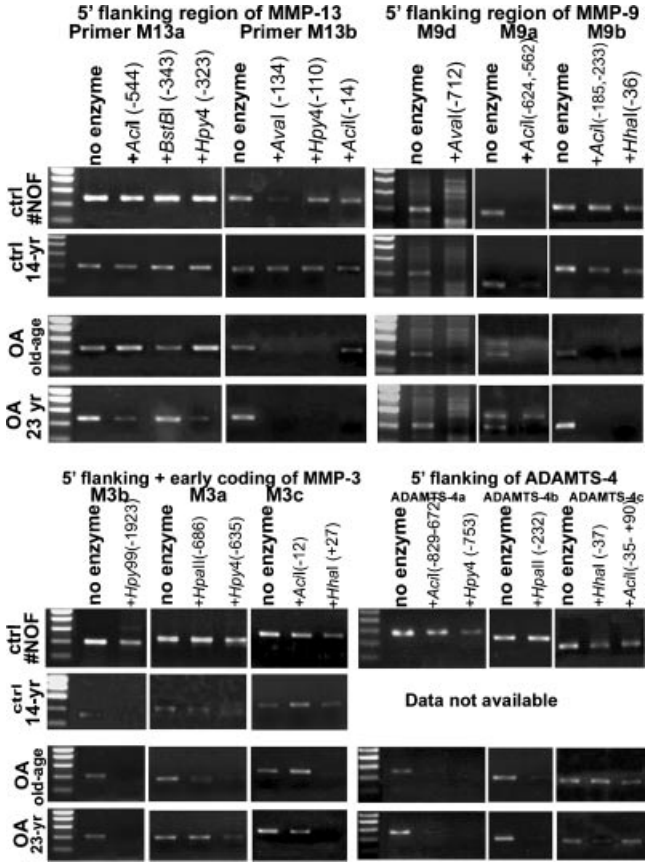

Figure 7. Methylation status at CpG sites in the promoters of MMP-3, MMP-9, MMP-13, and ADAMTS-4 for a typical #NOF patient, the 14-year-old control patient, a representative OA patient, and the 23-year-old DDH patient. The data for the #NOF patient and old-age OA patient are not new, but shown to enable comparison with the young patients. DNA was treated with the listed methylation-sensitive restriction enzymes, which cleave at the indicated locations if the CpG site(s) are not methylated. Hence presence of bands indicates methylation and vice versa. A weak band indicates absence of methylation in most, but not all chondrocytes. The first band of each primer pair shows the PCR for the DNA control, that is, not treated with enzyme. This band represents a check for DNA loading, to ensure that absence of a band is not due to an insufficient amount of DNA. *AciI* often cleaved several CpG sites within the amplicon of the PCR primers; these were assessed together. The DNA methylation pattern for the old-age #NOF patient was similar to that of the 14 year old, except for MMP-3. The DDH patient showed a similar loss of DNA methylation as the OA patient. The primers M9d show spurious bands; the relevant band is at 400 bp. Similarly, the primers used for the two OA samples show an additional band; the relevant is the lowest band at ~400 bp. For the #NOF patient and the 14 year old this primer pair was replaced by different primers bracketing the same *AciI*-cleavable region, hence the absence of spurious bands.

patients, except for MMP-3. The DNA methylation pattern in the DDH patient was similar, but not identical, to that previously observed in primary OA.[12]

DISCUSSION

Joint replacement operations not only give a new lease on life to elderly patients with OA, but their discarded femoral heads or knee joints provide researchers with articular cartilage. Non-OA cartilage becomes available when, as a result of osteoporosis, patients who had sustained a #NOF undergo hemi-arthroplasty. Patients with osteoporosis generally do not have OA,[9] which makes possible the use of cartilage from #NOF patients as control for studies of OA cartilage. This study determined whether it is valid to do so.

Age is a known risk factor for primary OA, but to what extent does age per se contribute to the disease process? To throw light on this question the cellular and epigenetic features of old-age primary OA were compared with those of a 23-year-old patient who had OA following failed treatment for DDH.

Differences between Young and Old-Age Control Cartilage

The first age-related change was a reduction in cell density from ~31 to ~12 cells/0.01 mm^2 in the 80–90-year-old cartilage; this suggests a net loss of ~60% of the cells over a period of six to seven decades. Cell density results from the difference between proliferation and apoptosis. Proliferation exceeds apoptosis during skeletal growth, but is reduced considerably after maturity. If cell density at skeletal maturity (at ~18 years) approximates that of the 14 year old and if chondrocytes in adult life divide infrequently, the age-related rate of cell death would be ~1% per year, a rate similar to the incidence of chondrocyte death estimated by Aigner et al.[18]

The second age-related feature was production of proteolytic enzymes by some chondrocytes in the superficial zone of #NOF cartilage. Of the four enzymes examined, only MMP-3 was expressed by chondrocytes in the 14 year old and only MMP-3 showed loss of DNA methylation in the MMP-3 promoter. Conceivably, remodeling of the growing cartilage requires only MMP-3, but not MMP-9, MMP-13, or ADAMTS-4. This inference is supported by reports that MMP-3 modulates remodeling in the physis of calves[19] and in articular and proliferating chondrocytes of fetal rabbits.[20] On the other hand, it is possible that low concentrations of inflammatory cytokines in the synovium of this spastic quadriplegia patient activated MMP-3 more than the other degradative enzymes.

Is Cartilage from Osteoporotic Patients a Suitable Control for OA?

Microscopic examination revealed variability both between patients and within the same femoral head. The observation that some chondrocytes of the superficial zone expressed proteases indicated caution in the indiscriminate use of #NOF cartilage as a control. However, because protease-positive chondrocytes were clearly confined to the superficial zone and to weight-bearing areas, cartilage from intermediate and deep zones of #NOF patients does indeed constitute suitable control cartilage, at least with regards to the parameters examined in the present study.

Cellular and Epigenetic Features of Young and Old-Age OA

The histology of the cartilage from the 23-year-old DDH patient was striking in showing how far the disease had advanced in this young patient. This was especially so

because the cartilage had been sampled near the femoral neck, where OA patients still have healthy cartilage, even if the cartilage at the weight-bearing region has been eroded. Although disease progression varies considerably from patient to patient, it generally takes decades for cartilage to become completely eroded. The age when arthritis started in the DDH patient is not known, but cartilage with an OARSI grade of 4 indicates a very advanced stage of the disease after at most two decades.

The fact that almost all chondrocytes, not only those in the superficial zone, were enzyme-producing cells is a further indication of how far the disease in this young patient had advanced. Because vast regions were devoid of cells, large numbers of chondrocytes had been eliminated, probably by "chondroptosis", which leads to autophagocytosis and regions that are acellular.[21] However, those chondrocytes that survived had formed clones, consistent with cell proliferation. This was as true for the diseased cartilage of the DDH patient cartilage as for the cartilage from primary OA. All cells in the clones of the DDH patient as well as aged OA patients were immunopositive for proteases which, upon release into the matrix, could contribute to degradation of the matrix. The expression of proteolytic enzymes in the chondrocytes of the DDH patient was always associated with loss of DNA methylation at specific CpG sites in the promoter regions, just as it had been for primary old-age OA, suggesting that age cannot be the sole explanation for the epigenetic "unsilencing" that occurs in OA.

Some protease-positive chondrocytes sited near the subchondral bone were three to five times larger than chondrocytes in a more distal site. This is consistent with hypertrophic differentiation, which has been proposed as a possible explanation for the changed OA phenotype, based on the observation that chondrocytes near the calcified cartilage of OA patients are immunopositive for type X collagen.[22,23] However, such an increase in cell size was only observed near the osteophyte, not in other cartilage sections from the DDH patient, nor have we observed it in primary OA cartilage. This suggests that the hypertrophy is related to the osteophyte formation by endochondral ossification rather than being a general phenomenon of OA cartilage.

There are several caveats that should be borne in mind. We only had access to one specimen of young healthy cartilage and one young OA patient, because these are only rarely available. Due to restrictions in the UK it is almost impossible to obtain postmortem femoral heads from young patients. With improved detection and correction of DDH in childhood, it is highly unlikely that we shall be able to obtain OA cartilage from another DDH patient. To the authors' knowledge, no other studies have investigated the histological or epigenetic characteristics of OA cartilage in a young patient. We believe that this study provides valuable data that increase our understanding of the patho-etiology of OA, even if based on only one patient.

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