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Histopathology of naturally occurring and surgically induced osteoarthritis in mice

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SUMMARY

Objective: The morphology of lesions in mouse models of osteoarthritis (OA) has not been comprehensively characterized, in part because current histological assessments of OA focus primarily on articular cartilage (AC). In the present study, sections of murine stifle joints with naturally occurring (aged animals) and surgically induced (destabilized medial meniscus, DMM) OA were examined using a newly developed histological grading scheme that includes quantitative measurements and semiquantitative grades to evaluate multiple joint tissues.
Design: The data collected was analyzed using Principal Components Analysis (PCA); factor scores for each joint were generated. Individual parameters and factor scores were compared between surgical groups and among age groups. For comparison, the original Mankin Histological-Histochemical Grading System (HHGS) also was applied.
Results: Overall, lesions were most severe in the medial tibial plateaus. Significant changes in AC and neighboring bone were identified in surgically induced models and in naturally occurring disease. Mean factor scores provided a comprehensive evaluation of joint changes. An important new finding was that chondrocyte cell death within the AC was a commonly identified lesion and its extent significantly increased with age. While the Mankin HHGS detected significant overall differences in OA severity between surgical groups, it was not sensitive in detecting age-related differences, nor did it provide information regarding changes in individual tissues.
Conclusion: These results demonstrate the utility of this newly developed murine OA grading scheme in identifying lesions in AC and in other joint tissues. Surgically induced changes were similar to those occurring naturally with aging.

Introduction

Accurate histological assessment of osteoarthritis (OA) severity in animal models is critical in studies that evaluate effectiveness of agents designed to prevent or reduce disease severity. The most commonly used OA histological grading scheme, the Mankin Histological-Histochemical Grading System (HHGS)[1], was initially developed in humans but is applied, either in its original form or with modifications, to evaluate OA severity in animals, including mice.[2–7] There are, however, limitations that prevent the Mankin HHGS, and other currently-used histological grading schemes, from accurately assessing OA severity in rodent models. Firstly, the Structure parameter in the Mankin HHGS relies on the ability to differentiate among the three zones of AC; however these are very difficult to consistently identify in rodents, particularly mice, in which the AC is less than 75 μm thick. These schemes also focus exclusively on changes within the AC and do not consider changes in other joint structures, such as bone and meniscus. Finally, schemes such as the Osteoarthritis Research Society International (OARSI) Osteoarthritis Cartilage Histopathology Assessment System (OOCHAS)[8] combine changes occurring in multiple different tissues into a single parameter, assuming that these changes are synchronous.

Due, in large part, to the aforementioned issues, very few studies have reproducibly and comprehensively characterized stifle (knee) joint changes associated with OA in mice in either naturally occurring or surgically induced models, despite the popularity of transgenic and surgical models. Histological characteristics of the stifle

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joint in several transgenic mouse models have been published[9–13]; however, these assessments commonly utilize the semiquantitative histological evaluations described above. The goals of the present study were to apply a newly developed histological grading scheme[14] to naturally occurring and surgically induced OA in mice to determine the ability of the newly developed scheme to identify changes in multiple joint tissues that are commonly associated with OA and to directly compare the ability of this scheme to identify OA lesion severity with that of the previously established Mankin scheme[1].

Materials & methods

Animal models of OA

The tissues evaluated in this study were from male C57Bl/6 mice in which OA either was surgically induced (destabilized medial meniscus, DMM) or occurred naturally (aged mice).

Surgically induced OA (DMM): Eleven mice, aged 2.5 months at the start of the experiment, underwent DMM ($n = 8$) or Sham ($n = 3$) surgery. One stifle served as either a sham (Sham) or surgery (DMM) site and the other was left as an unoperated control (Sham contralateral [where contralateral stifle was sham] or DMM contralateral [where contralateral stifle underwent DMM surgery] groups). After 2 months of free cage movement (at 4.5 months of age), the mice were sacrificed and hindlimbs were collected for evaluation. Sham joints were not utilized in this experiment. Sham contralateral joints were combined with DMM contralateral joints to form the Control group ($n = 11$ joints).

Fifteen mice, aged 3.5 months, underwent the same surgery outlined above (DMM $n = 12$, Sham $n = 3$). After 2 months of free cage movement (at 5.5 months of age), mice were sacrificed and hindlimbs were collected for evaluation. Sham contralateral joints were combined with DMM contralateral joints to form the Control group ($n = 15$ joints).

Naturally occurring OA: The contralateral (Control) joints described above were included in this analysis to represent joints from 4.5- ($n = 11$ joints) & 5.5- ($n = 15$ joints) month-old mice. One hind limb was also collected from ten 16-month old mice ($n = 10$ joints). Both hindlimbs were collected from eighteen 17-month-old mice ($n = 36$ joints), however three joints could not be used due to sectioning difficulties (therefore, $n = 33$ joints). Thirteen 23-month-old ($n = 26$ joints) mice were sacrificed and both limbs were collected.

Histological preparation and assessments

All stifle joints ($n = 115$ joints from 67 mice) were routinely fixed in 10% formalin, decalcified in 10% ethylenediaminetetraacetic acid (EDTA), processed, embedded intact into paraffin, and sectioned in a coronal plane. Sectioning, staining, grading, and measuring of these sections have been described previously in detail[14]. Representative sections (sections of high quality in a midcoronal location within 100 μm of each other) were evaluated. Two to four sections were stained with hematoxylin & eosin (H&E) or Safranin-O for evaluation. Evaluations were confined to the tibial plateaus. Measurements included AC area and thickness (AC thick); subchondral bone area (SCB area) and thickness (SCB thick); areas of chondrocyte cell death (CCD, defined as areas of AC occupied by two or more dead chondrocytes as determined by H&E staining) and percentages of CCD (CCD%) with respect to total AC area; number of viable chondrocytes (#chond); areas of abaxial (AbaxOP) and axial (AxOP) osteophytes; and total area (Men) and percentage of CCD within the meniscus (MenCCD%). Areas with full thickness cartilage loss were given "0" for area of CCD, "100%" for CCD%, and "0" for #chond. Lastly, articular cartilage structure (ACS), and Safranin-O staining (Saf-O) were evaluated using semiquantitative scores that ranged from 0 to 12. For comparison, the original Mankin HHGS grading scheme[1], was applied to both tibial plateaus of these same sections.

TUNEL staining

To determine if the dead chondrocytes observed were the result of apoptosis, one section from 12 different joints was used for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays using an *in situ* cell death detection kit (Chemicon International, USA). These included two joints with surgically induced OA (4.5 month DMM), seven joints with naturally occurring OA (17 & 23 month old joints), and three joints in which no OA or CCD was present (5.5 month Controls). In OA joints, sections were used in which at least one tibial plateau contained >25% dead chondrocytes. Murine thymus was used as a positive control. Slides were deparaffinized with xylene, rehydrated through a series of decreasing concentrations of ethanol, and were washed with tris-buffered saline (TBS). Antigen retrieval was achieved with citrate buffer. Slides were washed with TBS, endogenous peroxidase activity was blocked with 3% H_2O_2, and slides were washed again three times. Slides were incubated with the Equilibrium buffer for 10 s, and then incubated with terminal deoxynucleotidyl transferase (TdT) enzyme for 60 min at room temperature (RT). The slides were incubated with stop buffer for 10 min at RT, and then were washed three times with TBS. Anti-Digoxigenin-Peroxidase was applied to the slides and incubated at RT for 30 min. Slides were washed three times with TBS, and 3,3'-Diaminobenzidine (DAB) was added for 4 min. Slides were washed in distilled water and counterstained with Mayer's Hematoxylin (DAKO).

Statistics

All histological data from the newly developed scheme (continuous measurements and semiquantitative grades) from the medial tibial plateaus were subjected to Principal Components Analysis (PCA; SAS Proc Factor, Cary, NC) as previously described[14] to reduce 15 correlated OA parameters to five uncorrelated factors (principal components). The five factors generated by PCA were orthogonal linear transformations of the original observations and were each composed of a group of parameters that were closely related to one another. The five factors were renamed to reflect these relationships and included AC Integrity (Factor 1), Chondrocyte Viability (Factor 2), SCB (Factor 3), Meniscus (Factor 4), and Periarticular Bone (Factor 5) (Table I).

All factor scores and selected continuous measurements were subjected to analysis of variance (ANOVA) and post-hoc analyses (SPSS v.17). Tukey post-hocs were performed to address multiple comparisons in those analyses in which the overall results were significant, and those are the reported *P*-values unless otherwise noted. The semiquantitative grades and Mankin scores for each tibial plateau and total Mankin scores (summed Mankin scores from both plateaus) were evaluated using nonparametric statistical analyses.

Results

Medial vs lateral tibial plateaus

Histological lesions were most severe in the medial tibial plateaus in nearly all joints in both models, resulting in significant differences between medial and lateral tibial plateaus for multiple parameters. For this reason, data presented is from medial tibial plateaus unless otherwise noted. In general, the mean AC areas and