Gross, histologic, and gene expression characteristics of osteoarthritic articular cartilage of the metacarpal condyle of horses

Katie J. Smith, BVetMed; Alicia L. Bertone, DVM, PhD; Stephen E. Weisbrode, VMD, PhD; Michael Radmacher, PhD

Objective—To identify patterns and correlations of gross, histologic, and gene expression characteristics of articular cartilage from horses with osteoarthritis.

Animals—10 clinically normal horses and 11 horses with osteoarthritis of the metacarpal condyles.

Procedures—Metacarpophalangeal joints were opened and digitally photographed, and gross lesions were scored and quantified. Representative cartilage specimens were stained for histologic scoring. Total RNA from dorsal and palmar articular surfaces was processed on an equine gene expression microarray.

Results—Histologic scores were greater in both regions of osteoarthritic joints, compared with corresponding regions in control joints. Cartilage from the palmar aspect of diseased joints had the highest histologic scores of osteoarthritic sites or of either region in control joints. A different set of genes for dorsal and palmar osteoarthritis was identified for high and low gene expression. Articular cartilage from the dorsal region had surface fraying and greater expression of genes coding for collagen matrix components and proteins with anti-apoptotic function, compared with control specimens. Articular cartilage from the palmar region had greater fraying, deep fissures, and less expression of genes coding for glycosaminoglycan matrix formation and proteins with anti-apoptotic function, compared with cartilage from disease-free joints and the dorsal aspect of affected joints.

Conclusions and Clinical Relevance—Metacarpal condyles of horses with naturally occurring osteoarthritis had an identifiable and regional gene expression signature with typical morphologic features. (*Am J Vet Res* 2006;67:1299–1306)

Osteoarthritis is one of the most important causes of lameness in horses[1] and has been cited as the most important musculoskeletal disease of pleasure and performance horses in terms of economics and welfare.[1,2] The disease is typified by metabolic imbalance, excessive chondrocyte catabolism,[3-5] extracellular matrix macromolecule breakdown,[6,7] chondrocyte apoptosis,[8-10] and cartilage loss down to subchondral bone.[4]

The molecular characterization of osteoarthritis remains elusive because of the multifaceted nature of the disease[3] and the inherent limitations of the investigative techniques available. A number of genes involved in osteoarthritis have been studied intensively; however, use of traditional methods, including reverse transcriptase PCR[4,11-18] and in situ hybridization,[19,20] has resulted in a relatively narrow range of investigated genes. These classical approaches have focused on identifying and analyzing individual specific postulated mediators and typically require preexisting knowledge for selection of gene-specific primers. These methods have proven to be extremely productive; however, the degenerative process in the joint is complex, likely involving hundreds of gene products and interrelated pathways. A more global analysis of coordinated gene expression during osteoarthritis would be beneficial for a fuller understanding of the biological processes and to potentially identify novel mediators of disease.

The recent introduction of microarray technology provides a method for assessment of large-scale gene expression by measuring the expression of mRNA of thousands of genes.[21] Semiquantitative information regarding the expression of numerous genes can be obtained by measuring changes in mRNA expression. Expression of mRNA is unique to the corresponding protein produced, thereby giving an indication of the cellular activity at the time of tissue harvest.[22] The transcriptome is defined as the mRNA for specific genes transcribed at a certain time in tissues under normal or abnormal conditions.[23] Simultaneous transcriptome analysis of thousands of genes can provide information about genes whose functions are unknown and enhance our understanding of how genes interact to provide molecular control.[22] It is increasingly expected that new candidate biomarkers will arise from complex microarray approaches.

Laboratory-prepared cDNA microarrays have provided limited assessment of alterations in transcription

ABBREVIATIONS

MCP	Metacarpophalangeal
MC3	Third metacarpal
SOD	Superoxide dismutase
MMP	Matrix metalloproteinase

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From the Comparative Orthopedic Research Laboratories, Departments of Veterinary Clinical Sciences (Smith, Bertone) and Veterinary Biosciences (Weisbrode), College of Veterinary Medicine, and the Center of Biostatistics (Radmacher), The Ohio State University, Columbus, OH 43210. Dr. Smith's present address is New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348.
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Address correspondence to Dr. Bertone.

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profile in naturally occurring human osteoarthritis[24,25] and murine experimental models of rheumatic disease.[26] To our knowledge, the present study is the first to use annotated commercial-quality oligoarrays to correlate histologic characteristics and gross morphologic characteristics with alterations in the gene expression profile, offering the unique opportunity to statistically evaluate transcriptome changes in conjunction with quantified structural degeneration. Articular cartilage microarray experiments on the MCP joint were chosen because that joint has the largest number of traumatic and degenerative lesions of all joints in the appendicular skeleton of horses.[27] Strenuous exercise causes repetitive episodes of trauma,[28] initiating disease and alterations in articular cartilage matrix, including a decrease in proteoglycan concentration and disruption of the collagen framework.[29] The goal of the study reported here was to identify patterns and correlations of gross, histologic, and gene expression characteristics of articular cartilage from horses with osteoarthritis. We hypothesized that regional gross and histologic cartilage degeneration of the articular surface of the MC3 would correlate with differing expression profiles of genes, compared with control specimens.

Materials and Methods

Horses—Horses with osteoarthritis (n = 10) were purchased for research procedures approved by the institutional animal care and use committees. Horses were selected by use of clinical inclusion criteria, including forelimb lameness localized to the MCP joint ≥ 2/5 on the American Association of Equine Practitioners grading scale[30] and radiographic evidence of osteoarthritis in that joint, specifically osteophytes, entheseophytes, subchondral sclerosis, or lysis and joint space narrowing.[31] Control horses (n = 11) were euthanatized for a nonconflicting study[a] and were selected as similar-age horses without lameness and with visibly, palpably, and radiographically normal MCP joints.

Specimen collection—Immediately after euthanasia via overdose of pentobarbital (IV), the joints were opened and imaged with high-resolution digital photography (focal distance, 150 mm) to quantify and document the gross distribution of the lesions. Gross characteristics of osteoarthritis were evaluated for lesion extent, without knowledge of group assignment, by use of software,[b] and assigned numeric scores for severity, including visual assessment for the presence of score lines, surface fibrillations, cartilage erosions or ulcerations, and osteophytes, on the basis of the following scoring system: 0 = normal, 1 = scattered surface fibrillations, 2 = mild partial-thickness erosions, 3 = moderate partial-thickness erosions with full-thickness fissures, and 4 = severe full-thickness cartilage ulcerations extending to subchondral bone **(Figure 1)**.

Histologic grading—For histologic grading of severity of osteoarthritis, full-thickness cartilage specimens were cut from representative dorsal and palmar regions, processed, sectioned, and stained with H&E and toluidine blue.[19] Slides were assessed by investigators (ALB, KJS, and SEW) without knowledge of group or site. Mean scores and indices were determined for histomorphologic structure, hypocellularity, and histochemical staining intensity according to descriptions adapted from the Mankin[13,32] scoring system (range, 0 [normal] to 12 [severely abnormal]; maximal sum index, 36), as follows: structure (0 = normal, 1 = surface irregularities, 2 = irregularities down to the tangential layer, 3 = cleft to 50% depth of radial zone, 4 = cleft to 100% depth of radial zone); hypocellularity (percentage of empty lacunae; 0 = 0% cell reduction [normal], 1 = 1% to 25% cell reduction, 2 = 26% to 50% cell reduction, 3 = 51% to 75% cell reduction, 4 = 76% to 100% cell reduction [severe hypocellularity]); and matrix-staining intensity (0 = normal, 1 = ≤ 25% reduction, 2 = 26% to 50% reduction, 3 =51% to 75% reduction, 4 = 76% to 100% reduction in staining intensity).

Extraction of RNA—Immediately after specimens were harvested for histologic examination, the surface of MC3 was split frontally into dorsal and palmar halves. The entire dorsal and palmar surfaces were harvested via sharp curettage and snap-frozen separately in liquid nitrogen prior to storage at –80°C until required for RNA isolation. The RNA was extracted separately from each region with specimens individually processed to prevent inadvertent mixing of nuclear material from different regions or from different joints. Cartilage was ground under liquid nitrogen with a mortar and pestle,[16] avoiding sample thawing. Each milligram of milled cartilage powder was mixed with 10 mL of reagent[c] and homogenized with a rotor-stator tissue homogenizer for 1 minute followed by incubation for 5 minutes at 4°C.[33] Samples were centrifuged at 12,000 × g for 5 minutes at 4°C, and the clear supernatant was transferred to a new tube. Chloroform (0.2 mL) was added to each 1- mL aliquot of sample, and samples were shaken vigorously for 15 seconds. After samples were held for 3 minutes at 4°C, samples were centrifuged for 15 minutes at 4°C for phase separation. The colorless supernatant was transferred to a new tube, and isopropanol (0.5 mL) was added prior to incubation for 10 minutes at room temperature (approx 20°C). Samples were centrifuged for 10 minutes, and the RNA pellet was washed with 75% ethanol, dried, and resuspended in 50 µL of RNase-free water. The amount of nucleotide extracted was calculated by measuring UV absorbance at 260 to 280 nm. The RNA was assessed for quantity and integrity by use of capillary electrophoresis[d] to measure fluorescence of polynucleotides. The degree of fluorescence provided information on DNA or salt contamination sustained during extraction, as indicated by signal intensities of 28S and 18S rRNA. After extraction and preparation, 14 RNA specimens from the osteoarthritis group (dorsal, n = 6; palmar, 8) and 18 RNA specimens from the control group (dorsal, 10; palmar, 8) were suitable for placement on individual equine microarrays (32 total equine microarrays).

Preparation of RNA—The method used for RNA preparation has been detailed in the literature.[27,33] The RNA extracted from each specimen was processed individually, with 1 specimen hybridized with 1 gene chip. Total RNA (5 µg) was reverse transcribed into double-stranded cDNA by use of a polymerase.[c] Biotinylated cRNA was synthesized by use of a polymerase labeling kit[e] and fragmented prior to overnight hybridization at 45°C for 16 hours with the equine microarray gene chip.[f] Microarrays were washed and stained with streptavidin-phycoerythrin in accordance with an established protocol.[g] The equine microarray gene chip[f] has been validated for performance and gene expression by use of reverse transcriptase-PCR for select genes.[34,35] Light was emitted from the fluorescent reporter group only when bound to the probe. Light emitted from the perfect match oligoprobe, compared with the single base pair mismatched oligoprobe, was detected and analyzed by use of bioinformatics software.[h]

Statistical analysis—Quantitative data were analyzed via 2-factor ANOVA for disease (osteoarthritis and control) and region (dorsal and palmar). Semiquantitative data were

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analyzed by use of a Mann-Whitney U rank test with Bonferroni correction. Microarray probe-level data were analyzed with software.[36,i] Array normalization was performed with the invariant set procedure, and model-based expression indices were computed with the perfect-match-only model. Probe-set level data that were classified as array outliers were omitted and considered to be missing data in subsequent analyses. After model-based expression indices computation and log transformation of the values, data were imported into commercially available software[j] for statistical comparisons. Only probe sets that had a notable amount of variation in expression among specimens were considered for further analysis, and probe sets classified as absent for > 75% of the specimens were omitted. Specimens were clustered by use of hierarchal clustering, with mean linkage and 1 – Pearson correlation as the distance measurement. Results of statistical tests were considered significant at values of $P \leq 0.005$ to compensate for multiple comparisons. Genes were annotated for name and function by computer programs.[k,l]

Results

Macroscopic lesions—The median gross degeneration score for articular cartilage was significantly greater in the palmar region of osteoarthritic joints, compared with the dorsal region of osteoarthritic joints and also compared with the corresponding regions of control joints. Osteoarthritic specimens were all of grades 2 to 4, and control specimens were of grade 0 or 1. Clinically normal joints all had grossly normal cartilage (grade 0) in the dorsal region. The articular cartilage obtained from the palmar region of clinically normal joints and the dorsal region of osteoarthritis-affected joints had mild evidence of gross degeneration with surface fraying and score lines. Cartilage harvested from the palmar aspects of the diseased joints was severely degenerated with deep fissures, erosions, and ulcerations down to subchondral bone. Median gross score for articular cartilage was different ($P = 0.013$) between region (dorsal vs palmar) and between group (osteoarthritis vs normal).

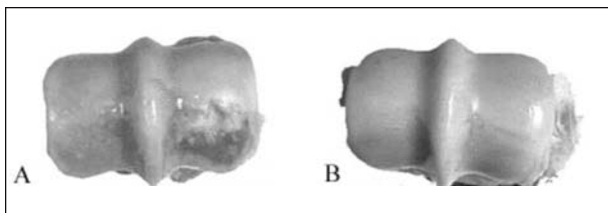

Figure 1—Digital photographs of the metacarpal condyles of a horse with osteoarthritis (A) and an age-matched control horse (B).

Histologic lesions—Histologic lesions were significantly greater in both regions of osteoarthritic joints, compared with corresponding regions in control joints. Cartilage from the palmar aspect of diseased joints consistently had the highest histologic scores (median index score, 28; range 11 to 31) with significantly ($P = 0.013$) greater indices than dorsal osteoarthritic sites or either region in control joints. In contrast, specimens harvested from the dorsal region of affected joints were characterized by mild to moderate degeneration, with obvious changes evident but of lower magnitude than the palmar region (median index score, 9; range, 7 to 16). The histologic index scores of cartilage from control joints were low (median index score, 0; range, 0 to 2), and there was mild degeneration in palmar regions (median index score, 3; range, 0 to 18).

Microarray analysis—Microarray quality characteristics, including signal intensity and percentage positive signal calls, were not different between regions or groups, indicating consistency of gene chip performance and tissue expression. Each array met internal standard requirements analyzed by the bioinformatic core before being included in further analyses.

Gene expression profiles clustered significantly by disease (osteoarthritis or control) and severity (mild or severe). One of the 2 main hierarchal clusters consisted almost entirely of control specimens **(Figure 2)**. A different set of genes for dorsal osteoarthritis (mild) and palmar osteoarthritis (more severe) was identified in association with greater and lesser gene expression **(Tables 1 and 2).**

Analysis revealed more evidence of anabolic pathways and anti-apoptotic mechanisms in dorsal specimens affected with osteoarthritis. Articular cartilage obtained from the dorsal region had > 2 times the expression of genes central to cartilage maintenance, compared with cartilage obtained from corresponding regions of control joints. These included genes encoding the macromolecules of the extracellular matrix (collagen types I and II), as well as significantly greater SOD-2 and genes coding for degradative enzymes, catabolic proteins, and transcriptional regulation (Table 1). Articular cartilage from specimens with palmar osteoarthritis predominantly yielded lesser expression, including that of genes involved in protein biosynthesis, anti-apoptotic function, and extracellular proteoglycan matrix formation. Expression of genes encoding collagen I and II was ≥ 2 times that in cartilage harvested from dorsal and palmar regions of osteoarthritic joints, compared with the equivalent regions in control specimens (Table 2).

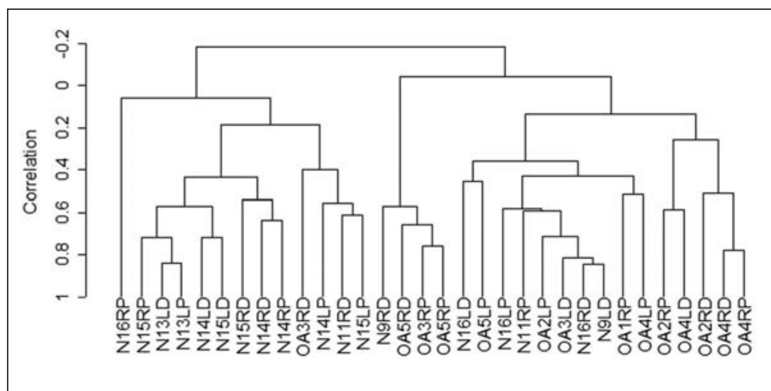

Figure 2—Dendrogram for microarray clustering analysis of RNA from metacarpal condyle articular cartilage of 14 specimens from horses with osteoarthritis and 18 specimens from control horses. N = Control. RP = Right palmar. OA = Osteoarthritis. LD = Left dorsal. LP = Left palmar. RD = Right dorsal.

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Table 1—Differential expression of genes in metacarpal condyle (dorsal region) articular cartilage specimens from horses with osteoarthritis.

GenBank accession No. of equine sequence	Gene ontology No.	GenBank human GenInfo No.	Sequence name of full or provisional annotation	Biological function	*P* value*	Factor difference*
U62528	5585	1480743	Collagen II	ECM structural protein	< 0.001	2.82
AF023169	5585	10947026	Collagen II α 1	ECM structural protein	< 0.001	2.6
AB070839	5584	15408576	Collagen I α 2	ECM structural protein	0.001	2.53
CD467650	6979	498259	SOD-2	Anti-apoptosis	0.004	2.00
BM734907	16021	19056240	Integral membrane protein 2C	Membrane structural component	0.028	1.95
X78077	5540	459438	Cartilage link protein 1	Hyaluronic acid binding	0.039	1.89
BM780416	4217	3869128	Cathepsin L2	Peptidase	0.014	1.7
AJ279008	6508	10185019	Cathepsin L	Peptidase	0.035	1.66
X69141	6694	435676	Squalene synthase	Steroid biosynthesis	0.011	1.65
AF307858	5200	11037762	Actin-related protein 3	Cytoskeleton constituent	0.007	1.64
U62529	30574	1480745	MMP 3 (stromelysin 1)	Collagen catabolism	0.012	1.59
BM781310	6955	4504410	Major histocompatibility complex II	Product of ECM catabolism	0.035	1.59
BI961112	6508	23110961	Cathepsin S	Peptidase	0.028	1.54
CD470622	45786	24660320	AXIN-1	Negative regulation of cell cycle	0.023	–2.2
CD469176	6916	16554596	Immediate early response 3	Anti-apoptosis	0.010	–2.14
CD466296	6446	37588989	Translation initiation factor 3	Transcription regulation	0.045	–2
CD465716	6334	31386984	H3 histone	Nucleosome assembly	0.004	–1.59

*Compared with control specimens.
ECM = Extracellular matrix.

Discussion

To the authors' knowledge, the present study was the first to quantitatively characterize metacarpal osteoarthritis by region via comparative, gross, histologic, and gene expression profiling. By use of macroscopic and histologic grading systems, it was established that specimens from clinically normal adult middle-aged horses had unaffected cartilage in the dorsal aspect of the joint and mild degenerative changes in certain palmar regions. In contrast, a marked difference in regional distribution of cartilage lesions was evident in condyles with osteoarthritis. Degenerative changes were most marked in the palmar region of affected condyles, consistent with severe osteoarthritis. Cartilage lesions in the dorsal region were evident but of a markedly lesser magnitude and were characteristic of mild osteoarthritis.

Radiographic assessment, in conjunction with clinical and surgical findings, has suggested involvement of MC3, specifically, the palmar condyle region, but this has remained minimally studied. In contrast, histologic grading systems[13,32] have been used extensively in humans and veterinary species to classify the severity of disease in affected cartilage,[37] but are limited to the microscopic areas retrieved. This disadvantage is particularly important in diseased specimens that are highly heterogeneous or have regional variability in lesions, as in the MCP joint.[38] This focal nature of osteoarthritis is thought to suggest a role for biomechanical loading, superimposed on age-related changes, in the distribution of lesions throughout the joint. The injury progressively results in morphologic changes and matrix degradation, not only in the directly injured area but also in the surrounding cartilage.[7,14] Furthermore, the biomechanical and biochemical properties of articular cartilage adjacent to the lesion are substantially weaker than that of grossly normal cartilage from remote, unaffected parts of the joint.[29,38,39] Previous studies[38,40,41] of affected MCP joints have focused on the biochemical degeneration of the articular surface of the first phalanx. A distribution pattern of site-related changes in the biochemical composition of the cartilage of the first phalanx has been identified,[40-42] with the medial side more frequently affected than the lateral side.[43] It has been postulated that the dissimilarity in response between dorsal and palmar regions may be related to differences in the transmission and dissipation of force experienced with sudden loading during exercise.[43] The medial to lateral distribution pattern is thought to be caused by asymmetrical loading of the articular surface caused by the eccentric position of the center of gravity of the horse in relation to the axis of the limb.

The effect of the location of diseased cartilage has been evaluated in experimental animal models other than those involving horses by use of traditional methods to investigate location-specific changes in the expression of a limited number of genes and proteins pertaining to cartilage metabolism.[11,14] In the present study, specimens from the mildly affected dorsal region

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and severely affected palmar region had significantly different gene expression profiles, compared with cartilage from equivalent regions in normal joints. Articular cartilage from the dorsal region was predominantly characterized by greater expression of genes for extracellular matrix macromolecules. In addition, there was significant expression of the gene encoding SOD-2, which has an important role in reduction of apoptosis caused by several classes of oxidative stress inducers.[44] Expression of the SOD-2 gene indicates the chondrocyte's attempts to maximize structural viability by reducing programmed cell death and may imply a survival response by the chondrocyte, early in the stage of disease, as described in other species.[14] Other genes of greater expression encoded for proteolytic enzymes (cathepsin L and L2, cathepsin S, and MMP 3) and proteins involved in anabolic processes (squalene synthase, integral membrane protein 2C, and cartilage link protein 1). Our results were consistent with the findings of other investigators and indicated high activity

Table 2—Differential expression of genes in metacarpal condyle (palmar region) articular cartilage specimens from horses with osteoarthritis.

GenBank accession No. of equine sequence	Gene ontology No.	GenBank human GenInfo No.	Sequence name of full or provisional annotation	Biological function	*P* value*	Factor difference
U62528	5585	1480743	Collagen II	ECM structural protein	0.003	2.3
AF023169	5585	10947026	Collagen II α 1	ECM structural protein	0.026	2.06
AB070839	5584	15408576	Collagen I α 2	ECM structural protein	0.025	2.0
BM781118	19904	19129350	Tyrosine 3-monooxygenase 5-monooxygenase activation protein	Protein domain specific binding	0.019	1.7
AB035080	5509	7959047	Beta-casein	Calcium ion binding	0.011	1.56
BM734930	6979	19056263	SOD	Anti-apoptosis	0.03	1.54
BI961670	6412	16319873	Ribosomal protein L19	Catalyze protein synthesis	0.0089	–2.34
CD466576	6446	31387844	Translation initiation factor 3	Transcription regulation	< 0.001	–2.29
BM781189	6412	19129421	Ribosomal protein L18a	Protein biosynthesis	0.014	–2.28
BM734946	6334	19056279	H1 histone	Nucleosome assembly	0.009	–2.17
BI961854	8467	16320057	Heparan sulfate (glucosamine) 3-O-sulfotransferase	ECM formation	0.001	–2.07
CD469176	6916	31390444	Immediate early response 3	Anti-apoptosis	0.007	–2.04
AB043676	6457	12082133	Heat shock protein 90 beta	Protein folding	0.007	–1.97
BM735117	6916	19056450	B-cell chronic lymphocytic leukemia	Anti-apoptosis	0.001	–1.93
CD469014	3723	31390282	Poly(A) binding protein cytoplasmic 1	RNA binding	0.011	–1.93
BI961689	8092	16319892	Syndecan 4 (amphiglycan)	Cytoskeletal protein binding	0.011	–1.91
BM735084	6412	19056417	Ribosomal protein L13	Protein biosynthesis	0.014	–1.89
CD536783	6444	31579198	Nascent polypeptide associated complex α	Polypeptide translocation, protein biosynthesis	0.009	–1.72
CD536349	7165	31578764	Guanine nucleotide binding protein	Signal transduction	0.007	–1.71
BM780648	6526	19128880	Argininosuccinate synthetase	Arginine biosynthesis, limits NO autotoxicity	0.007	–1.7
BM734510	6412	19055843	Ribosomal protein L13a	Protein biosynthesis	0.013	–1.66
CD465716	6334	31386984	H3 histone	Nucleosome assembly	< 0.001	–1.62
CD466435	8284	31387703	DEAD (Asp-Glu-Ala-Asp) box	Positive regulation cell proliferation	0.002	–1.56
BM735460	4713	19056793	Tyrosine kinase	Protein kinase activity	0.01	–1.56
BM735062	6511	19056395	NEDD8 (ubiquitinlike protein)	Ubiquitin-dependent protein catabolism	0.002	–1.55
BI961165	6414	16319368	Ribosomal protein P1	Translational elongation	0.015	–1.52
CD528854	6412	31567476	Ribosomal protein L12	Protein biosynthesis	0.013	–1.51

*Compared with control specimens. *See* Table 1 for key.

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of anabolic pathways in mild or moderate disease simultaneous with high activity of pathways for matrix degradation. In a developmental osteoarthritis cartilage explant model,[18] chondrocytes have high expression of genes for type II collagen; aggrecan; biglycan MMP-1, -3, and -13; and TIMP-1. The present study of specimens with mild osteoarthritis supported findings of other investigators that early degeneration is accompanied by greater expression of collagenase activity[13] and expression of genes associated with matrix macromolecules.[25] Furthermore, it was possible to verify that in early osteoarthritis, among all the proteases, MMP 3 had the strongest expression, which is in accordance with previous work.[17] Matrix metalloproteinease 13 is detectable only in late-stage specimens, suggesting that early stages are characterized more by degradation of other matrix components than by degradation of type II collagen fibers.[24] The results presented here suggest that mild osteoarthritis, as is typical of the dorsal region of diseased joints, involves proteolysis in conjunction with enhanced transcriptional activity of chondrocytes as an attempt to maintain the integrity of the matrix.

In contrast to the profile of cartilage affected with mild osteoarthritis, the expression profile of severely affected cartilage had less transcriptional activity, with most differences in gene expression representing lesser gene expression. Results indicated significantly less expression of genes encoding transcriptional proteins, including H3 histone and translation initiation factor 3. Several ribosomal proteins also had lower expression. Lower expression of a biosynthetic matrix enzyme (heparin sulfate 3-O-sulfotransferase) was consistent with the overall lower extracellular proteoglycan matrix formation detected histochemically. Lower expression of genes with anti-apoptotic function was detected (B-cell chronic lymphocytic leukemia) in addition to immediate early response protein 3, which have an inhibitory effect on programmed cell death by directly interacting with various proteins involved in control of apoptotic pathways,[44] and DEAD box, involved in the positive regulation of cell proliferation. Greater collagen II expression was still present, as in mild osteoarthritis of the dorsal region, which may suggest that progression into later stage osteoarthritis is initially a failure of chondrocyte anabolic activity, as well as sustained resistance to senescence, and not loss of ability to produce type II collagen. This profile represented overall lower transcription, less ability to generate extracellular glycosaminoglycan matrix protein, and less chondrocyte ability to prevent cell death. It has been proposed that in the later, more severe stages of disease, the biosynthetic machinery of the chondrocyte is unable to keep up with anabolic demands and a net depletion of extracellular matrix subsequently occurs. Our results confirmed lower expression of genes for proteins with anti-apoptotic properties, adding further evidence that cell death is a feature of the pathogenesis of osteoarthritis late in the stage of disease.

The present findings on gene expression suggest potential therapeutic targets for molecular treatments of osteoarthritis that could be beneficial. Research of molecular mechanisms involved in the progression of osteoarthritis has identified a small number of known molecular mediators associated with osteoarthritis and has contributed to the subsequent development of new protein and gene-based approaches.[45] Initial experimental studies of the effect of protein and gene therapy strategies in the treatment of osteoarthritis have yielded encouraging results.[2] Protein therapies such as interleukin-1 receptor antagonist[m] can ameliorate disease but requires continuous injections. Gene transfer offers the capability to achieve sustained, localized presentation of bioactive proteins, or gene products, to sites of tissue damage,[46] with the emphasis predominantly on the genes whose products enhance synthesis of the cartilage matrix or inhibit its breakdown. Results of the present study suggest that anti-apotoptic genes and genes trophic to cell health may have the most impact for long-term joint health, particularly in chronic or severe osteoarthritis. Genes that enhance collagen II production may be less effective.

The contribution of the chondrocyte as a source of inflammatory signals in osteoarthritis has been unclear. Our data suggest that the chondrocyte may not be the primary source for inflammatory cytokines in the progression of osteoarthritis. The classical inflammatory proteins that have been implicated in osteoarthritis include inducible nitric oxide synthetase, tumor necrosis factor-α, cyclooxygenase-2, and interleukin-β and were not differentially expressed in the present study. Inflammatory pathways are important in naturally occurring disease.[28] Two of the few studies[24,25] that used microarrays to evaluate chondrocyte gene expression in naturally occurring osteoarthritis did not detect substantial changes in inflammatory mediators, supporting our findings in equine osteoarthritis. In consideration of the experimental conditions in which inflammatory mediators from chondrocytes have been identified, our results may be more indicative of the native state.

This study revealed location-specific morphologic and gene expression patterns of the MC3 condyles of horses. Results of profile analysis indicated that several biological processes, including inflammatory, anabolic, catabolic, apoptotic, and degradative pathways, contribute to the pathogenesis of osteoarthritis at different stages of disease. Mild osteoarthritis was typified by an increase in genes encoding collagen, chondrocyte anabolism, and anti-apoptotic proteins. More severe osteoarthritis was characterized by a decrease in transcription, maintenance of proteoglycan matrix macromolecules, and the ability of the chondrocyte to regulate apoptosis, but persistence of increased collagen expression. In naturally occurring osteoarthritis cartilage, chondrocyte-derived inflammatory mediators were not a featured phenotype. Further characterization of these genes and pathways will advance our understanding of the basic mechanisms responsible for the initiation and progression of osteoarthritis and may aid the development of novel therapies.

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a. Bertone AL, Orban J, Grande DA, et al. Articular cartilage and subchondral bone repair using a biodegradable polymer matrix and instrumentation system (abstr), in *Proceedings*. 51st Annu Meet Orthop Res Soc 2005;1803.

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b. Scion Image Beta, version 4.02. Frederick, Md. Available at: www.scioncorp.com. Accessed Jan 11, 2005.
c. Trizol solution, Life Technologies, Gaithersburg, Md.
d. Bioanalyzer 2100, Agilent, Palo Alto, Calif.
e. Superscript II, Invitrogen, Carlsbad, Calif.
f. GeneChip, Affymetrix Inc, Santa Clara, Calif.
g. EukGE-WS2, Affymetrix Inc, Santa Clara, Calif.
h. GeneChip Operating System, version 1.2, Affymetrix Inc, Santa Clara, Calif.
i. dChip, Department of Biostatistics, Harvard School of Public Health, Harvard University, Boston, Mass. Available at: www.dchip.org/. Accessed Jan 21, 2005.
j. BRB-ArrayTools of the National Cancer Institute, version 2.1, National Cancer Instittue, Biometric Research Branch, Bethesda, Md. Available at: linus.nci.nih.gov/BRB-ArrayTools.html. Accessed Jan 4, 2005.
k. BlastN, TimeLogic DeCypher system, TimeLogic Corp, Carlsbad, Calif. Available at: www.ncbi.nih.gov. Accessed Jan 21, 2005.
l. National Council Biotechnology Information (NCBI) Web site. Available at: www.geneontology.org/. Accessed Jan 4, 2005.
m. Kineret IL-1 receptor antagonist, Amgen Inc, Thousand Oaks, Calif.

References

1. McIlwraith CW. General pathobiology of the joint and response to injury. In: McIlwraith CW, Trotter GW, eds. *Joint disease in the horse*. Philadelphia: WB Saunders Co, 1996;40–69.
2. Frisbie DD, Ghivizzani SC, Robbins PD, et al. Treatment of experimental equine osteoarthritis by in vivo delivery of the equine interleukin-1 receptor antagonist gene. *Gene Ther* 2002;9:12–20.
3. Malemud CJ, Islam N, Haqqi TM. Pathophysiological mechanisms in osteoarthritis lead to novel therapeutic strategies. *Cells Tissues Organs* 2003;174:34–48.
4. Young AA, Smith MM, Smith SM, et al. Regional assessment of articular cartilage gene expression and small proteoglycan metabolism in an animal model of osteoarthritis. *Arthritis Res Ther* [serial online]. 2005;7:R852–R861. Available at: arthritis-research.com/content/7/4/R852. Accessed Dec 27, 2005.
5. Wang J, Verdonk P, Elewaut D, et al. Homeostasis of the extracellular matrix of normal and osteoarthritic human articular cartilage chondrocytes in vitro. *Osteoarthritis Cartilage* 2003;11:801–809.
6. Pelletier JP, Martel-Petellier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. *Arthritis Rheum* 2001;44:1237–1247.
7. Hembry RM, Dyce J, Driesang I, et al. Immunolocalization of matrix metalloproteinases in partial-thickness defects in pig articular cartilage. A preliminary report. *J Bone Joint Surg Am* 2001:83-A:826–838.
8. Lo MY, Kim HT. Chondrocyte apoptosis induced by collagen degradation: inhibition by caspase inhibitors and IGF-1. *J Orthop Res* 2004;22:140–144.
9. Kuhn K, D'Lima DD, Hashimoto S, et al. Cell death in cartilage. *Osteoarthritis Cartilage* 2004;12:1–16.
10. Schuerwegh AJ, Dombrecht EJ, Stevens WJ, et al. Influence of pro-inflammatory (IL-1 alpha, IL-6, TNF-alpha, IFN-gamma) and anti-inflammatory (IL-4) cytokines on chondrocyte function. *Osteoarthritis Cartilage* 2003;11:681–687.
11. Dumond H, Presle N, Pottie P, et al. Site specific changes in gene expression and cartilage metabolism during early experimental osteoarthritis. *Osteoarthritis Cartilage* 2004;12:284–295.
12. Gehrsitz A, McKenna LA, Soder S, et al. Isolation of RNA from small human articular cartilage specimens allows quantification of mRNA expression levels in local articular cartilage defects. *J Orthop Res* 2001;19:478–481.
13. Tchetina EV, Squires G, Poole AR. Increased type II collagen degradation and very early focal cartilage degeneration is associated with upregulation of chondrocyte differentiation related genes in early human articular cartilage lesions. *J Rheumatol* 2005;32:876–886.
14. Le Graverand MP, Eggerer J, Vignon E, et al. Assessment of specific mRNA levels in cartilage regions in a lapine model of osteoarthritis. *J Orthop Res* 2002;20:535–544.
15. Martin I, Jakob M, Schafer D, et al. Quantitative analysis of gene expression in human articular cartilage from normal and osteoarthritic joints. *Osteoarthritis Cartilage* 2001;9:112–118.
16. Fehr JE, Trotter GW, Oxford JT, et al. Comparison of Northern blot hybridization and a reverse transcriptase-polymerase chain reaction technique for measurement of mRNA expression of metalloproteinases and matrix components in articular cartilage and synovial membrane from horses with osteoarthritis. *Am J Vet Res* 2000;61:900–905.
17. Bau B, Gebhard PM, Haag J, et al. Relative messenger RNA expression profiling of collagenases and aggrecanases in human articular chondrocytes in vivo and in vitro. *Arthritis Rheum* 2002; 46:2648–2657.
18. Lee JH, Fitzgerald JB, DiMicco MA, et al. Mechanical injury of cartilage explants causes specific time-dependent changes in chondrocyte gene expression. *Arthritis Rheum* 2005;52:2386–2395.
19. Gelse K, Soder S, Eger W, et al. Osteophyte development—molecular characterization of differentiation stages. *Osteoarthritis Cartilage* 2003:11;141–148.
20. Chubinskaya S, Kuettner KE, Cole AA. Expression of matrix metalloproteinases in normal and damaged articular cartilage from human knee and ankle joints. *Lab Invest* 1999;79:1669–1677.
21. Lequerre T, Coulouarn C, Derambure C, et al. A new tool for rheumatology: large-scale analysis of gene expression. *Joint Bone Spine* 2003;70:248–256.
22. Burgess JK. Gene expression studies using microarrays. *Clin Exp Pharmacol Physiol* 2001;28:321–328.
23. Sandell LJ. Genes and gene expression. *Clin Orthop Relat Res* 2000; 379:S9–S16.
24. Aigner T, Zien A, Gehrsitz A, et al. Anabolic and catabolic gene expression pattern analysis in normal versus osteoarthritic cartilage using complementary DNA-array technology. *Arthritis Rheum* 2001;44:2777–2789.
25. Aigner T, Zien A, Hanisch D, et al. Gene expression in chondrocytes assessed with use of microarrays. *J Bone Joint Surg Am* 2003;85-A(suppl 2):117–123.
26. Thornton S, Sowders D, Aronow B, et al. DNA microarray analysis reveals novel gene expression profiles in collagen-induced arthritis. *Clin Immunol* 2002;105:155–168.
27. Pool RR. Pathologic manifestations of joint disease in the athletic horse. In: McIlwraith CW, Trotter GW, eds. *Joint disease in the horse*. Philadelphia: WB Saunders Co, 1996;87–104.
28. Palmer JL, Bertone AL. Joint structure, biochemistry and biochemical disequilibrium in synovitis and equine joint disease. *Equine Vet J* 1994;26:263–277.
29. Buckwalter J. Articular cartilage injuries. *Clin Orthop Relat Res* 2002;Sep:21–37.
30. American Association of Equine Practitioners Web site. Lameness guide. Available at: www.aaep.org. Accessed Jan 12, 2005.
31. Simmons EJ, Bertone AL, Weisbrode SE. Instability-induced osteoarthritis in the metacarpophalangeal joint of horses. *Am J Vet Res* 1999;60:7–13.
32. Mankin HJ, Dorfman H, Lippiello L, et al. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *J Bone Joint Surg Am* 1971;53:523–537.
33. Baelde HJ, Cleton-Jansen AM, van Beerendonk H, et al. High quality RNA isolation from tumours with low cellularity and high extracellular matrix component for cDNA microarrays: application to chondrosarcoma. *J Clin Pathol* 2001;54:778–782.
34. Gu W, Bertone AL. Generation and performance of an equine-specific large-scale gene expression microarray. *Am J Vet Res* 2004;65:1664–1673.
35. Zachos TA, Bertone AL. Gene-mediated osteogenic differentiation of stem cells by bone morphogenetic proteins-2 or -6. *J Orthop Res* 2006;24:1279–1291.
36. Li C, Wong WH. DNA-Chip analyzer (dChip). In: Parmigiani G, Garrett ES, Irizarry R, et al, eds. *The analysis of gene expression data: methods and software*. Springer, NY: Springer Inc, 2003;120–141.
37. van der Sluijs JA, Geesink RG, van der Linden AJ, et al. The reliability of the Mankin score for osteoarthritis. *J Orthop Res* 1992;10:58–61.

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38. Brama PA, TeKoppele JM, Bank RA, et al. Topographical mapping of biochemical properties of articular cartilage in the equine fetlock joint. *Equine Vet J* 2000;32:19–26.

39. Strauss EJ, Chen CT, Goodrich LR, et al. Biochemical and biomechanical properties of lesion and adjacent articular cartilage after chondral defect repair in an equine model. *Am J Sports Med* 2005;3:1647–1653.

40. Brommer H, Brama PA, Barneveld A, et al. Differences in the topographical distribution of articular cartilage degeneration between equine metacarpo- and metatarsophalangeal joints. *Equine Vet J* 2004;36:506–510.

41. van der Harst MR, DeGroot J, Kiers GH, et al. Biochemical analysis of the articular cartilage and subchondral and trabecular bone of the metacarpophalangeal joint of horses with early osteoarthritis. *Am J Vet Res* 2005;66:1238–1246.

42. Brommer H, van Weeren PR, Brama PAJ, et al. Quantification and age-related distribution of articular cartilage degeneration in the equine fetlock joint. *Equine Vet J* 2003;35:697–701.

43. Chateau H, Degueurce C, Jerbi H, et al. Normal three dimensional behaviour of the metacarpophalangeal joint and the effect of uneven foot bearing. *Equine Vet J Suppl* 2001; 33:84–88.

44. Epperly MW, Bernarding M, Gretton J, et al. Overexpression of the transgene for manganese superoxide dismutase (MnSOD) in 32D cl 3 cells prevents apoptosis induction by TNF-alpha, IL-3 withdrawal, and ionizing radiation. *Exp Hematol* 2003;31:465–474.

45. Trippel SB, Ghivizzani SC, Nixon AJ. Gene-based approaches for the repair of articular cartilage. *Gene Ther* 2004;11:351–359.

46. Zhang X, Mao Z, Yu C. Suppression of early experimental osteoarthritis by gene transfer of interleukin-1 receptor antagonist and interleukin-10. *J Orthop Res* 2004;22:742–750.

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