Osteoarthritis and Cartilage 27 (2019) 294–303

Osteoarthritis and Cartilage

Regional gene expression analysis of multiple tissues in an experimental animal model of post-traumatic osteoarthritis

G.E. Salazar-Noratto † ‡, N. De Nijs †, H.Y. Stevens ‡ §, G. Gibson ‡ ||, R.E. Guldberg ‡ § *

† *Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, United States*
‡ *Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, United States*
§ *George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, United States*
|| *School of Biological Sciences, Georgia Institute of Technology, United States*

ARTICLE INFO

Article history:
Received 16 March 2018
Accepted 8 October 2018

Keywords:
Osteoarthritis
Animal model
Gene expression
Articular cartilage
Osteophyte
Synovium

SUMMARY

Objective: To characterize local disease progression of the medial meniscus transection (MMT) model of post-traumatic osteoarthritis (OA) at the molecular level, in order to establish a baseline for therapeutic testing at the preclinical stage.
Design: Weight-matched male Lewis rats underwent MMT or sham surgery on the left limb with the right leg as contralateral control. At 1 and 3 weeks post-surgery, tissues were harvested from different areas of the articular cartilage (medial and lateral tibial plateaus, and medial osteophyte region) and synovium (medial and lateral), and analyzed separately. RNA was extracted and used for microarray (RT-PCR) analysis.
Results: Gene expression changes due to surgery were isolated to the medial side of the joint. Gene changes in chondrocyte phenotype of the medial tibial plateau cartilage preceded changes in tissue composition genes. Differences in inflammatory markers were only observed at the osteophyte region at 3 weeks post-surgery. There was surgical noise in the synovium at week 1, which dissipated at week 3. At this later timepoint, meniscal instability resulted in elevated expression of matrix degradation proteins and osteogenic markers in the synovium and cartilage.
Conclusion: These results suggest feedback interactions between joint tissues during disease progression. Regional tissue expression differences found in MMT joints indicated similar pathophysiology to human OA, and provided novel insights about this degeneration model. The examination of gene expression at a localized level in multiple tissues provides a well-characterized baseline to evaluate mechanistic effects of potential therapeutic agents on OA disease progression in the MMT model.

Introduction

Osteoarthritis (OA) involves the degradation of articular cartilage, subchondral bone remodeling, and inflammation of the synovium. There are currently no FDA-approved disease-modifying OA drugs in the market. While global focus is centered on tissue engineering and regenerative medicine with targeted therapeutics, these have yet to successfully complete phase III clinical trials for OA, despite having shown positive results in preclinical studies[1–5].

There is a need for full characterization of pre-clinical models, with a view to comparison to human OA pathophysiology and to establish a baseline for therapeutic testing in these systems. Pre-clinical animal models of OA include experimentally accelerated but naturally occurring joint degeneration, transgenic mouse models, and surgically or chemically induced OA[2]. Unfortunately, none of these models can fully replicate the features and symptoms of human OA. Nevertheless, the impact of preclinical research will depend largely on the choice of the most appropriate model of OA for the specific process under investigation[2].

The rat medial meniscus transection (MMT) model is a frequently used model of post-traumatic OA, and is of high clinical relevance. The transection of the meniscus alters the mechanical

* Address correspondence and reprint requests to: R.E. Guldberg, University of Oregon, Phil & Penny Knight Campus for Accelerating Scientific Impact, 6231 University of Oregon, Eugene, OR 97403-6231, United States. Tel: 541-346-2120.
E-mail addresses: giuliana.sn@gatech.edu (G.E. Salazar-Noratto), nica.de.nijs@gatech.edu (N. De Nijs), hazel.stevens@ibb.gatech.edu (H.Y. Stevens), greg.gibson@biology.gatech.edu (G. Gibson), guldberg@uoregon.edu (R.E. Guldberg).

https://doi.org/10.1016/j.joca.2018.10.007
1063-4584/

stability of the knee joint and induces OA-like progressive damage: cartilage degeneration involving chondrocyte and proteoglycan loss, osteophyte and focal lesion formation, collagen degradation and cartilage fibrillation[6]. The MMT model is known for localized degeneration primarily restricted to the medial tibial plateau. However, despite being the industry standard for therapeutic testing, local molecular events in disease progression have not been thoroughly characterized in this model and assessment of therapeutic outcomes is largely limited to morphological analyses as endpoint readouts[6].

To date, a single study has reported on changes in the articular cartilage transcriptome of the rat MMT model. It concluded that the model paralleled key features of OA pathology, namely articular cartilage extracellular matrix (ECM) remodeling, angiogenesis, and chondrocyte cell death[7]. In this study, articular cartilage from both medial and lateral sides of the tibial plateaus and femoral condyles were collected from each animal, and then the cartilage tissue from five animals was pooled to create a single sample[7]. Cartilage collection from the entire joint and pooling of samples is standard for gene expression analyses in OA small animal models[7–9]. Yet this practice may mask identification of localized events in such models. With advances in microarray RT-PCR technology, we are now able to analyze tissues of much lower cell concentration.

The objective of this study was to characterize the gene expression changes in MMT, sham and control groups for each side of the articular cartilage of the tibial plateau, for the osteophyte region, and for each side of the synovium. This study is the first to elucidate localized disease progression in the rat MMT model and in osteophyte and synovial tissues in an OA preclinical model. This study represents a significant step towards the use of this model in therapeutic development and testing. It is important to determine the molecular mechanism of the therapeutic in question, and due to the localized nature of this disease model, it is important to characterize effects of a therapeutic acting on different regions and tissues of the joint. This work provides a baseline in disease-modifying changes in the MMT model.

Method

MMT model

The Georgia Institute of Technology Institutional Animal Care and Use Committee approved all experimental animal procedures (#A14023). 48 male weight-matched (275–300 g) Lewis rats were surgerized: A small incision was made through the skin on the medial aspect of the femoro-tibial joint of the left limb. The medial collateral ligament was exposed by blunt dissection and transected to visualize the joint space and meniscus. In the MMT group, the meniscus was transected, while in the sham group, it was exposed but left intact. MMT and sham surgeries were randomized. The naïve right limbs were used as contralateral baseline controls.

Rats were euthanized at 1 and 3 weeks post-surgery, and tissues were harvested and analyzed in a blind manner. Nine animals were used for gene expression and three for histology per group.

Microarray gene expression

From each leg, three samples of cartilage were collected: the medial tibial plateau, the lateral tibial plateau, and the osteophyte region at the joint margin of the medial side of the tibia [Supplementary Fig. 1(A)]. Osteophyte tissue was only collected from MMT and sham groups due to insufficient tissue in naïve controls. Moreover, two samples of synovial membrane were also collected for all groups, from the medial and lateral sides of the joint, distal of the meniscus and directly adjacent to the tibial plateau. Each sample was stored in RNAlater (Ambion). Total RNA was extracted, quantified, and converted to cDNA (Supplementary Material).

RNA expression was quantified using the real-time PCR Fluidigm Dynamic Array Integrated Fluidic Circuits (Fluidigm), which included 40 genes [TaqMan Gene Expression Assays, Applied Biosystems, Supplementary Fig. 1(B)] that were selected from the human OA literature. Rat universal cDNA (Gene Scientific) and ultrapure water (Thermo) were used as positive and negative controls.

Details about the statistical analysis are described in Supplementary Material. Briefly, Ct values were normalized to housekeeping genes, and outliers were removed by the Quantile Range Outlier test. Principal Component Analysis (PCA) and hierarchical clustering analysis were performed in JMP Genomics V9.0 (SAS Institute, NC). Volcano plots were used to compared two groups for multiple comparisons (JMP Genomics). For each gene expression, two-way ANOVA was conducted with the appropriate *post-hoc* tests (JMP Genomics and GraphPad V5, CA).

Immunohistochemistry

To validate gene expression results, immunofluorescent staining of full-joint histological sections was carried out for collagen type 2 (sc-52658), mmp13 (ab75606), and osteopontin (sc-21742) (Supplementary Material).

Results

Articular cartilage

Tibial plateau

There were at least a half dozen innate significant gene expression differences between the medial and lateral sides of the tibial plateau, in the control group, at both 1 and 3 weeks post-surgery (Supplementary Fig. 2). There were no significant differences in gene expression for the lateral side among surgical groups at any timepoint, indicating that the lateral side is not affected by surgery at 1 week and 3 weeks post-surgery [Supplementary Fig. 3(A)]. Changes in gene expression in the medial side of the tibial plateau were, however, affected by the surgery [Supplementary Fig. 3(B)].

Medial tibial plateau

A clustering analysis showed that the gene expression profiles of control and sham samples were similar enough to be grouped into one cluster, while the MMT samples belonged to a different cluster entirely [Fig. 1(A)]. This was confirmed with a PCA [Fig. 1(B)]. There were no temporal differences within each cluster. Overall, expression of 14 genes was significantly different in the MMT group, when compared to control and sham groups [Fig. 2(A), Supplementary Fig. 4]. There were no significant differences between the control and sham groups.

Chondrocyte hypertrophy genes were differentially regulated beginning at 1 week in the MMT group: *Frzb*, *Grem1*, *Col10a1*. *Frzb* (Frizzle-related protein) and *Grem1* (Gremlin 1) are Wnt-pathway antagonists, and were underexpressed at weeks 1 and 3. The Wnt pathway is associated with articular chondrocyte dedifferentiation[13]. *Col10a1* (type 10 collagen) was also underexpressed in the MMT group, observed at both week 1 and 3.

Gene expression of key cartilage ECM proteins, *Col2a1* (type 2 collagen) and *Acan* (aggrecan), was not significantly different among groups. However, *Fn1* (fibronectin) and *Col1a2* (type 1 collagen) were overexpressed by week 3 in the MMT model.

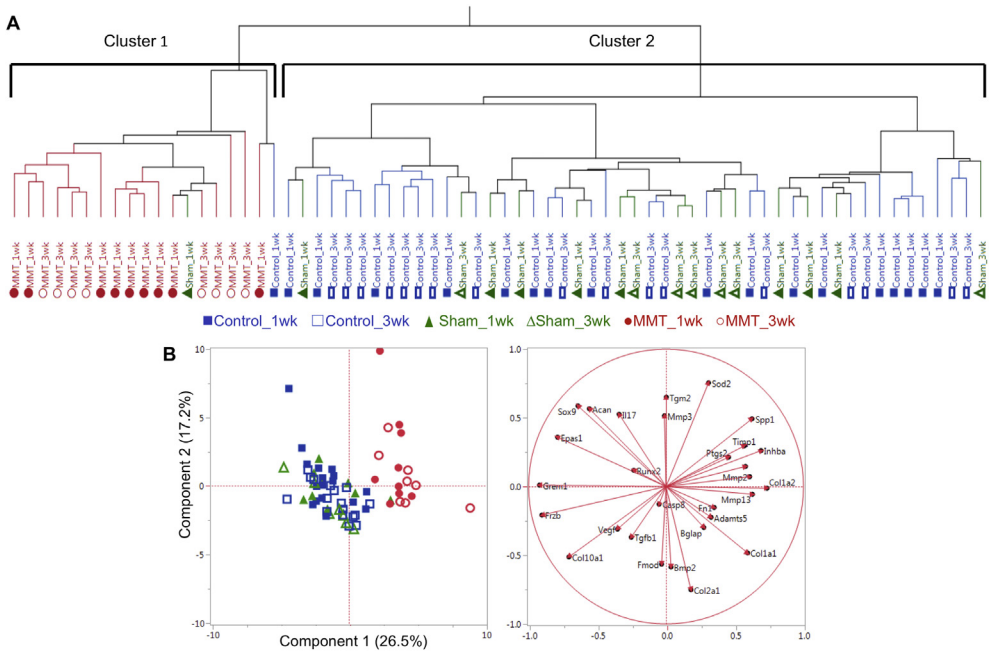

Fig. 1. A. Dendogram shows clustering of groups based on their gene expression profile. Control at week 1 and 3 cluster with sham at week 1 and 3, while MMT forms another group altogether. **B.** PCA plots confirm clustering of control and sham groups away from the MMT group. There are no temporal differences within groups.

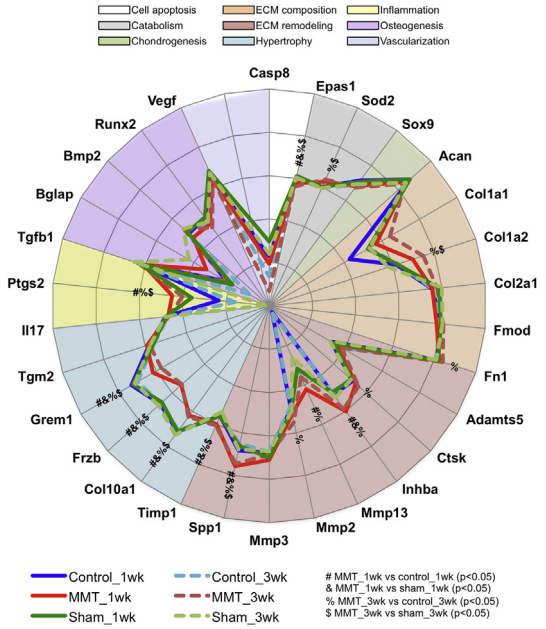

Fig. 2. Radar plot of averaged (mean) ΔCt expression of individual genes in the unoperated, control medial tibial plateau cartilage (control), the MMT medial tibial plateau cartilage (MMT) and the sham medial tibial plateau cartilage (sham), at different timepoints. Statistical comparisons were carried out for each individual gene as described in the methods. # indicates significant ($P < 0.05$) difference between MMT and control at week 1; & between MMT and sham at week 1; % between MMT and control at week 3; and $ between MMT and sham at week 3. There were not statistical differences between timepoints within surgical groups.

Matrix degradation proteins and their inhibitors were also differentially expressed in the MMT group. *Mmp* (metalloproteinase)-13 is considered the primary collagenase in OA, and it was overexpressed at weeks 1 and 3, while *Mmp2* and *Ctsk* (cathepsin k) were elevated at week 3 only. *Timp1* and *Inhba* are inhibitors of these matrix degradation proteins, and they were both overexpressed at weeks 1 and 3. *Timp1* is a metalloproteinase inhibitor which acts on Mmps (relevant to this study: Mmp2, Mmp3, Mmp9, Mmp12, Mmp13). *Inhba* has shown to inhibit aggrecanase-mediated cleavage of aggrecan in human and rat articular cartilage[7].

Spp1 (osteopontin) is a matrix remodeling marker and was overexpressed as early as week 1 in the MMT model. *Spp1* has been implicated in a number of physiological and pathological events, including maintenance or reconfiguration of tissue integrity during inflammatory processes[14].

Known catabolic factors in OA were dysregulated in the MMT model. *Epas1* encodes hypoxia-inducible factor-2alpha (HIF-2α), a known catabolic factor in the osteoarthritic process. In this study, however, we observed an under-expression of *Epas1* at weeks 1 and 3. *Sod2* (superoxide dismutase 2) is also a marker for catabolism in the articular cartilage, and it was overexpressed in the MMT group at weeks 1 and 3.

Lastly, *Ptgs2* (prostaglandin-endoperoxide synthase 2 encoding cyclooxygenase 2 [COX-2]) is an inflammatory response gene[7]. *Ptgs2* was significantly overexpressed in the MMT group at weeks 1 and 3.

Osteophyte region

There was significantly more RNA extracted from tissue collected from the medial joint margin (osteophyte) region in the MMT than the sham at 3 weeks [Fig. 3(B)]. This difference in RNA concentration was not observed for any other tissue, for either groups or timepoints. A PCA showed that, regardless of surgical group, tissue from this osteophyte region was distinctively different from that which had been collected from the articular tibial plateau [Fig. 3(A)]. We observed significant overexpression of osteogenic markers and underexpression of chondrogenic markers (data not shown). Given that the tibial plateau cartilage is dissimilar to the osteophyte tissue, we proceeded with comparisons exclusively within the osteophyte tissue of sham vs MMT at the different

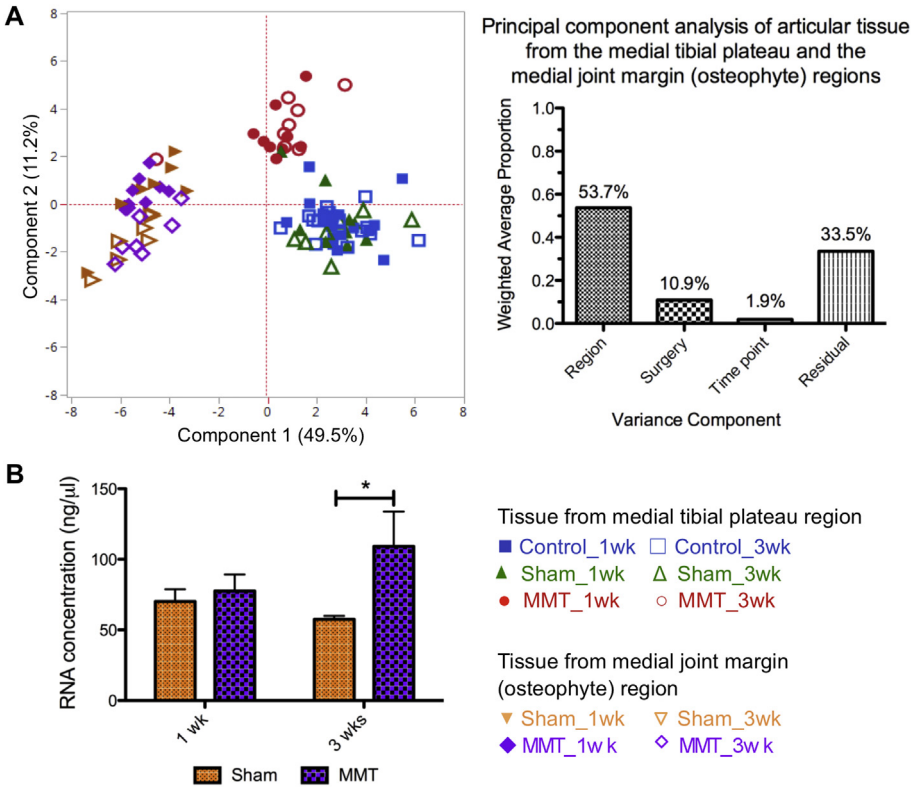

Fig. 3. A. PCA of tissue from the medial tibial plateau region (control, sham and MMT groups) and osteophyte region (sham and MMT groups) for all timepoints. The significant difference in gene expression profiles is mainly due to the region from which tissue was collected (53.7%). Tissue from the osteophyte (sham and MMT) is fundamentally different from the tissue from the articular cartilage of the medial tibial plateau. **B.** There is more tissue (and therefore RNA extracted) from the MMT group at the osteophyte region at week 3. Concentrations from nanodrop are graphed as means ± standard error of the mean (SEM). * indicates significant difference ($P < 0.05$) between the two groups.

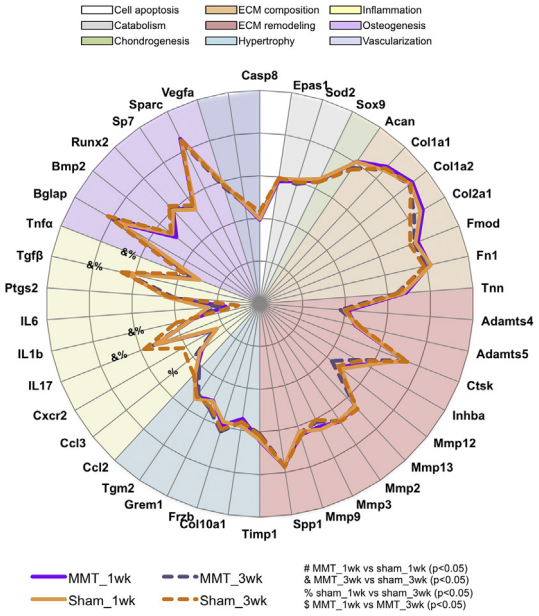

Fig. 4. Radar plot of averaged (mean) ΔCt expression of individual genes in the sham osteophyte tissue (sham) and the MMT osteophyte tissue (MMT), at different timepoints. Statistical comparisons were carried out for each individual gene as described in the methods. # indicates significant ($P < 0.05$) difference between MMT and sham at week 1; & between MMT and sham at week 3; % between week 1 and 3 for the sham group; and $ between week 1 and 3 for the MMT group.

timepoints. We found that five genes were differentially expressed between these two groups [Fig. 4, Supplementary Fig. 5].

The inflammatory genes *IL* (interleukin)-*1b*, *IL17*, *Tgbβ* (transforming growth factor-β) and *Tnfα* (tumor necrosis factor-α) were significantly underexpressed in the MMT group at week 3 compared to the sham. In the sham group, their expression at week 1 was comparable to that of the MMT, but at week 3, these genes were significantly overexpressed compared to the sham at week 1 and MMT at week 3. *Ccl3* (c-c-motif chemokine-3), another gene involved in inflammation, showed a similar trend; however, no differences were observed at week 3.

Synovium

In the control group, medial and lateral sides of the synovium had one significantly differentially expressed gene [Supplementary Fig. 6(A)]. Unlike the cartilage samples, however, there were significant differences in the lateral side samples among surgical procedures (data not shown), though we note that latent unknown variables beyond surgery type, microarray plate number (batch effect), and timepoint contribute to a large residual variance [Supplementary Fig. 6(B)]. On the other hand, on the medial side, changes observed in gene expression among groups are largely driven by the surgery type [Supplementary Fig. 6(C)]. Therefore, we focus only on the differences in the medial side of the synovium.

Medial synovium

Our cluster analysis showed that there were temporal differences in the sham group [Fig. 5(A)]. Sham samples at week 1 cluster with the MMT samples (both timepoints). At week 3, however,

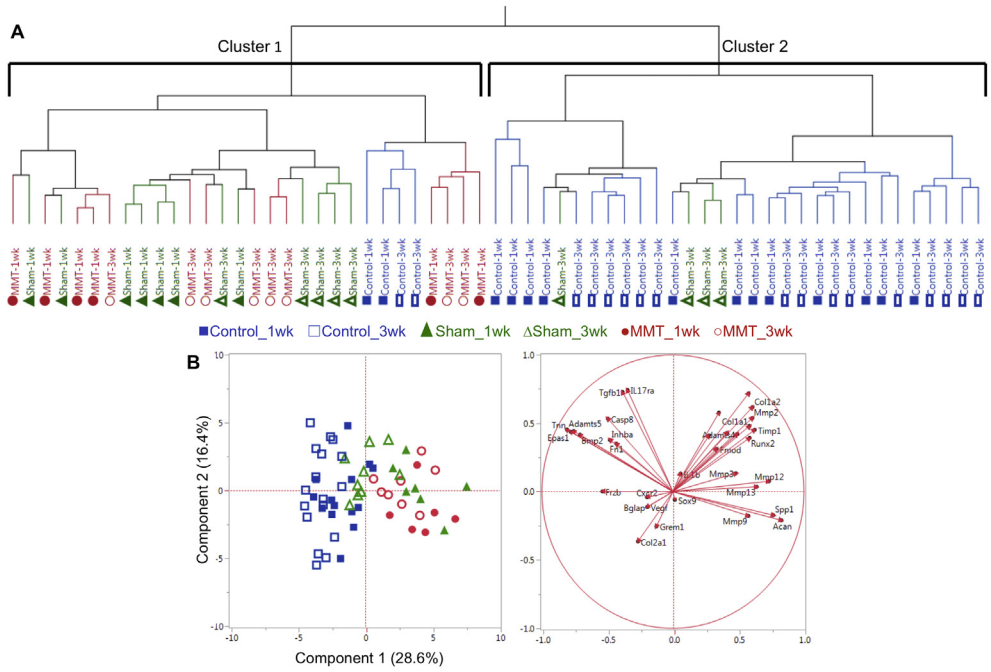

Fig. 5. A. Dendogram shows clustering of groups based on their gene expression profile. Sham at week 1 clusters with MMT at week 1 and 3, while at 3 weeks post-surgery, sham clusters with both MMT and control groups. **B.** PCA confirms this clustering and shows that this difference is largely driven by ECM remodeling genes and osteogenic markers. These results suggest a surgical effect that dominates at week 1.

sham samples are equally split between the control cluster and the MMT cluster. The PCA further confirms this clustering and shows that expression of Mmps is a key driver of principal component 1 [Fig. 5(B)]. There were 16 genes that were differentially expressed among groups [Fig. 6, Supplementary Fig. 7].

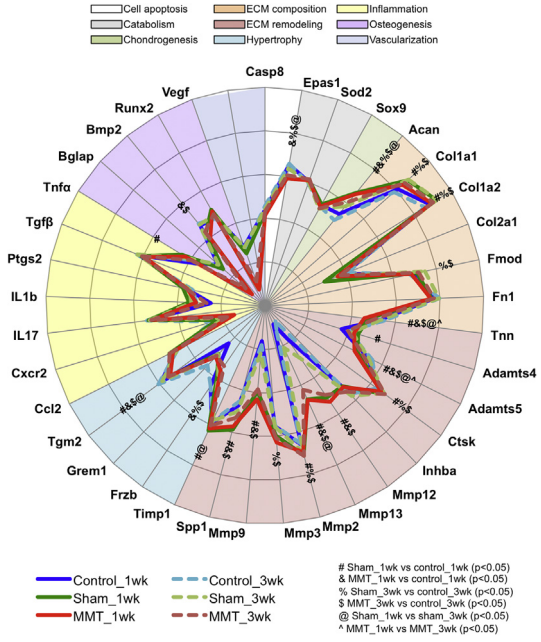

Fig. 6. Radar plot of averaged (mean) ΔCt expression of individual genes in the unoperated, control medial synovial membrane (control), the sham medial synovial membrane (sham) and the MMT medial synovial membrane (MMT), at different timepoints. Statistical comparisons were carried out for each individual gene as described in the methods. # indicates significant ($P < 0.05$) difference between sham and control at week 1; & between MMT and control at week 1; % between sham and control at week 3; $ between MMT and control at week 3; @ between week 1 and 3 within sham group; and ^ between week 1 and 3 within MMT group.

In general, surgery (MMT and sham) had an effect on genes related to the ECM. *Acan* was overexpressed in the surgery groups at week 1 and 3, compared to the control groups. *Fmod* (fibromodulin) was overexpressed in both surgery groups at week 3 only. While *Tnn* (tenascin) was significantly underexpressed at week 1 for the surgery groups, it reached the level of the control group at week 3 for the sham group but remained significantly lower for the MMT group.

Compared to the control group, the synovium had a sustained significantly elevated expression of matrix degradation proteins in the MMT group. *Mmp3*, *Mmp9*, *Mmp12*, and *Mmp13* were significantly higher in the MMT group at week 1 and remained so by week 3. *Mmp2* and *Ctsk* were significantly higher only at week 3 in the MMT group. In the sham surgery, these genes were also elevated at week 1 but most declined to the control level by week 3. *Mmp2*, *Mmp9*, *Mmp12*, *Mmp13*, *Ctsk*, *Adamts4*, *Spp1* and *Timp1* were overexpressed (vs control) at week 1, and all, with the exception of *Mmp2* and *Ctsk*, declined to expression levels comparable to the control group by week 3. *Adamts5* was underexpressed in the MMT group (vs control) at week 1 and 3, while again in the sham group it was significantly lower at week 1 but recovered to the control level by week 3.

Osteogenic markers were also dysregulated in the synovium. *Col1a1* and *Col1a2* were overexpressed in both surgery groups at week 1 (vs control), but remained elevated only in the MMT group at week 3. *Frzb* and *Epas1* were underexpressed in the surgery groups (vs control) as early as week 1, but remained low for the sham as well as the MMT at week 3. *Tgm2* (transglutaminase-2) was underexpressed at week 1 for both surgery groups (vs control), yet the sham group recovered to the control level by week 3. Tgm2 is modulated by inflammatory cytokines, and regulates chondrocyte hypertrophic differentiation and ECM mineralization[15,16].

Protein expression

Immunostaining (Fig. 7) of type 2 collagen showed morphological differences in the medial articular cartilage at week 3,

confirming damage to this area due to MMT surgery. Mmp13 and osteopontin were overexpressed in the synovium and articular cartilage of the tibial plateau at week 3 post-surgery, confirming our gene expression results. We hypothesize that they were not detected at week 1 due to the natural delay between translation and transduction and the need for protein accumulation for immunolocalization.

Discussion

Localized gene expression

Regional analysis of the tibial plateau shows that the lateral and medial sides have a significantly different gene expression profile, which is not surprising given the differences in anatomy between these two sides. While the lateral side of the tibial plateau articular cartilage is not affected by surgery, MMT surgery creates localized damage in the medial tibial plateau[6], and analysis of this specific region allow us to pinpoint changes that might otherwise be lost by the pooling of tissue from both sides of the tibial plateaus and femoral condyles; a standard practice in gene expression studies[7–9]. Our findings are summarized schematically in Fig. 8.

Localized gene expression analysis is particularly important in the synovium, as we showed that the lateral side is affected but these changes have confounding and/or lurking variables. Each side of the synovium is affected differently and has significantly different gene expression profiles; therefore, it is imperative that they are not pooled when analyzed in order to avoid misinterpretation of results.

Articular cartilage of the medial tibial plateau

This study is the first to report gene expression findings specifically for the medial tibial plateau of the MMT model. Here, we showed that the MMT model replicated several pathologies observed in human OA. Our findings also suggest that changes in chondrocyte phenotype may precede changes in ECM in this animal model, indicating that the response to destabilization may be firstly to readdress tissue composition and secondly to remodel.

Chondrocyte phenotype changes begin as early as week 1 in the MMT model. As Wnt antagonists, *Frzb* and *Grem1* function as natural brakes on hypertrophic differentiation of articular cartilage[17]. Therefore, under-expression of *Frzb* and *Grem1* in the MMT group points toward a de-differentiation of chondrocytes into the hypertrophic state. *Col10a1* was also under-expressed in the MMT model. Although *col10a1* is a hypertrophic marker associated with endochondral ossification during growth and fracture healing and might, therefore, be expected to be overexpressed, previous studies have also shown that its gene expression level is actually reduced in the OA disease state in articular cartilage of animal models[18].

While chondrocyte phenotypic changes were discernible at week 1, changes in the ECM composition (elevated *Fn1* and *Col1a2*) were not significant until week 3. Although often considered an earlier sign of OA[19,20], the MMT model did not show significant

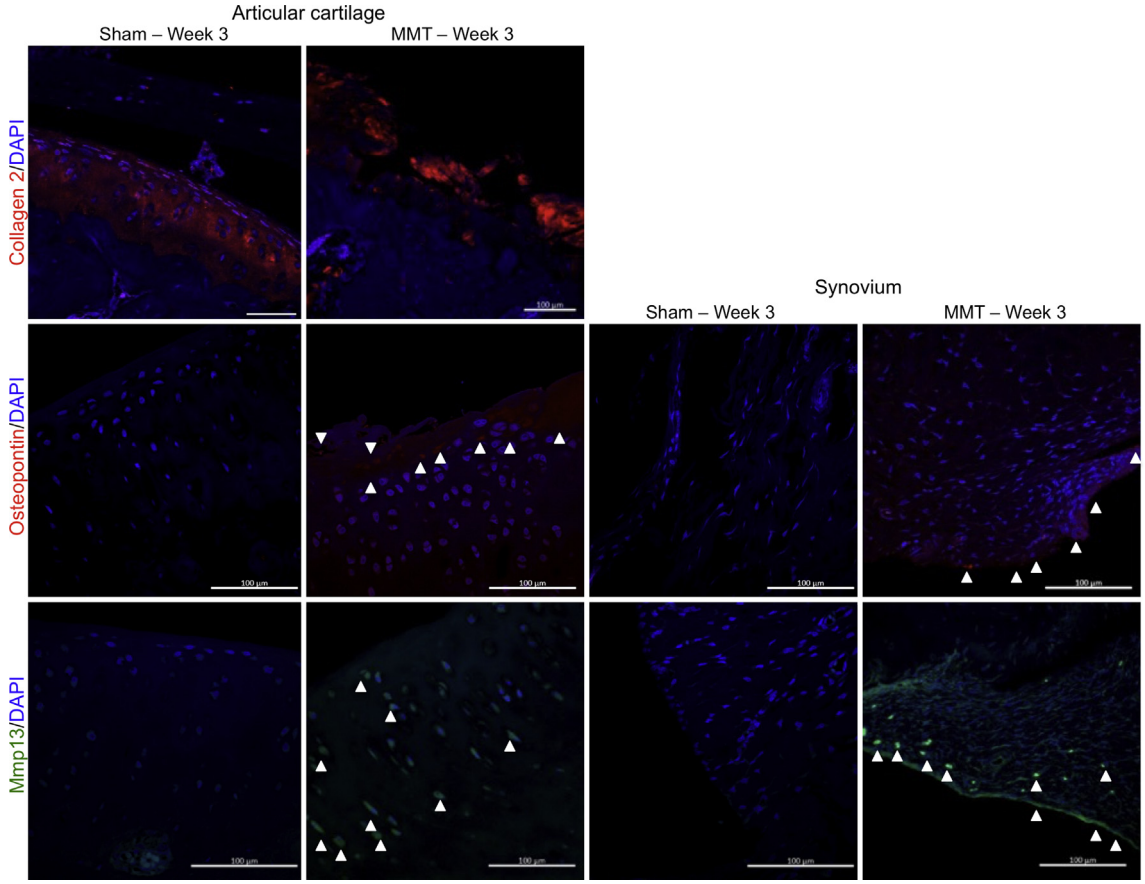

Fig. 7. Fluorescent immunohistochemistry of collagen type 2, Mmp13, and osteopontin of the articular cartilage and the synovium at week 3. Collagen 2 staining was used to visualize the articular cartilage, in which fibrillation and lesion formation is apparent in the MMT group at 3 weeks post-surgery. White triangles point at positive staining of Mmp13 and Osteopontin. At 3 weeks post-surgery, Mmp13 and osteopontin were detected in the medial articular cartilage and synovium of the MMT group and absent in the sham group.

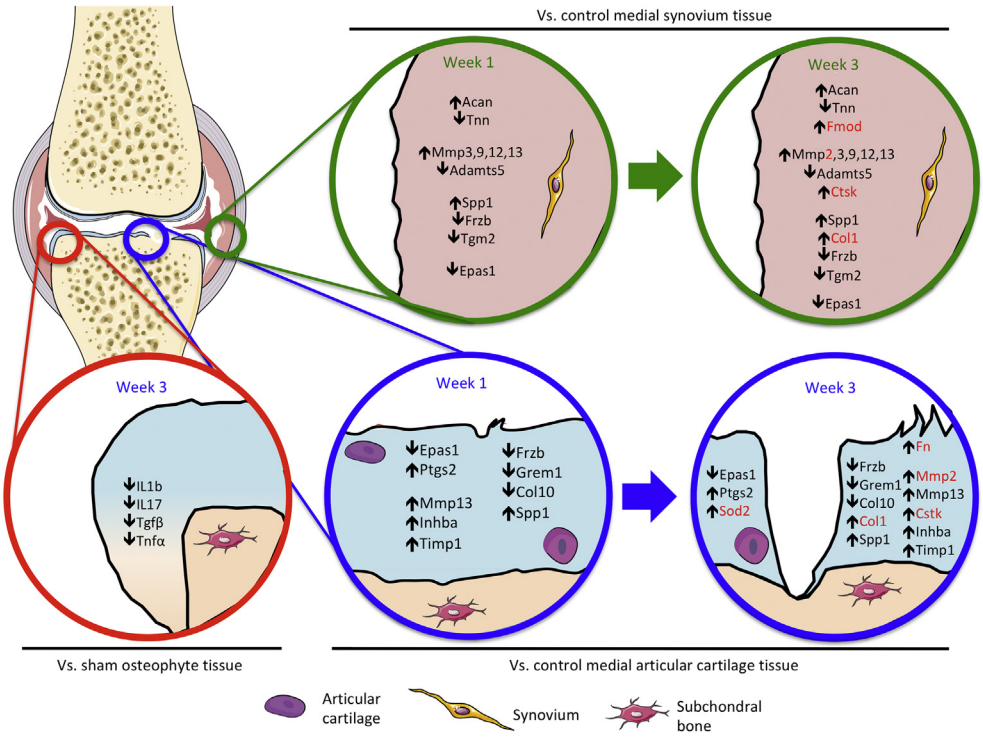

Fig. 8. Summary of localized gene expression results for the MMT surgical group. Blue circles show statistically significant different genes between the MMT and control articular cartilage of the medial tibial plateau, at 1 and 3 weeks post-surgery; green circles between the MMT and control distal medial synovial membrane at 1 and 3 weeks post-surgery; and red circle between the MMT and sham osteophyte tissue at 3 weeks post-surgery. Arrows indicate over- or underexpression of genes. Red indicates differences within MMT group (for the indicated tissue) between 1 and 3 weeks post-surgery.

differences in *Fn1* expression until week 3. This suggests that, in the MMT model, changes in chondrocyte phenotype precede changes in the ECM of the articular cartilage. This is further supported by the fact that *Acan* and *Col2a1* were not yet significantly different from the control group by week 3. In human OA, the exact timing of activation of chondrocytes is not understood[21]. Changes in ECM molecules could act as an initiator, as a consequence of aging, to drive activation of chondrocytes and a change in their phenotype[21–24]. Alternatively, chondrocyte hypertrophy-like changes in OA may be responsible for the active production of cartilage-degrading enzymes, which alter the cartilage ECM composition and structure[25–28].

Overexpression of metalloproteinase and aggrecanase inhibitors *Timp1* and *Inhba* (respectively) in the MMT group as early as week 1 suggests a protective mechanism in the model. This may be further corroborated by the downregulation of *Epas1* in the MMT group. A previous study has shown that chondrocyte-specific knockout of *Epas1* in mice suppressed destabilization of the medial meniscus (DMM)-induced chondrocyte apoptosis and inhibited OA cartilage destruction[29]. We, therefore, propose that downregulation of *Epas1* in this case may be part of a protective mechanism in the MMT model.

Higher expression of the catabolism marker *Sod2* at weeks 1 and 3 indicates a homeostatic imbalance within the chondrocytes. Oxidative stress and dysregulated chondrocyte mitochondrial function contribute not only to impaired matrix synthetic function and viability, but also to molecular inflammatory processes and matrix catabolism in OA[30]. Ptgs2, which we have shown to be overexpressed in the MMT, is an inflammatory responsive gene and is regulated by nitric oxide[31].

Altogether, analysis of the medial tibial plateau articular cartilage shows that sham behaves similarly to the control group and that MMT surgery replicates several human OA pathomechanisms. The pathomechanistic implications outlined in this study should be taken into account when using this model to test and/or explore therapeutic mechanisms. It is important to understand the microenvironment that is encountered by the therapy of interest upon delivery, so that the therapeutic mechanisms are thoroughly investigated and tested properly.

Osteophyte tissue

At the joint margins, osteophytes form in human and experimental OA[32,33]. While osteophytes have been reported and quantified at week 3 in the MMT model[6], they are non-detectable via morphological analysis at week 1. In this study, we analyzed and compared osteophyte tissue from sham and MMT groups at 1 and 3 weeks post-surgery, and we showed that we are able to detect gene expression differences between the sham and MMT groups at 3 weeks only.

It is known that there are morphological differences in the osteophyte region between the MMT and sham groups at 3 weeks post-surgery, including increased mineralization and tissue formation[6]. However, we were not able to detect differences in these markers between the sham and MMT groups. Interestingly, we detected significant differences only in genes related to inflammation. Recent studies have reported evidence of the effects of inflammatory mediators on cellular differentiation[34]. In fact, Tnfα, IL1β, and NF-κB stimulate osteogenesis at low concentrations and inhibit it at high concentrations[34–39]. We, therefore, believe that the observed downregulation of inflammation genes in the MMT group is actually indicative of a pre-ossification phase of this region at week 3.

It is worthwhile mentioning that, while gene expression related to chondrogenic, chondrocyte hypertrophy and osteogenic markers

is not different between the sham and MMT groups at any timepoint, there are signs of cell proliferation in the MMT group at week 3. The tissue from this region of the joint is fundamentally different than the tibial plateau, and so, we postulate that morphological differences between the sham and MMT at 3 weeks post-surgery are due to there simply being more tissue in the MMT model at this timepoint. To test this hypothesis, thorough profiling of markers related to proliferation in this area, as well as extended timepoints of analyses, will be required.

Distal medial synovial membrane

Surgical effect (sham and MMT) dominates the gene expression profile of the medial synovium at week 1, and it is not until week 3 that this surgical noise dissipates and meniscal instability starts to have a pronounced effect on the medial synovium. This is not wholly surprising as the synovium is cut to access and visualize the meniscus in both sham and MMT surgeries. This MMT characteristic is of the upmost importance when considering experimental design of a new therapy, and it reemphasizes the importance of a sham group in a treatment targeting synovium tissue in this model.

Overall, we observed an increase in matrix degradation and remodeling proteins in the synovium that remained elevated in the MMT at week 3. We also showed an overexpression of most osteogenic markers and underexpression of Wnt antagonists in the MMT. These findings suggest a feedback loop with the articular cartilage. As in human OA, the synovium is affected by the mechanical instability of the model and may exacerbate the changes to the articular cartilage. We, therefore, postulate that the joint space acts in concert in the MMT model.

Inflammation in the MMT model

Surprisingly, apart from *Ptgs2*, our genes related to inflammation were not differentially regulated, except in the osteophyte region. We believe that this may have been due to our harvesting timepoint. Collecting tissues at earlier timepoints may have shown a detectable signal difference between unoperated and operated legs. However, for the purpose of this study, we wanted to minimize noise due to surgery. It was, therefore, interesting that we still detected evidence of surgery in the synovium at week 1.

The MMT model is a mechanically induced model and it is generally not associated with substantial inflammation, unlike the MIA model[40]. Nevertheless, it is worth pointing out that even though we do not observe changes in genes generally associated with inflammation at our timepoints, we may be observing the consequences of their dysregulation. In OA, *IL1* (pro-inflammatory) is synthesized by chondrocytes and capable of inducing the expression of *Mmps* and other catabolic genes[41]. A variety of inflammatory mediators and growth factors, including *IL1*, *Tnfα* and *Pdgf* (platelet-derived growth factor), stimulate *Spp1* transcription[14]. In fact, *Spp1* has both pro- and anti-inflammatory actions[14]. We have shown changes in the expression of several *Mmps* and *Spp1* in the articular cartilage and synovium in this study due to MMT surgery. Whether these changes are indeed downstream of inflammatory processes remains to be answered, and evaluating the model at earlier timepoints would be a useful start for future directions.

Study limitations

There are some limitations to this study that should be considered for future work. Namely, gene expression changes were evaluated against a pre-determined array of 40 genes. While a comprehensive literature review was conducted to determine the array, there may be other genes which may be important in disease development in this model that have not been tested. Additionally, only two timepoints were explored in this work, and there may be molecular changes that were not captured by these two snapshots. Despite these limitations, this work provides a baseline for localized molecular changes in different regions of different tissues within the joint space of the rat MMT model of post-traumatic OA.

Study significance

While the MMT model replicates several key aspects of human OA[42–45], this study is the first to elucidate localized molecular events and discuss their relevance to human OA, and the first to characterize the synovium and osteophyte—key tissues involved in OA development. These findings provide a basis to assess whether the MMT model is appropriate to test the therapeutic in question. Additionally, the different gene expression profiles of the different regions of each tissue (e.g., medial vs lateral articular cartilage) emphasize the importance of evaluating local molecular mechanisms during therapeutic testing. It is important to understand how drugs may affect MMT-affected regions (e.g., medial articular cartilage) and non-affected regions (e.g., lateral articular cartilage) in order to fully elucidate their mechanisms of action and potential benefits and pitfalls. Finally, this work highlights the significance of including a sham group in future studies, especially for comparisons with the synovium and osteophyte tissue.

Authors' contributions

Giuliana E. Salazar-Noratto, Nica De Nijs. Hazel Y. Stevens, Greg Gibson, and Robert E. Guldberg designed the study; acquired, analyzed, and interpreted the data; and wrote and approved the submission of this article.

Competing interests

The authors have no conflicts of interest to disclose.

Funding sources

This study was supported by the National Science Foundation Graduate Research Fellowship Program (# DGE-1148903) and a Pediatric Research Alliance seed grant from a research partnership between Children's Healthcare of Atlanta and the Georgia Institute of Technology. The study sponsors had no role in the study design, collection, analysis, or interpretation of data; or in the writing of the manuscript and decision to submit for publication.

Acknowledgments

The authors would like to acknowledge the core facilities at the Parker H. Petit Institute for Bioengineering and Bioscience at the Georgia Institute of Technology for the use of their shared equipment, services and expertise, especially Dalia Afarat for her technical assistance with the Fluidigm BioMark machine in the Genomics core facility.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.joca.2018.10.007.

References

1. Krzeski P, Buckland-Wright C, Bálint G, Cline GA, Stoner K, Lyon R, *et al*. Development of musculoskeletal toxicity without clear benefit after administration of pg-116800, a matrix metalloproteinase inhibitor, to patients with knee osteoarthritis: a randomized, 12-month, double-blind, placebo-controlled study. Arthritis Res Ther 2007;9(5):R109.

2. Thysen S, Luyten FP, Lories RJU. Targets, models and challenges in osteoarthritis research. Dis Model Mech 2015;8(1):17–30.
3. Cohen SB, Proudman S, Kivitz AJ, Burch FX, Donohue JP, Burstein D, *et al*. A randomized, double-blind study of AMG 108 (a fully human monoclonal antibody to il-1r1) in patients with osteoarthritis of the knee. Arthritis Res Ther 2011;13(4):R125.
4. Clutterbuck AL, Asplin KE, Harris P, Allaway D, Mobasheri A. Targeting matrix metalloproteinases in inflammatory conditions. Curr Drug Targets 2009;10(12):1245–54.
5. Catterall JB, Cawston TE. Drugs in development: bisphosphonates and metalloproteinase inhibitors. Arthritis Res Ther 2003;5(1):12–24.
6. Thote T, Lin ASP, Raji Y, Moran S, Stevens HY, Hart M, *et al*. Localized 3d analysis of cartilage composition and morphology in small animal models of joint degeneration. Osteoarthritis Cartilage 2013;21(8):1132–41.
7. Wei T, Kulkarni NH, Zeng QQ, Helvering LM, Lin X, Lawrence F, *et al*. Analysis of early changes in the articular cartilage transcriptisome in the rat meniscal tear model of osteoarthritis: pathway comparisons with the rat anterior cruciate transection model and with human osteoarthritic cartilage. Osteoarthritis Cartilage 2010;18(7):992–1000.
8. Appleton CT, Pitelka V, Henry J, Beier F. Global analyses of gene expression in early experimental osteoarthritis. Arthritis Rheum 2007;56(6):1854–68.
9. Endo J, Sasho T, Akagi R, Muramatsu Y, Watanabe A, Akatsu Y, *et al*. Comparative analysis of gene expression between cartilage and menisci in early-phase osteoarthritis of the knee-an animal model study. J Knee Surg 2018;31(7):664–9.
13. Hwang SG, Ryu JH, Kim IC, Jho EH, Jung HC, Kim K, *et al*. Wnt-7a causes loss of differentiated phenotype and inhibits apoptosis of articular chondrocytes via different mechanisms. J Biol Chem 2004;279(25):26597–604.
14. Denhardt DT, Noda M, O'Regan AW, Pavlin D, Berman JS. Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. J Clin Investig 2001;107(9):1055–61.
15. Cecil DL, Terkeltaub R. Transamidation by transglutaminase 2 transforms s100a11 calgranulin into a procatabolic cytokine for chondrocytes. J Immunol 2008;180(12):8378–85.
16. Yin X, Chen Z, Liu Z, Song C. Tissue transglutaminase (tg2) activity regulates osteoblast differentiation and mineralization in the saos-2 cell line. Braz J Med Biol Res 2012;45(8):693–700.
17. Leijten JC, Emons J, Sticht C, van Gool S, Decker E, Uitterlinden A, *et al*. Gremlin 1, frizzled-related protein, and dkk-1 are key regulators of human articular cartilage homeostasis. Arthritis Rheum 2012;64(10):3302–12.
18. Bluteau G, Conrozier T, Mathieu P, Vignon E, Herbage D, Mallein-Gerin F. Matrix metalloproteinase-1, -3, -13 and aggrecanase-1 and -2 are differentially expressed in experimental osteoarthritis. Biochim Biophys Acta 2001:147–58.
19. Chevalier X. Fibronectin, cartilage, and osteoarthritis. Semin Arthritis Rheum 1993;22(5):307–18.
20. Lust G, Burton-Wurster N, Leipold H. Fibronectin as a marker for osteoarthritis. J Rheumatol 1987;14 Spec No:28–9.
21. van der Kraan PM, van den Berg WB. Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration? Osteoarthritis Cartilage 2012;20(3):223–32.
22. Goldring MB, Otero M, Plumb DA, Dragomir C, Favero M, El Hachem K, *et al*. Roles of inflammatory and anabolic cytokines in cartilage metabolism: signals and multiple effectors converge upon mmp-13 regulation in osteoarthritis. Eur Cell Mater 2011;21:202–20.
23. Marcu KB, Otero M, Olivotto E, Borzi RM, Goldring MB. Nf-kappab signaling: multiple angles to target oa. Curr Drug Targets 2010;11(5):599–613.
24. Loeser RF. Age-related changes in the musculoskeletal system and the development of osteoarthritis. Clin Geriatr Med 2010;26(3):371–86.
25. Shlopov BV, Gumanovskaya ML, Hasty KA. Autocrine regulation of collagenase 3 (matrix metalloproteinase 13) during osteoarthritis. Arthritis Rheum 2000;43(1):195–205.
26. Wang X, Manner PA, Horner A, Shum L, Tuan RS, Nuckolls GH. Regulation of mmp-13 expression by runx2 and fgf2 in osteoarthritic cartilage. Osteoarthritis Cartilage 2004;12(12):963–73.
27. Song RH, Tortorella MD, Malfait AM, Alston JT, Yang Z, Arner EC, *et al*. Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5. Arthritis Rheum 2007;56(2):575–85.
28. Plaas A, Osborn B, Yoshihara Y, Bai Y, Bloom T, Nelson F, *et al*. Aggrecanolysis in human osteoarthritis: confocal localization and biochemical characterization of ADAMTS5-hyaluronan complexes in articular cartilages. Osteoarthritis Cartilage 2007;15(7):719–34.
29. Ryu JH, Shin Y, Huh YH, Yang S, Chun CH, Chun JS. Hypoxia-inducible factor-2alpha regulates fas-mediated chondrocyte apoptosis during osteoarthritic cartilage destruction. Cell Death Differ 2012;19(3):440–50.
30. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis. Nat Rev Rheumatol 2015;11(1):35–44.
31. Yang S, Kim J, Ryu JH, Oh H, Chun CH, Kim BJ, *et al*. Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. Nat Med 2010;16(6):687–93.
32. Xu Z, Chen T, Luo J, Ding S, Gao S, Zhang J. Cartilaginous metabolomic study reveals potential mechanisms of osteophyte formation in osteoarthritis. J Proteome Res 2017;16(4):1425–35.
33. Hashimoto S, Creighton-Achermann L, Takahashi K, Amiel D, Coutts RD, Lotz M. Development and regulation of osteophyte formation during experimental osteoarthritis. Osteoarthritis Cartilage 2002;10(3):180–7.
34. Jenner F, Jpma A I, Cleary M, Heijsman D, Narcisi R, van der Spek PJ, *et al*. Differential gene expression of the intermediate and outer interzone layers of developing articular cartilage in murine embryos. Stem Cells Dev 2014;23(16):1883–98.
35. Cho HH, Shin KK, Kim YJ, Song JS, Kim JM, Bae YC, *et al*. Nf-kappab activation stimulates osteogenic differentiation of mesenchymal stem cells derived from human adipose tissue by increasing taz expression. J Cell Physiol 2010;223(1):168–77.
36. Hess K, Ushmorov A, Fiedler J, Brenner RE, Wirth T. TNFalpha promotes osteogenic differentiation of human mesenchymal stem cells by triggering the NF-kappaB signaling pathway. Bone 2009;45(2):367–76.
37. Nam D, Mau E, Wang Y, Wright D, Silkstone D, Whetstone H, *et al*. T-lymphocytes enable osteoblast maturation via IL-17F during the early phase of fracture repair. PLoS One 2012;7(6):e40044.
38. Sonomoto K, Yamaoka K, Oshita K, Fukuyo S, Zhang X, Nakano K, *et al*. Interleukin-1beta induces differentiation of human mesenchymal stem cells into osteoblasts via the wnt-5a/receptor tyrosine kinase-like orphan receptor 2 pathway. Arthritis Rheum 2012;64(10):3355–63.
39. Yeh LC, Zavala MC, Lee JC. Osteogenic protein-1 and interleukin-6 with its soluble receptor synergistically stimulate rat osteoblastic cell differentiation. J Cell Physiol 2002;190(3):322–31.

40. Teeple E, Jay GD, Elsaid KA, Fleming BC. Animal models of osteoarthritis: challenges of model selection and analysis. AAPS J 2013;15(2):438–46.
41. Goldring MB, Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol 2011;23(5):471–8.
42. Baragi VM, Becher G, Bendele AM, Biesinger R, Bluhm H, Boer J, *et al.* A new class of potent matrix metalloproteinase 13 inhibitors for potential treatment of osteoarthritis: evidence of histologic and clinical efficacy without musculoskeletal toxicity in rat models. Arthritis Rheum 2009;60(7):2008–18.
43. Janusz MJ, Bendele AM, Brown KK, Taiwo YO, Hsieh L, Heitmeyer SA. Induction of osteoarthritis in the rat by surgical tear of the meniscus: inhibition of joint damage by a matrix metalloproteinase inhibitor. Osteoarthritis Cartilage 2002;10(10):785–91.
44. Moore EE, Bendele AM, Thompson DL, Littau A, Waggie KS, Reardon B, *et al.* Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. Osteoarthritis Cartilage 2005;13(7):623–31.
45. Willett NJ, Thote T, Lin AS, Moran S, Raji Y, Sridaran S, *et al.* Intra-articular injection of micronized dehydrated human amnion/chorion membrane attenuates osteoarthritis development. Arthritis Res Ther 2014;16(1):R47.