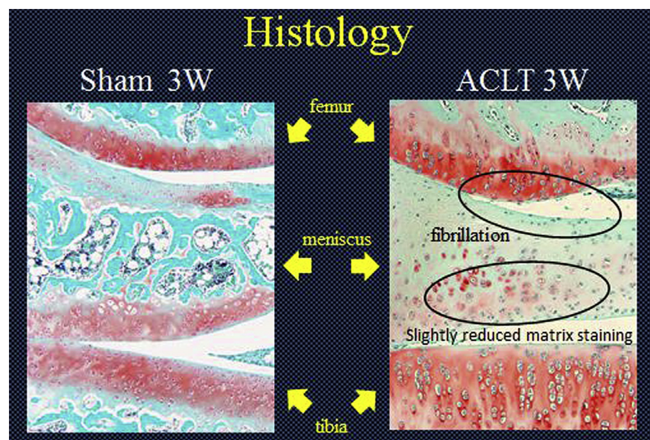
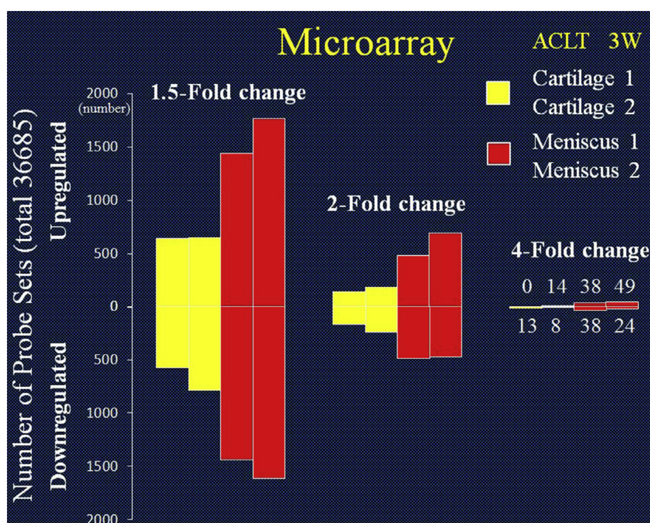
We have taken advantage of ENCODE consortium which provides information about tissue-specific histone modifications, particularly enrichment of mono-methylation of histone H3 lysine 4 (H3K4me1) and acetylation of histone H3 lysine 27 (H3K27Ac) associated with active enhancer regions to explore the presence of novel enhancers in the Acan gene. We selected 8 cis acting sequences including the previously described enhancer at -11kb (Han and Lefebvre, 2008), based on evolutionarily conserved sequences and chromatin modification.
Results: We cloned all eight isolated regions upstream of the silent hsp68 minimal promoter and the lacZ reporter gene, We show that four of these sequences express in cartilage in transgenic mice at E15.5. Three of these are new enhancers that are comparable in their temporal and spatial expression to that already described enhancer at -11kb. Moreover, we show that these sequences can differentiate between chondrocytes and other aggrecan expressing cells.
Some of these enhancers contain potential binding sites for Sox9, consistent with its described role as a regulator of Acan expression.
Conclusions: We have made several lines of several nocel enhancers in the Acan gene and we are investigating their role in maintenance of adult cartilage. The requirement for multiple enhancers are discussed and potential use of these sequences to target specific tissues is being investigated.

234
COMPARATIVE ANALYSIS OF GENE EXPRESSION BETWEEN CARTILAGE AND MENISCI IN EARLY-PHASE EXPERIMENTAL OSTEOARTHRITIS

J. Endo, Sr., S. Takahisa, A. Watanabe, S. Yamaguchi, R. Akagi, Y. Muramatsu, S. Mukoyama, Y. Akatsu, J. Katsuragi, T. Fukawa, Y. Yamamoto, H. Hoshi, K. Takahashi. *Chiba Univ., Chiba, Japan*

Purpose: Pathogenesis of knee osteoarthritis (OA) is still largely unknown but secondary OA gaining much interest in terms of pathogenesis and intervention. Primal initial trauma such as ligament tear, resulting in joint instability, unnatural articulation, causes eventual development of histopathologic abnormality as secondary OA. Some researchers stressed the importance of meniscal degeneration in OA development. Whether the association between meniscal and cartilage degeneration in OA is purely due to the mechanical property of the meniscus, or whether meniscal cells secrete enzymes and inflammatory mediators that contribute to degeneration of other joint tissues, are unclear. It would not be too much to say the integrity of the meniscus is vital in maintaining healthy articular cartilage and prevention of degenerative changes in the knee. Despite the clear link between meniscal deterioration and the development of OA, there have been relatively few studies investigating the molecular mechanisms of meniscal degeneration compared with cartilage. In this study, we analyzed the gene expression using microarray technique for the purpose of researching OA pathogenesis. We focus on evaluating molecular changes in very early phase of OA in ACLT in rats in order to further our understanding of the relationship between cartilage and meniscal degradation.
Methods: OA was surgically induced in 10 week old male Wister rats by ACLT surgery. Articular cartilage from the femoral condyles and bilateral menisci were separately dissected of 6 ACLT and 6 sham operated rats. Each specimen composed of 3 knees was pooled for averaging and yielding enough RNA for subsequent analyses. Consequently, total of 8 RNA samples (2 ACLT and 2 sham femoral cartilage, 2 ACLT and 2 sham medial menisci) were extracted. Affymetrix® 3'GeneChips arrays were used to assess genome-wide changes in gene expression. Probe sets were filtered using the criterion of a minimum 1.5-fold change in differential gene expression between ACLT operated and sham operated in both cartilage and meniscus, respectively. Subsequently, the genes over 1.5-fond change were analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) for cluster analysis (http://david.abcc.ncifcrf.gov/). Five rats in each group were underwent the same surgical protocol and sacrigficed 3 weeks after surgery for histological and immunohistochemical analysis.
Results: Histologically, 3 weeks after ACLT operated rats showed slight fibrillation in bilateral surface of cartilage and menisci. The proteoglycan content appeared almost normal in ACLT operated rats since no loss of Safranin O staining was observed comparing to sham operated rats in both of cartilage and menisci. Of the 36,685 transcripts detectable by microarray, 641 (1.74%) and 651 (1.77%) transcripts in ACLT cartilage, 1439 (3.92%) and 1773 (4.83%) transcripts in ACLT menisci were up-regulated by more than 1.5 fold change comparing to sham operated, respectively. Cluster analysis using DAVID showed genes related to OA were found in ACLT menisci such as response to stimulus, angiogenesis, and apoptosis. Representative proteases including Adamts1,2,3,4, Mmp1,2,12,13,14,19, and extra cellular matrix genes including Versican(Vcan), lumican (Lum), syndecan 1 (Sdc1), and prostaglandin endoperoxide synthase2 (Ptgs2) were up-regulated in menisci but were not up-regulated in cartilage. Expression of type II collagen (Col2a1) was not affected in both cartilage and menisci in ACLT operated rats as well as sham operated rats. Immunohistochemical analysis revealed that MMP-13, Adamts-4 and type II collagen-related neoepitope (C2C) were present in bilateral menisci of ACLT operated rats, however no staining was found in cartilage.
Conclusions: We evaluated molecular changes in very early phase of OA by microarray analysis in terms of the relationship between cartilage and meniscal degradation in surgically-induced OA models in rats. It has been unclear in the previous studies that meniscal and cartilage degeneration in OA is purely due to the mechanical property of the menisci, or cellular reaction of meniscal cells where secretion of enzymes or inflammatory mediators that contributes to degeneration of other joint tissues. Our results indicated that molecular changes occurred in menisci preceded those occurred in cartilage in very early phase of surgically-induced OA models. And this suggested the possibility of interventions targeting menisci in early phase of OA might be a new strategy to prevent OA development.





Cluster analysis (1.5-Fold change)

The Database for Annotation, Visualization and Integrated Discovery (*DAVID*)

Cartilage	E	N	Meniscus	E	N
1 Sensory perception	5.36	22	1 ECM components	5.65	81
2 Growth factor	1.73	5	2 Ligand activity	5.18	26
3 Cell signal	1.68	12	3 Ossification	4.31	13
4 Peptidase activity	1.1	5	4 Response to stimulus	4.22	39
5 Cell cycle	0.89	7	5 Angiogenesis	3.98	20
6 Cell proliferation	0.82	3	6 Osteoblast	2.79	7
7 ligand activity	0.75	6	7 Apoptosis	2.61	40

E: Enrichment score
N: Number

Fold changes of previously implicated in OA-related genes, in ACLT operated cartilage and meniscus compared with sham-operated samples.

MMPs

	Cartilage	Meniscus
Mmp 2	0.9	1.5
Mmp 3	1	1.3
Mmp 7	1	0.9
Mmp 8	1.1	0.6
Mmp 9	0.9	0.4
Mmp 10	0.8	1.4
Mmp 11	0.9	1.4
MMP 12	1.4	3.5
Mmp 13	0.9	1.9
Mmp 16	0.9	1.7
Mmp 14	0.8	1.5
Mmp 15	1.1	1.3
Mmp 16	0.9	1.4

Adamts

	Cartilage	Meniscus
Adamts1	1.2	2.2
Adamts2	0.9	1.5
Adamts3	1	0.8
Adamts4	0.8	1.7
Adamts5	0.9	0.7
Adamts9	0.8	0.9
Adamts12	1.1	1.2
Adamts14	0.9	1.1
Adamts18	0.6	0.8
Adamts19	1.1	0.8

Others

	Cartilage	Meniscus
Vcan	1.1	2.4
Sdc1	0.9	1.5
Lum	0.9	1.5
Timp2	1	1.3
Ptgs2	0.6	1.9
Ptgs1	0.9	0.8
Timp4	0.8	0.6
Timp3	1.1	1.1
Ptges	0.9	1.3

235
STRAIN DIVERSITY OF ARTICULAR CARTILAGE DEGENERATION INDUCED BY OVARIECTOMY AND FORCED RUNNING IN MICE

J. Yamada †, K. Tsuji ‡, K. Miyatake †, Y. Matsukura †, A. Kahaer †, I. Sekiya §, T. Muneta †. † *Dept. of joint surgery and sports medicine Tokyo Med.&dental Univ., Tokyo, Japan;* ‡ *Dept. of Cartilage Regeneration Tokyo Med.&dental Univ., Tokyo, Japan;* § *Ctr. for stem Cell and Regenerative Med. Tokyo Med.&dental Univ., Tokyo, Japan*

Purpose: Generally, osteoarthritis (OA) is a multifactorial disease with factors such as humoral factors, mechanical stress and racial disparities. In mice, it is a possibility that the different susceptibility to osteoarthritis due to differences in strains. We previously reported a non-invasive experimental OA model, in which we showed that synergistic effects of extensive treadmill exercise and ovariectomy (OVX) on articular cartilage degeneration in Balb/c mice(refer Miyatake OARSI2012). Histological and immunohistochemical analysis indicated that exercise and OVX synergistically induced synovitis. In this study, to examine the effect of racial and ethnic disparities on articular cartilage homeostasis in mice, we employed the same experimental protocol and examined articular cartilage degeneration in C57BL/6J mice. We investigated the differences between the two groups

Methods: We employed the same protocol with previously reported. Briefly, 28 female C57BL/6J mice (8 weeks) were randomly divided into OVX or SHAM groups. Moreover we divided them into Cage or Run groups. Run groups were subjected to treadmill exercise for 6 weeks for :60km. While Cage groups were left in cage ad libitum. After 6 weeks, both left and right knee joints were harvested. Left knee joints were sliced into 5μm-serial sagittal sections for histology and immunohistochemistry. To evaluate the loss of proteoglycan from articular cartilage in safranin-O stain, we developed modified Mankin scoring system. To further compare the severity of articular cartilage damage between the four groups, we analyzed type II collagen expression in articular cartilage. H&E slides were used to evaluate synovial activation by scoring thickening of the synovial lining and cellular influx into joint cavity and synovium. Further we evaluated macrophage infiltration in F4/80 stain. Right knee joints were subjected for μCT analyses. Both metaphysial and epiphyseal bone volume per tissue volume (BV/TV) were calculated using Tri/3D-BONE software. We compared the results of Balb/c and C57BL/6J mice.

Results: Safranin-O dyeability of articular surface was quite similar between the 4 groups (Fig.1a). Modified Mankin score indicated no significant difference between the 4 groups (Fig.1b). To further compare the severity of articular cartilage damage, we analyzed type II collagen expression in articular cartilage (Fig.1c). As shown in the figure, type II collagen expression in both femoral and tibial articular cartilage was comparable between the 4 groups. Fig.2a shows H/E staining of sagittal sections of the knee joint. The synovium became thickened and a density of cells was high in SHAM+Run, OVX+Cage and OVX+Run groups. The synovitis score of SHAM+Run, OVX+Cage and OVX+Run groups were significantly higher than that of SHAM+Cage group in Fig.2b. As shown in Fig.2a, F4/80 immunostaining of the joint tissue revealed that synovial hyperplasia and macrophage infiltration into synovial membrane were significantly enhanced in the SHAM+Run and OVX+Run groups(black arrows). Micro CT pictures showed no apparent alteration in the bone shape and osteophyte formation between the four experimental groups (Fig.3a). In parallel with previous reports, we observed significant reduction in BV/TV in OVX+Cage group in epiphysis (Fig.3b). Forced running for 6 weeks partially reversed BV/TV in the OVX mice (Fig.3b,3c).In this study, the effects of exercise and OVX on bone homeostasis were on the same line between the two mice lines.But we showed no synergistic effects on extensive treadmill exercise and OVX on articular cartilage degeneration in C57BL/6J mice. We investigated the differences between the Balb and C57 mice. Firstly, we focused on the difference of bone parameters. The BV/TV of Balb mice was higher 1.27-fold as compared to that of C57 mice in metaphysis as with previous reports. Moreover the subchondral bone plate of Balb mice was significantly higher 1.04-fold as compared to that of C57 mice in only OVX+Run group. The difference in bone parameters of both mice may affect some on the phenotype of articular cartilage. Secondary, we noted the influence of the difference of inflammatory response. The synovitis score of each group was higher in Balb mice about 1.07 ~ 1.90 fold as compared to that of C57 mice. Further the F4/80 score of OVX-Run group was higher in Balb mice 2.14-fold as compared with C57 mice. The different sensitivity in inflammatory response in synovial tissues may have some impacts on articular cartilage.

Conclusions: Our experimental model suggests that cartilage metabolism is partially dependent on the strain differences.

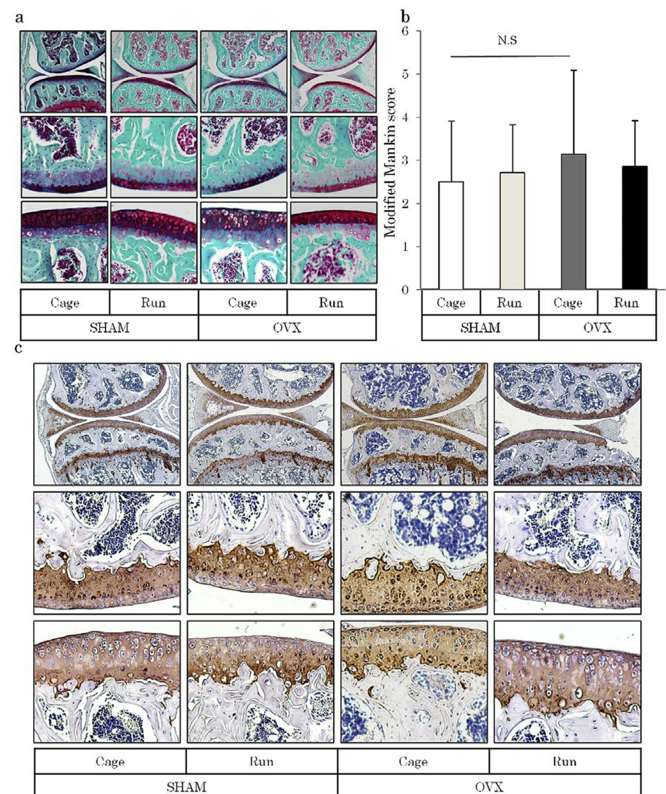


Fig. 1.