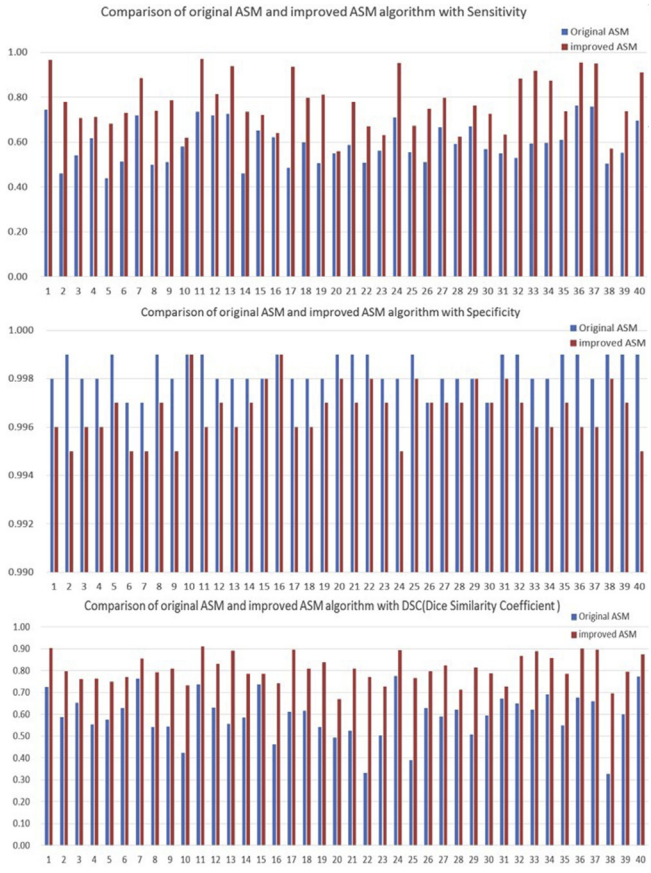

Figure 3. Comparison of ASM and proposed method.

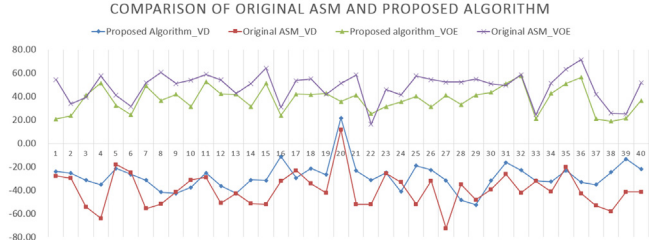

Figure 4. Comparison of ASM and proposed method using VOE and VD.

Conclusions: This study proposed a fully automated segmentation algorithm based on ASM that combines the 3D shape context to obtain a better knee cartilage extraction, and its performance was compared with the original ASM. For 3D shape context, a block approach was applied to adapt to bone shape construction. This consideration provides faster convergence, better matching and produces a better bone mean shape which influences the results of bone segmentation. Profile based BCI extraction method is applied to extract BCI. By extracting profiles and a threshold value which was obtained from experiments, the proposed approach achieves to be more efficient than those which only consider neighbor intensity.

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IDENTIFICATION OF A NOVEL TARGET, FOXA2, IN THE ONSET AND DEVELOPMENT OF OSTEOARTHRITIS

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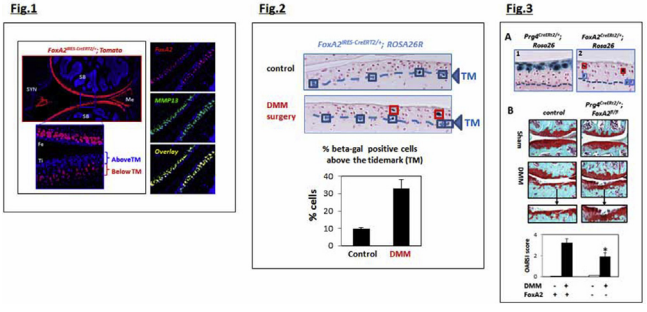
Purpose: The proposed studies are anticipated to establish whether FoxA2 is a potential target for the treatment of OA, and also to provide insights into mechanisms underlying OA initiation.
Methods: described in Results.

Results: In OA, irreversible degradation of the "permanent" articular cartilage by MMP13 is a key pathological event. Since I found that FoxA factors control MMP13 expression in skeletal development, it seemed possible they would to do the same in OA. Thus, we evaluated the expression of FoxA2 in both healthy and OA cartilage using FoxA2IRES-CreERT2/+ Tomato reporter mice. In healthy articular cartilage, the majority of cells positive for FoxA2 expression (red) are located on the tidemark (TM) or below, in the calcified zone of the articular cartilage (Fig.1). We also looked for co-localization of FoxA2 and MMP13 in articular cartilage by performing immunofluorescence for MMP13 on sections from FoxA2IRES-CreERT2/+;Tomato mice and found that FoxA2 positive cells (red) and MMP13 positive cells (green) overlap in the hypertrophic zone (overlay yellow) (Fig.1).
Next, we looked at expression of FoxA2 in OA articular cartilage using the Destabilization of the Medial Meniscus (DMM) murine model of OA. We performed DMM surgery on FoxA2IRES-CreERT2/+; Rosa26LacZ reporter mice, injected the mice with tamoxifen, and stained the knee joints for beta-galactosidase activity. We found that the number of LacZ FoxA2-positive cells located ABOVE the tidemark in the operated knee articular cartilage is 3.4-fold higher than the number of FoxA2-positive cells observed above the tidemark in the control knee (Fig.2). This suggests that induced expression of FoxA2 above the tidemark correlates with the localization of induced MMP13 expression in this murine model of OA.
Since FoxA2 expression is induced in murine OA cartilage, we next asked whether FoxA2 is necessary for cartilage degradation, and examined whether the loss of FoxA2 in superficial articular cartilage would alter the progression of the disease in the DMM surgical model. To delete FoxA2 specifically in the chondrocyte population above the tidemark, we employed a mouse line that contains a tamoxifen-inducible CRE recombinase (CreERT2) driven by the Prg4 (Lubricin) regulatory sequences. Tamoxifen treatment of Prg4GFPCreERt2/+; Rosa26LacZ mice induces recombination at the Rosa26lacZ locus and labels the cells in the top 3 cell layers of the articular cartilage (Fig.3A). Using this line, we specifically removed FoxA2 from the superficial cells of articular cartilage by injecting 6-week old Prg4CreERt2-GFP;FoxA2fl/fl mice with either tamoxifen or corn oil followed by DMM or sham surgery. At 16 weeks post surgery, knee joints were processed for Safranin O/Fast green staining and scored for cartilage degradation using the OARSI histological scale. While control (corn oil treated) Prg4+/CreERt2-GFP; FoxA2fl/fl mice (with intact FoxA2) displayed significant cartilage destruction following DMM surgery (OARSI score of 3.2), tamoxifen treated Prg4+/CreERt2-GFP; FoxA2fl/fl; mice had considerably less (42%) cartilage destruction (OARSI score of 1.9) (Fig.3B). These findings demonstrate that loss of FoxA2 in articular cartilage can slow the progression of cartilage destruction following surgically induced joint destabilization.
I next asked whether overexpression of FoxA2 in murine articular cartilage cells is sufficient to accelerate cartilage degradation. To drive exogenous FoxA2 expression in articular chondrocytes, I have generated mice containing the FoxA2 transgene driven by a reiterated reverse tetracycline transactivator (rtTA) response element (TREtight-FoxA2). I performed surgical destabilization of the medial meniscus on either the triple transgenic Prg4CRE;rtTA;TgFoxA2 mice or their control Prg4CRE;rtTA littermates both treated with Doxycycline. Control mice, lacking the FoxA2 transgene, that have undergone surgery developed mild symptoms of the disease (OARSI score 1.37±0.33). In contrast, triple transgenic mice that have undergone surgery developed far more



cartilage damage with more lesions on the articular cartilage (OARSI score 2.63±0.43).
Conclusions: We have found that conditional deletion of FoxA2 in a murine model of osteoarthritis prevents disease progression, while overexpression of FoxA2 accelerates progression of the disease. Taken together these preliminary results suggest that inhibiting FoxA factors could represent a potential therapeutic intervention to ameliorate the progression of OA.

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CHARACTERIZATION OF RAT OSTEOARTHRITIS MODEL: CORRELATION BETWEEN WEIGHT BEARING, SERUM BIOMARKERS, IMAGING AND HISTOLOGY

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Purpose: The objectives of this work were to assess the pathophysiology of articular cartilage and subchondral bone in the rat medial meniscal tear model (MMT) of osteoarthritis and to evaluate the application of various endpoints and assessment techniques. Standard methods, including histology and serum biomarkers were compared and contrasted with weight bearing and microCT assessment to determine the diagnostic and prognostic value of such endpoints for in studies designed to evaluate efficacy and safety of anti-arthritis medicines.
Methods: MMT surgery was performed on male Lewis rats were to induce OA. Biomarkers of bone and cartilage metabolism (Osteocalcin, P1NP, CTX I, TRAP5b, CTX-II) were evaluated in the serum. A Dynamic Weight Bearing (DWB) system was utilized to measure functional capacity of the musculoskeletal system and 3-point bending method was used to assess femur strength. Micro-CT (mCT) and contrast (EPIC) mCT were used to evaluate bone geometry and articular cartilage morphology. Fluorescent labels (calcein and alizarin red-S) were used to assess bone remodeling by histomorphometry in undemineralized bone sections. Articular cartilage and bone morphology was evaluated using histology (H&E, Toluene blue, Safranin-O, Cathepsin -K).
Results: DWB data showed distinctive difference in weight bearing capacity between control and OA rats and provided the substrate for bone loss evidenced by micro-CT in the tibia of an operated hind limb. Contrast enhanced 3D mCT data clearly demonstrated degradation of articular cartilage covering the medial tibial plateau in MMT rats, and these data correlated extremely well with cartilage histology by traditional paraffin histology. Dynamic bone histomorphometry showed increased subchondral bone formation beneath the damaged cartilage in the tibias of MMT rats, as well as, active osteophyte formation at the medial aspect of the tibial plateau. Serum biomarkers of bone and cartilage metabolism correlated well with mCT and histology.
Conclusions: DWB data indicate that the joint damage is partially dependent upon weight bearing capacity of injured limb, while mCT data and dynamic histomorphometry clearly demonstrate that different bone compartments within the same limb react differently to MMT surgery; bone loss at tibial metaphysis and bone formation of the subchondral bone at tibial epiphysis. Contrast enhanced EPIC-mCT imaging provides a reproducible method to assess 3D distribution of GAGs in the articular cartilage of laboratory rats. Contrast imaging data are complementary to histological findings and can be utilized to guide histological assessment. Serum biomarkers along with imaging have abundant translational value when preclinical data are extrapolated to the clinical milieu.

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MEDIAL CARTILAGE LESIONS DETECTED BY 3.0-TESLA MRI ARE THE RISK FACTOR FOR THE PROGRESSION OF EARLY-STAGE MEDIAL KNEE OSTEOARTHRITIS

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Purpose: It is currently impossible to prevent the progression of the knee osteoarthritis (OA). OA has been believed that cartilage is the primary and most important source of lesions that lead to the disease. However, in addition to cartilage, the importance of all parts of the joint, including the bone, meniscus, ligaments, muscle, and synovium and the crucial role of inflammation in these parts that were previously considered to be irrelevant in this condition are now recognized to be important for OA. The symptoms, especially pain, are also known to be one of the risk factor for the progression of OA. It is impossible to detect the OA-related early changes of the cartilage using radiograph. A magnetic resonance imaging (MRI) is currently underway to facilitate the understanding the pathophysiology of the early stage of knee OA, as it can detect not only bone but also other structural changes occurred in OA. In this study, we examined the factors in patients with early-stage knee OA among the radiographic- and MRI-detected structural changes of the knee joint and the clinical symptoms which were associated with the radiographic OA progression after three years of follow up.
Methods: Among the one-hundred thirty-two early stage medial knee OA patients with K/L grade 1 or 2 who visited our hospital from January 2009 to December 2011 for medial knee pain, thirty-five patients who were able to follow up for three years were registered. At baseline, the knee radiographs, JKOM score, a patient-oriented outcome measure, and 3.0-Tesla knee MRI were performed. The progression of knee OA was evaluated by radiographs taken at three years from the baseline. OA-related structural changes in the knee joint evaluated by MRI were semi-quantified using the Whole-Organ Magnetic Resonance Imaging Score (WORMS). The subjects were divided into two groups by the presence or absence of the progression of the disease (P; progress group, n=22; NP; non-progression group, n=13). When the K/L grade of the patients were progressed one or more grades after 3 years, they were defined as P. Difference between the two groups was determined by Mann-Whitney U test, and logistic regression analysis was performed using SPSS ver.19.0.
Results: No significant differences of the medial knee joint space width (JSW) and femoro-tibial angle (FTA) by radiographs at baseline were observed between NP and P (p=0.73 and 0.31, respectively). Among the MRI-detected joint changes induced by knee OA and evaluated by WORMS, the medial cartilage lesion score in P (4.70) was only significantly increased in comparison to that in NP group (1.62) at baseline (p=0.01). At three years after follow up, the JSWs of the patients in P were significantly decreased in comparison to those of the patients in NP (p=0.007). The MRI-detected medial cartilage lesions showed the significant negative association with the radiographic medial knee joint space width (JSW) after three years of follow up (r=-0.382, p=0.02). The logistic regression analysis adjusted for age and BMI revealed that among the radiographic OA-related parameters, such as JSW and FTA, pain VAS, JKOM score and the MRI-detected eight parameters, the medial cartilage lesion was only associated with the knee OA progression (OR; 5.02, 95%CI: 1.05 to 23.96, p=0.043).
Conclusions: When the structural changes of knee OA were precisely evaluated by 3TMRI and WORMS, the cartilage damage was found to be the most important factor for the progression of the early-stage knee OA.

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BONE MARROW LESIONS DETECTED BY DIFFERENT MAGNETIC RESONANCE SEQUENCES AS POTENTIAL BIOMARKERS FOR KNEE OSTEOARTHRITIS: COMPREHENSIVE TISSUE LEVEL ANALYSIS

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Purpose: Osteoarthritis (OA) is well recognised as a multifactorial disease of the whole joint with significant involvement of the subchondral bone. Epidemiological studies have demonstrated a strong association between MRI-detected bone marrow lesions (BMLs) and the severity of symptoms and structural degeneration in knee OA. Thus, BMLs appear to have diagnostic and prognostic value, and potentially represent a therapeutic target for knee OA. What BMLs represent at the cartilage-subchondral bone tissue level is poorly described and it is not known whether different MRI sequences detect the same tissue characteristics. Therefore, the aim of this study was to characterise the cartilage-subchondral bone morphometric features corresponding to BMLs detected using two different MRI sequences for a cohort of knee OA individuals.