Targeted disruption of cartilage-specific microRNA140 promotes osteoarthritis-like pathology

INTRODUCTION: Osteoarthritis (OA), the most prevalent aging-related joint disease, is characterized by an imbalance of articular cartilage degradation and deficient new extracellular matrix synthesis. MicroRNAs (miRNAs) are a class of non-coding RNAs that negatively regulates gene expression by promoting mRNA degradation and/or repressing translation through partial sequence-specific interactions with the 3’ untranslated regions (UTRs) of specific mRNA targets. MiRNAs show tissue specific expression patterns, suggesting that these miRNAs play a crucial role in tissue specific physiological or developmental processes and also in human diseases. We previously observed that miR-140 has an expression pattern suggestive of a role in chondrocyte differentiation and found that reduced miR-140 expression in human OA cartilage and in response to IL-1B stimulation may contribute to the abnormal gene expression pattern characteristic of OA [1]. Reduced miR-140 expression in human OA cartilage prompted us to determine whether miR-140 functions in cartilage homeostasis. The objective of this study was to define the in vivo function of the chondrocyte specific miR-140 in cartilage homeostasis.

MATERIALS AND METHODS: All animal experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee at the Scripps Research Institute. To examine the functions of miR-140, we created a mouse deleted for miR-140 and collected knee joints and chondrocytes from wild-type and miR-140-/- mice. To quantify OA-like pathological changes in articular cartilage, mouse knee joints were harvested from 1, 3, 6 and 8 month-old mice. We used a validated histological scoring system [2] based on Safranin O staining and evaluated expression of cartilage related genes by immunohistochemistry. In vitro proteoglycan catabolism was analyzed in cultured femoral head cartilage explants from miR-140-/- mice. Mouse chondrocytes were prepared from rib cartilage of postnatal day 3 (P3) mice by digesting with collagenase. Mouse chondrocytes were cultured in DMEM with 10% FBS. DNA microarray analysis was performed using Affymetrix mouse genome 430 2.0 array. RNA in DMEM with 1% DNA microarray analysis was performed using Affymetrix mouse genome 430 2.0 array. RNA samples collected from cultured chondrocytes of wild-type and miR-140-/- mice at P3. Microarray data were summarized by Robust Multichip Average (RMA) method and statistical analysis was performed using NIA Array Analysis. Double-strand (ds) RNA oligos representing mature sequences that mimic endogenous miR-140 and Silencer Negative Control siRNA #1 (Ambion) were transfected into chondrocytes at 5nM concentration with Lipofectamine 2000 (Invitrogen). A fragment of the 3’UTR of Adams-5 including the predicted miR-140 binding site was cloned downstream of luciferase2 gene, and the luciferase activity was measured. Two-tailed independent Student’s t-test or nonparametric Wilcoxon signed-rank tests were used for statistical analysis.

RESULTS AND DISCUSSION:
OA-like pathology in miR-140-/- mice MiR-140-/- mice were born at normal and were fertile. Skeletal development during embryogenesis in miR-140-/- mice appeared normal (Fig. 1A). Postnatally, miR-140-/- mice manifested a mild skeletal phenotype, with short stature and craniofacial deformities characterized by a short snout and domed skull. The structure and shape of knee joints, including articular cartilage, menisci and ligaments was normal in miR-140-/- mice at 1 month of age. Interestingly, miR-140-/- mice develop aging-related OA-like pathology. Knee joints from 6-month-old mice showed reduced Safranin O staining, indicative of proteoglycan loss, in femoral condyles and tibial plateaus. By 8 months there was cartilage degradation, with proteoglycan loss, roughened articular surface, and fibrillation, changes that were not observed in age-matched wild-type mice (Fig. 1B). To quantify OA-like pathological changes in articular cartilage, we used a validated histological OA scoring system based on Safranin O staining and the extent of tissue erosion. OA scores of miR-140-/- mice were significantly higher than those of wild-type mice (Fig. 1C), supporting the hypothesis that miR-140 is a novel regulator of cartilage homeostasis and its loss contributes to OA cartilage degradation.

CONCLUSION: MiR-140 disruption in vivo induced early onset OA-type changes in articular cartilage, which was associated with increased Adams-5 expression. Our results indicate that Adams-5 is directly regulated by miR-140, suggesting that miR-140 plays an important role in regulating the balance between extracellular matrix formation and degradation. This represents one of the first studies to show that a miRNA is required for tissue homeostasis and that its loss contributes to aging-related pathology.

REFERENCES:

Poster No. 436 • 56th Annual Meeting of the Orthopaedic Research Society