drocytes. The proliferative ability of chondrocytes was evaluated by measuring the incorporation of BrdU into newly synthesized DNA. The incorporation of BrdU in cultured BACs was observed under a confocal laser microscope and quantified using ELISA. Proteoglycan (PG) synthesis was assayed by monitoring [35S] sulfate incorporation. The newly synthesized PGs present within the cells were measured by assessing the incorporation of [35S] sulfate into cetylpyridinium chloride precipitable material. Changes in bFGF expression in mRNA and protein levels were investigated using the quantitative real-time PCR method (reverse delta-delta Ct method) and the western blotting analysis. Furthermore, changes in p53 expression were assessed with the real-time PCR and the western blotting. Effects of ox-LDL on Phospholylation of p53 also assessed.

Results: Ox-LDL treatment increased a ratio of SA β-gal-positive cells and the intensity of the stain in a dose-dependent manner within 24 hrs, whereas native LDL treatment did not. The ox-LDL-induced increase in the SA β-gal staining was significantly attenuated by pretreatment of the anti-LOX-1 blocking antibody (TS20). Addition of ox-LDL suppressed BrdU incorporation into cultured BACs in a dose-dependent manner, but native LDL did not. Pretreatment with TS20 recovered the ox-LDL-induced suppression of BrdU incorporation. Ox-LDL significantly suppressed PG synthesis by BACs in dose- and time- dependent manner. Pretreatment with TS20 significantly reversed the suppression in PG synthesis caused by ox-LDL. bFGF expression was also suppressed by ox-LDL addition in a dose dependent manner in both mRNA level and protein level. Ox-LDL upregulated p53 mRNA and protein expression and increased an amount of phospholylated p53.

Conclusions: Epidemiologic studies have shown that age is the chief risk factor for atherosclerotic diseases and osteoarthritis. Both endothelial cells in atherosclerotic lesions and chondrocytes in OA cartilage show attributes of cell senescence, and cell senescence and aging of the tissue are strongly correlated in both diseases. The data presented in this study show that ox-LDL binding to LOX-1 induces SIPS of chondrocytes. We previously demonstrated that ox-LDL binding to LOX-1 increases oxidative stress in chondrocytes by producing intracellular reactive oxygen species, which may be attributable to induction of SIPS in chondrocytes. Ox-LDL may play some roles in progression of osteoarthritis by inducing chondrocyte premature senescence.

034

REDUCTION IN ARTICULAR CARTILAGE LESIONS IN OLDER ADULT MICE OVEREXPRESSING CATALASE TARGETED TO MITOCHONDRIA

R.F. Loeser[1], M.A. McNulty[2], P.M. Treuting[3], C.S. Carlson[2]
[1]Wake Forest Univ. Sch. of Med., Winston Salem, NC; [2]Univ. of Minnesota Coll. of Vet. Sci., St. Paul, MN; [3]Univ. of Washington, Seattle, WA

Purpose: Increased levels of reactive oxygen species with aging may contribute to age-related diseases including OA. In this study, we tested the hypothesis that overexpression of the anti-oxidant enzyme catalase, targeted to mitochondria, would reduce OA severity in mice.

Methods: Mouse stifle (knee) joints were obtained from male transgenic mice (C57BL/6 background) that overexpress human catalase localized to the mitochondria (MCAT). MCAT (n=12) and male C57BL/6 wild-type controls (n=11) from 3 age groups were studied: young adult (10 months old), older adult (18-21 months), and very old adult (33 months). Paraffin embedded stifle joints were serially sectioned in a coronal plane. Two representative midcoronal sections were selected for evaluation and stained with hematoxylin & eosin (H&E) and Safranin-O stains. Sections were scored by an observer, blinded to groups, for articular cartilage structure (ACS) changes (0-12), Safranin-O staining (0-12), size of osteophytes, % area of chondrocyte death and morphometric measures of articular cartilage and subchondral bone area and thickness. Separate and combined results for the medial and lateral tibial plateaus were analyzed by ANOVA.

Results: Examination of combined results for wild-type and MCAT mice revealed that the young adult mice had minimal to no OA lesions with significantly ($p<0.001$) lower ACS and Saf-O scores, less cell death, and better morphometric measures than the two older groups of mice which did not differ significantly from each other. In the young adult mice, there were no significant differences between MCAT and wild-type mice in any of the measures. Because the two older groups had similar OA severity scores, the results in these two groups were combined in order to increase the numbers for analysis of differences between MCAT and wild-type. The sum of the ACS scores (med+lat) was significantly (p=0.04) lower in the MCAT (11.8 ± 1.5) vs wild-type mice (17.4 ± 1.8) (Fig. 1) as were the Saf-O scores (5.6 ± 1.7 for MCAT and 10.2 ± 0.8 for wt). The remainder of the measures did not differ significantly between groups but the trend was for better scores in the MCAT mice.

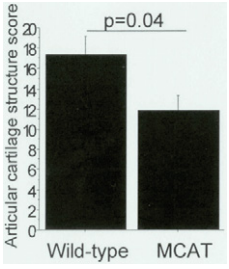

Figure 1

Conclusions: Naturally occurring OA-like lesions appear with aging in male C57BL/6 mice becoming prevalent by 18-21 months of age. Overexpression of catalase targeted to the mitochondria did not prevent lesions from developing but did significantly reduce OA severity measured by articular cartilage structure changes and loss of Safranin-O staining. These results support a role for mitochondrial reactive oxygen species in age-related OA.

035

EFFECTS OF INORGANIC PYROPHOSPHATE ON CHONDROCYTE RESPONSE WHEN ENCAPSULATED IN 3D SYNTHETIC HYDROGELS

N. Bishop[1], M. Kissler[1], M. Husa[2], R. Terkeltaub[2], S. Bryant[1]
[1]Univ. of Colorado, Boulder, CO; [2]VA Med. Ctr. San Diego, San Diego, CA

Purpose: Synthetic hydrogels are attractive for culturing cells in 3D where the hydrogel structure and chemistry are readily controlled. Specifically, poly(ethylene glycol) (PEG) hydrogels are being explored as a platform for cartilage tissue engineering where the gel environment maintains the chondrocyte phenotype and promotes cartilage matrix production. Here, 3D PEG hydrogels were employed as a model system to study the role of inorganic pyrophosphate (PPi) on chondrocyte function. Extracellular levels of PPi in cartilage have been reported to increase with age and osteoarthritis and are closely associated with calcification of cartilage. In this study, we sought to test the hypothesis that high levels of PPi decrease cell proliferation and tissue production, which occur with age, by chondrocytes encapsulated in PEG hydrogels.

Methods: Articular chondrocytes were isolated from the patellar-femoral groove of adult steers. A solution of cells at 4×10^6 cells per