Table 1

Cartilage measure variable	Meniscus volume measure (mm^3)		
	AH	PH	Total
CLTPD	–	<0.0001	–
ILTPD	–	0.0005	–
IMTMTH	–	–	<0.0001
WLTACS	<0.0001	<0.0001	<0.0001
WLTCAAB	<0.0001	<0.0001	<0.0001
WLTFVCL	–	–	<0.0001
WLTPD	–	0.0003	–
WLTSBA	0.0003	–	–
WLTVCL	–	–	<0.0001
WMTACS	–	–	<0.0001
WMTCAAB	–	–	<0.0001
WMTSBA	–	–	<0.0001
WMTVCL	–	–	0.0002

Cartilage measure variable	Cartilage measure description
CLTPD	% area of subchondral bone denuded of cartilage – lateral tibia (center) (cLT.dAB%) [%]
ILTPD	% area of subchondral bone denuded of cartilage – lateral tibia (internal) (iLT.dAB%) [%]
IMTMTH	mean cartilage thickness – medial tibia (internal) (iMT.ThCtAB) [mm]
WLTACS	area of cartilage surface – lateral tibia (LT.AC [cm^2]
WLTCAAB	area of subchondral bone covered by cartilage – lateral tibia (LT.cAB) [cm^2]
WLTFVCL	cartilage volume – lateral tib-fem compartment (LFTC.VC) [m^3]
WLTPD	% area of subchondral bone denuded of cartilage – lateral tibia (LT.dAB%) [%]
WLTSBA	total area of subchondral bone – lateral tibia (LT.tAB) [cm^2]
WLTVCL	volume of cartilage – lateral tibia (LT.VC) [mm^3]
WMTACS	area of cartilage surface – medial tibia (MT.AC) [cm^2]
WMTCAAB	area of subchondral bone covered by cartilage – medial tibia (MT.cAB) [cm^2]
WMTSBA	total area of subchondral bone – medial tibia (MT.tAB) [cm^2]
WMTVCL	volume of cartilage – medial tibia (MT.VC) [mm^3]

respond similarly, perhaps even in unison, to changes due to OA. The current study analyzed meniscus and cartilage relationships for subjects with a wide range of radiographic and symptomatic features of OA. Further work will focus on characterization of the interactions between meniscus and articular cartilage and stratification of associations with respect to clinical measures of OA severity such as KL grade.

032

A REDUCTION IN SIRT1 LEVELS IN OA ARTICULAR CARTILAGE IS ASSOCIATED WITH AN INCREASE IN PTP1B, MMP13 AND CHONDROCYTE APOPTOSIS

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Purpose: Osteoarthritis is a multi-factorial disease that results from an imbalance between cartilage anabolism and catabolism. This imbalance results from the over-expression of pro-inflammatory mediators, which induce the expression of matrix-degrading enzymes. Elevation in chondrocyte apoptosis is also evident in OA cartilage, contributing to matrix damage. Since OA is a disease associated with aging, the role of the SirT1 protein in chondrocyte biology was examined since SirT1 is recognized to be a longevity factor. SirT1 is a lysine deacetylase that can prolong lifespan in a variety of organisms by inhibiting the onset of diseases of aging. Here we examine the role of SirT1, and its regulatory factors, in a number of critical features of OA. We find that SirT1 displays aspects of an anti-OA protein, consistent with its function in prolonging lifespan.
Methods: Human articular cartilage samples were obtained from OA patients undergoing total knee arthroplasty while normal samples were obtained from cadeavers (NDRI). Cartilage was embedded sectioned and processed for immunohistochemistry using antibodies for SirT1, AROS, DBC1, PTP1B and MMP13. Additionally, chondrocytes isolated from cartilage samples were cultured in vitro and used for the generation of RNA for analysis of gene expression and for generation of protein extracts for immunoblotting, using the antibodies just mentioned. Human chondrocytes were Amaxa transfected with a SirT1 expression plasmid.
Results: In human chondrocyte cell culture we find that overexpression of SirT1 enhances expression of cartilage specific matrix genes (collagen 2, aggrecan) while repressing expression of matrix degrading metalloproteinases (MMP3, 8, 13, Lee et al., 2009 submitted). Further, Sirt1 was found to be a potent inhibitor of apoptosis in human chondrocytes through repression of the protein tyrosine phosphatase 1B (PTP1B, Gagarina et al, 2009 submitted), an extremely potent pro-apoptotic protein in chondrocytes. Examination of tissue sections revealed that SirT1 levels were significantly downregulated in OA cartilage compared to normal cartilage, while PTP1B and MMP13 levels were elevated in OA tissue. Proteins known to associate with Sirt1 and regulate its enzymatic activity (Aros and DBC1) were also examined in chondrocytes and cartilage sections. Aros (a Sirt1 activator) was down regulated in OA samples while DBC1 (a SirT1 repressor) was upregulated in OA. These data are consistent with the finding that SirT1 enzyme activity is significantly decreased in OA chondrocytes
Conclusions: We show that longevity protein SirT1 is a powerful regulator of cartilage matrix and MMP gene expression and chondrocyte apoptosis in human chondrocytes. Further we show that SirT1 is down regulated in OA cartilage, which correlates well with an elevation in MMP and PTP1B levels and an increase in apoptosis. These data suggest that SirT1 has features of an anti-osteoarthritic protein, consistent with its positive effects on aging.

033

OXIDIZED LDL INDUCES STRESS-INDUCED PREMATURE SENESCENCE IN CULTURED BOVINE ARTICULAR CHONDROCYTES

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Purpose: In addition to replicative senescence with critical telomere shortening, stress-induced premature cell senescence (SIPS) occurs by which cells without discernible attrition of telomeres show a growth arrest. There are some stressors already identified include DNA damage, oxidative stress, suboptimal culture conditions, and oxidized LDL (ox-LDL). Both modes of senescence are associated with suppressed cell proliferation, impaired physiological cell function and a distinct pattern of gene expressions and may contribute jointly to the pathogenic process of chronic diseases in vivo. The purpose of this study was to investigate whether ox-LDL induces SIPS of cultured bovine articular chondrocytes (BACs) through the lectin-like ox-LDL receptor-1 (LOX-1) expressed on the chondrocyte.
Methods: Chondrocytes were isolated from articular cartilage of 10-month-old cows by enzymatic digestion and cultured in DMEM supplemented with 10 % FBS at 37 °C in a humidified and hypoxic atmosphere (5% O2 and CO2) to avoid cell senescence caused by oxidative stress. Senescence-associated β-galactosidase (SA β-gal) activity was detected by cytochemical staining of chon-

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

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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.

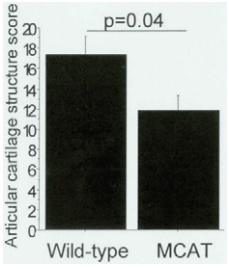

Figure 1

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

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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