Fig. Molecular networks generated from the genes differentially expressed by age. The molecular networks generated from genes differentially expressed in meniscus by age (top: up-regulated genes; bottom: down-regulated genes) using GRANITE. The colors are based on the database from which the network is extracted. ellipse = protein; square = transcription factor

79 DECIPHERING THE ROLE OF 75KDA SIRT1 FRAGMENT IN OSTEOARTHRITIS

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Objective: SirT1 is a nuclear NAD-dependent deacetylase associated with cell metabolism and survival. In joints, recent evidence shows that it regulates cartilage homeostasis and is involved in the inflammatory phase of osteoarthritis (OA). It is widely held that the inflammatory cytokines tumor necrosis factor alpha (TNFa), and interleukin 1 b (IL-1b) may elicit chondrocyte death associated with the deterioration of articular cartilage (AC) in OA. Exposing human chondrocytes to both cytokines leads to cathepsin B-mediated cleavage of the nuclear full-length SirT1 (FLSirT1; 110kDa) to generate a stable but enzymatically inactive 75kDa fragment of SirT1 (75SirT1). Here we aim to elucidate the biological and biochemical role of 75kDa SirT1 fragment in-vivo and in-vitro

Methods: Using immunoblot analyses we aimed to detect cathepsin B, FLSirT1 and/or 75SirT1 in OA and normal human chondrocytes. Confocal imaging of SirT1 was used to monitor its subcellular trafficking following TNFα stimulation of human chondrocytes. Coimmunofluorescent staining for cathepsin B, mitochondrial cytochrome oxidase subunit IV and lysosome-associated membrane protein 1 together with SirT1 was performed. Human chondrocytes were tested for apoptosis by fluorescence-activated cell sorter analysis and immunoblotting for caspases 3 and 8. Human chondrocyte mitochondrial extracts were obtained and analyzed for 75SirT1-cytochrome C association.

In-vivo assays of heterozygous haploinsufficient (SirT1+/-) and wild-type (WT; SirT1+/+) 129/J mice aged 1 or 9 months were systematically compared for musculoskeletal features, scored for osteoarthritis (OA) severity, and monitored for chondrocyte apoptosis in articular cartilage. Protein extracts from articular chondrocytes were isolated and immunoblotted for SirT1 and active caspase 3.

Results: Confocal imaging, immunoprecipitation assays and immunoblot analyses following TNFα challenge of human chondrocytes exhibited that 75SirT1 was generated and exported to the cytoplasm wherein it colocalized with the mitochondrial membrane and associates with cytochrome C. Additionally, levels of cathepsin B and 75SirT1 were elevated in OA versus normal chondrocytes. Subjecting human chondrocytes to TNFa and a cathepsin B inhibitor (ALLN), enhanced levels of apoptotic cell and cleaved caspase 3.

Immunoblot assays comparing medial tibial plateau articular cartilage (MTP-AC) of heterozygous haploinsufficient (SirT1+/-) and wild-type (WT; SirT1+/+) 129/J mice revealed reduced levels of FLSirT1 in both strains at 9 months compared to their 1 month equivalent. WT 9-month-old strains presented the 75SirT1 fragments in immunoblot assays, whereas age-matched SirT1+/- mice possessed undetected 75SirT1 levels. Interestingly, 9 month-old SirT1+/- mice exhibited increased OA severity and augmented levels of apoptosis compared with age-matched WT mice.

Conclusion: Despite 75SirT1 being enzymatically impaired our results show that it is relatively stable and participates in promoting chondrocyte survival following exposure to proinflammatory cytokines or during articular cartilage maturation.

80 ASYMPOTOMATIC INDIVIDUALS WITH RADIOGRAPHIC EVIDENCE OF KNEE OSTEOARTHRITIS WALK WITH DIFFERENT KNEE MECHANICS PATTERNS THAN SYMPTOMATIC INDIVIDUALS WITH THE SAME RADIOGRAPHIC EVIDENCE

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