EVALUATING CATHEPSIN ACTIVITY AND SIRT1 CLEAVAGE IN EXPERIMENTAL OSTEOARTHRITIS

L. Ben-Aderet, G. Blum, M. Dvir-Ginzberg; Hebrew Univ. of Jerusalem, Jerusalem, Israel

Purpose:

SIRT1 is a NAD-dependent protein deacetylase that regulates cartilage matrix gene expression and was found to be impaired in protein levels and enzymatic activity in OA vs. normal cartilage. Stimulation of chondrocytes with pro-inflammatory factors induces site-specific cleavage of full-length SIRT1 (110kDa) to generate an inactive variant (75SIRT1;75kDa). This research aims to profile variations in SIRT1 cleavage fragments and cathepsin B and S activities to determine the inflammatory and catabolic state of cartilage and susceptibility to develop Osteoarthritis (OA).

Methods:

OA derived articular chondrocytes were obtained from total knee arthroplasty. Following isolation and propagation, primary human chondrocytes where either plated (2D) or encapsulated in three dimensional (3D) alginate micro-beads and cultured in DMEM culture media for 2-weeks. Chondrocytes were treated or untreated with 2 ng/ml IL-1β and 50 ng/ml TNF-α for 24h. Following stimuli, cell extracts and media were obtained and analyzed for SIRT1 cleavage or for cathepsin activity using activity based probes (ABPs), which covalently bind active cathepsin B and S and exert a fluorescent signal. Further, media from round 4-mm-diameter human articular cartilage explants, subjected to cyclic mechanical loading (60 N, 0.1 Hz, 1 h) in the presence or absence of 2 ng/ml IL-1ß and 50 ng/ml TNF-α, were analyzed similarly. Finally, regions of intact cartilage (IC) vs. degenerative cartilage (DC) were cryo-sectioned and stained with fluorescent ABPs to detect temporal changes in cathepsin B and S activities as a function of articular cartilage degeneration.

Results:

Chondrocyte lysates from monolayer 2D and 3D cultures, exhibited reduced protein levels of full length SIRT1 (110kDa), with enhanced levels of 75SIRT1 and a 35kDa SIRT1-responsive fragment, when subject to proinflammatory stimuli. Conditioned media from 2D chondrocytes exhibited 3-fold enhancement in 75SIRT1 and 35kDa SIRT1-responsive fragment with full-length SIRT1 being beneath the limit of detection, under proinflammatory conditions. These data correlate with enhanced mRNA expression levels of cartilage degrading enzymes upon cytokine stimuli in both 2D and 3D chondrocytes (i.e. average increase of 160-fold for MMP13; 3-fold for ADAMTS5, 6-fold for cathepsin B, and

50-fold for cathepsin S), and reduced expression for cartilage structural genes (i.e. average reduction of 2-fold for both COL2A1 and ACAN). Analysis of cathepsin activity with ABPs in cell lysates, showed 2-5 fold enhancement of enzymatic activity of cathepsin S with insignificant changes in cathepsin B activity. In addition, conditioned media obtained from plated chondrocytes showed enhanced cathepsin S activity with undetected cathepsin B activity. While both cathepsins showed augmented activity in freshly isolated chondrocytes from IC and DC, surprisingly, only cathepsin S was observed in supernatant media following collagenase treatment in DC regions of OA cartilage, indicating active cathepsin S is secreted by chondrocytes and contributes to extracellular matrix degradation.

Conclusions:

In summary, our in-vitro data indicate that the 75SIRT1 fragment is augmented in lysates and conditioned media of plated chondrocytes under proinflammatory conditions. As well, cathepsin activity, especially cathepsin S, is augmented in chondrocytes, subject to proinflammatory stimuli. These data are further supported by the enhanced activity of cathepsin S in degenerated cartilage matrix. Hence these findings may provide a basis for early detection of OA susceptible individuals, based on enhanced cathepsin activity and SIRT1 cleavage, generated in regions of articular cartilage prone to damage.