Characterization Of Senescent Intervertebral Disc Cells: Secretion Of Catabolic Factors And Proteolysis Of Matrix Aggrecan

Kevin Ngo, MS, Gwendolyn Sowa, MD, PhD, James D. Kang, MD, Nam Vo, PhD.
University of Pittsburgh, Pittsburgh, PA, USA.

Disclosures:  K. Ngo: None. G. Sowa: None. J.D. Kang: None. N. Vo: None.

Introduction: Aging is the largest single risk factor for intervertebral disc degeneration (IDD), as disc matrix proteoglycan (PG) is invariably and progressively depleted with age. Dysregulated disc cells are thought to drive age-associated disc PG loss through a combination of reduced capacity to synthesize matrix PG and increased production of proteolytic enzymes to breakdown matrix. The link between aged degenerative discs and cellular senescence has been previously observed in human discs, but it remains unknown if senescent disc cells are phenotypically different from their non-senescent counterpart in terms of matrix homeostasis. Our previous studies have established that stress-induced senescence of human disc cells resulted in decreased PG synthesis and increased production of several key catabolic factors such as IL-6, IL-8, MMP-1, and MMP-3. The goal of this study is to further explore matrix homeostasis characteristics of senescent disc cells, specifically how senescent disc cells affect aggrecan proteolytic degradation.

Methods: To induce complete senescence, human nucleus pulposus (hNP) and annulus fibrosis (hAF) cells isolated from surgical specimen were serially treated twice, each time with 0.5mM H2O2 for 2 hours, followed by incubation for 4 days in fresh media (F-12, 10% FBS, 1% PS). Cellular senescence was measured by SA-β-GAL assay. Aggrecan fragments in conditioned media of H2O2-induced senescent cell and untreated non-senescent cell culture were assayed by Western blot using Anti-G1 antibodies (Abcam ab36861). Inflammation and MMP antibody arrays were used to assess proteins levels in conditioned media (Ray Bio Tech AAH-INF-3 and AAH-MMP-1). DMMB assay was performed to measure total GAG content.

Results: Most of the hNP cells (>90%) were stained positive for SA-β-GAL following H2O2 treatment, confirming full induction of senescence. Western analysis revealed greater levels of ADAMTS- and MMP-generated proteolytic aggrecan fragments as a result of cleavage in the aggrecan interglobular domain (IGD) in the conditioned culture media of H2O2-induced senescent hNP cells compared to those of nonsenescent hNP cells. In contrast, the levels of aggrecan IGD proteolytic fragments were relatively unchanged in the conditioned media of senescent hAF cell culture compared to nonsenescent AF cell culture (Fig.1). Antibody array experiment showed elevated levels of many pro-inflammatory cytokines (IL-6, IL-8, PDGF-BB, GCSF), chemokines (EOTAXIN-2, IP-10, RANTES) and MMPs (MMP-3, MMP-10, TIMP-2) in the conditioned media of senescent hNP cells (data not shown). The total GAG content decreased in senescent hAF and hNP cell cultures, the latter was statistically significant while the former was not (Fig. 2).

Discussion: In this study we use the strong oxidant H2O2, to induce senescence of human disc cells in order to characterize their matrix metabolism. H2O2-induced senescent disc cells exhibited greatly perturbed matrix homeostasis, showing decreased capacity for new PG synthesis as well as enhanced expression and secretion of key inflammatory factors. This provides new insights into the pathogenesis of disc degeneration.
cytokines, chemokines, and matrix proteinases. These are the catabolic factors which constitute the hallmark feature of senescent fibroblasts previously termed senescence associated secretory phenotype (SASP). Age-dependent accumulation of senescent cells in various organs is thought to disrupt tissue structure and function, and promote aging due at least in part to their SASP. Our study showed that stress-induced senescent human disc cells also acquired SASP-like phenotype which may have profound catabolic effects on neighboring cells and the extracellular matrix.

Consistent with this idea is the observation of enhanced aggrecanolysis and decreased total GAG content in senescent disc cell culture.

**Significance:** Disc senescent cells contribute to perturbed extracellular matrix homeostasis through the acquisition of SASP, reduced matrix synthesis, increased matrix degradation.

**Fig.1:** Western analysis of Aggrecan proteolytic fragments in conditioned media of H$_2$O$_2$-induced senescent and non-senescent human disc cell culture. (A) Representative western blot for detection of Aggrecan (Anti-Agc) in hNP and hAF senescent and negative control conditioned media samples. ADAMTS-generated and MMP-generated fragments indicated. (B) Quantification of (A) using densitometry represented as relative ratio of volumes for senescent to control CM. Dotted line represents ratio of 1 denoting no change. Error bars represent SEM (n=3).
Fig. 2: Oxidative-stress induced senescent human NP cells show decreased total GAG content compared to untreated, non-senescent control. Normalized to DNA. Dotted line represents ratio of 1 denoting no change. Significance code: ** p <0.001. Error bars represent SEM (n=3).