The Role of Fatty Acid Metabolism In Intervertebral Disc Degeneration and Senescence

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INTRODUCTION: Intervertebral disc degeneration (IDD) is an age-related disorder, affecting more than 90% of the population above 50 years of age. The manifestations of IDD are disc prolapse, spinal nerve decompression and lower back pain. Despite being one of the leading causes of disability, the treatment options for IDD are only palliative if surgical removal of degenerated segments is not required. The intervertebral disc (IVD) loses its structural integrity due to a reduction in extracellular matrices, produced by the cells in IVD. It was reported that IDD involves accumulation of senescent cells, characterized by permanent cell-cycle arrest with metabolic changes and a pro-inflammatory secretome called senescence-associated secretory phenotype (SASP). Accumulation of SASP induces further senescence in neighboring cells non-autonomously and, therefore, may aggravate IDD over time. It remains largely undefined what drives these phenotypic changes leading to senescence. Several studies have associated changes in lipid profile with aging and senescence. Fatty acid synthase (FASN), a critical enzyme in lipid biosynthesis, and its main product, palmitic acid (PA), are found to be overexpressed in various models of aging and plasma coupled with SASP. We, therefore, hypothesize that changes in FASN activity could contribute to IDD by regulating senescence. In this study, we aimed to investigate what is the link between FASN activity, palmitic acid, and cell senescence. We firstly tested if an exposure to PA could promote senescence in human nucleus pulposus (NP) cells. Thereafter, we used a FASN inhibitor to assess whether it can alleviate cell senescence in IDD.

METHODS: Human subjects were recruited with informed consent under IRB and local ethics committee approval. IVD specimens were collected from patients undergoing disc excision and spinal fusion due to symptomatic IDD or scoliosis. The specimens were used for immunohistochemical staining of FASN in IDD samples vs. scoliotic controls. Primary cells were isolated from IDD samples and selected based on proliferative capacity and basal level of activity of senescence-associated β-galactosidase (SA-β-gal), known to be increased in senescent cells. NP cells were treated with different concentrations of PA in the form of PA:BSA conjugate for 1 day and/or 100nM FASN inhibitor (iFASN) for 1 or 3 days. PA:BSA ratio did not exceed 6:1. To assess the development of senescence, β-galactosidase activity was analyzed by histochemical cell staining kit (sigma, cat no: CS0030-1KT). Quantitative PCR (qPCR) was performed using Power SYBR Green PCR mix (Thermo Fisher Scientific) to detect expression of genes responsible for cell cycle arrest and SASP production (*p21, MMP12, IL6, IL8*). Gene expressions of target genes were normalized to GAPDH. One-way ANOVA (Graphpad Prism ver9.5.1) was performed to statistically evaluate differences between pairs of groups. p value < 0.05 was considered statistically significant.

RESULTS: Immunostaining showed an increase of FASN expression in the IVD of the patients with symptomatic IDD, suggesting an increased fatty acid synthesis in IDD (Figure 1A). Senescent cells were identified in the NP populations collected from IDD patients. As indicated by the SA-β-gal activity staining, basal level of senescence is different among the patients and it increases with cell passage (Figure 1B, C). QPCR analysis showed consistent increase of SASP marker expression 24 h post PA treatment (n=3) (Figure 2A). Notably, 24h treatment with a range of 100uM-400uM PA resulted in significant upregulation of proinflammatory cytokines *IL6* and *IL8* in 100-1000 fold change in a dose-dependent manner. As senescent cells were found present in the human primary NP cell culture (as confirmed by SA-β-gal assay), we treated the cells with FASN-inhibitor and examined any changes in the expression of senescence markers. FASN inhibition did not elicit significant changes in most senescence markers, however, there was a decrease in expression of gene coding catabolic enzyme matrix metalloproteinase 13 (*MMP13)* and increase in expression of collagen II (*COL2A1*), a one of the functional markers of NP cells (Figure 3A). We hypothesized that the accumulation of PA in cells could be countered by co-treatment with a FASN inhibitor (iFASN). However, such co-treatment, on the contrary, showed increase in the expression of cell cycle inhibitor p21 protein, suggesting that inhibition of FASN aggravated the effect of PA treatment (Figure 3B).

DISCUSSION: Our findings confirm the existence of senescent cells in degenerative IVD tissues, which might contribute to the IDD development. We showed that treatment with PA could induce inflammation, indicated by significant increase in expression of pro-inflammatory factors, *IL6* and *IL8*, which may explain cell apoptosis after treatment. *IL6* and *IL8* participate in SASP and have been associated with chronic low back pain in multiple reports. Inflammatory response could be induced at a relatively low dosage of PA, indicating the importance of lipid profile balance in IVD homeostasis. Inhibition of fatty-acids producing enzyme, FASN, caused an anabolic (induction of *COL2A1*) and anti-catabolic (suppression of *MMP13*) in NP cells, supporting that FASN activity might be involved in IDD. However, simultaneous treatment of NP cells with FASN inhibitor and PA appeared to aggravate senescence. This might be due to an increased uptake of PA or other metabolites because of inhibited PA synthesis. The impact of fatty acid intake and metabolism on IDD progression warrants further investigation.

SIGNIFICANCE: Our findings indicate the role of fatty acid metabolism in IDD pathogenesis, suggesting a potential impact of diet on its manifestation and clinical management.

IMAGES AND TABLES:

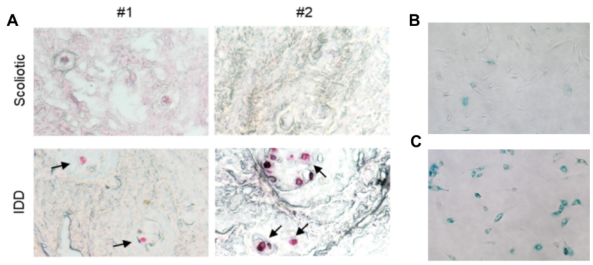

Figure 1. (A) Immunohistochemistry detected elevated intracellular Fasn expression in nucleus pulposus (NP) tissues in IDD vs scoliotic controls. (B) Human nucleus pulposus cells with low basal level of SA-β-gal activity, passage 2. (C) Human nucleus pulposus cells with a relatively high level of SA-β-gal activity, passage 9.

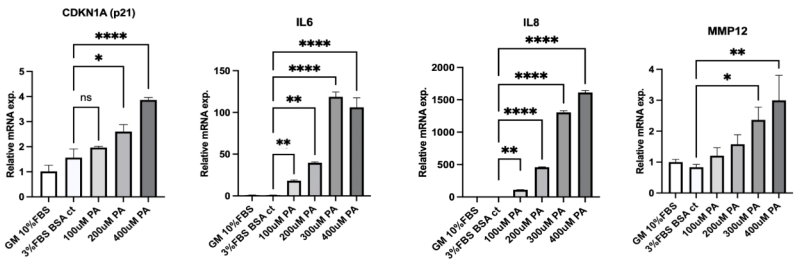

Figure 2. Primary NP cells were treated with different concentrations of PA for 24h. PA was delivered using fatty acid free bovine serum albumin as a carrier in a form of PA:BSA conjugate. The expression levels of the senescence markers *CDKN1A, IL6, IL8* and *MMP12* were analysed by RT-qPCR and normalised to *GAPDH*.

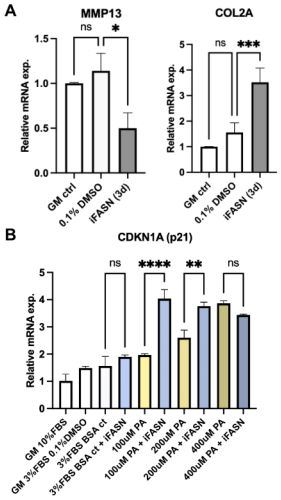

Figure 3. (A) Primary NP cells were treated with iFASN for 3 days (dissolved in DMSO). The expression levels of *MMP13* and *COL2A1* were analysed by RT-qPCR and normalised to *GAPDH*. (B) Primary NP cells were treated with different concentrations of PA for 24h and 100nM of iFASN simultaneously. PA was delivered in a form of PA:BSA conjugate. iFASN was delivered at the same time with PA.