DNA Damage in Intervertebral Disc Degeneration: CRISPR Modulation of ZNF865 (ZNF865)

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INTRODUCTION: Low back pain is the world's leading cause of years lived with disability. As such, low back pain creates a tremendous economic burden of over $100 billion in the United States annually with degenerative disc disease accounting for 40% of all instances. In the degenerative intervertebral disc (IVD), the pH decreases from 7.1-7.4 to as low as 6.2 in severely degenerated discs, a relatively understudied phenomenon in the degenerative disc. Furthermore, along with these pH changes, cells in the degenerative disc begin to exhibit signs of senescence. Senescent cells have recently been associated with disc degeneration and pain through the senescence-associated secretory phenotype (SASP). Further, cellular senescence has been postulated to be a response to DNA damage in many other cells, tissues, and organs, but the exact mechanism of this relationship in the IVD remains unknown. We have recently discovered the gene ZNF865 (BLST) as a regulator of cellular senescence with involvement in intervertebral disc degeneration; however, its functional mechanism and interactions have not been extensively characterized. Here, we investigate differences in DNA damage in healthy and degenerative human intervertebral disc cell populations as well as the ability of low pH to induce DNA damage in healthy human IVD cells. Further, we quantify the ability of ZNF865 to regulate DNA damage in human IVD cells in both directions utilizing CRISPRa and CRISPRi.

METHODS: Human IVD samples were obtained from surgical waste tissue from deidentified trauma or IVD replacement patients. Nucleus pulposus (NP) cells were isolated from the samples and plated onto tissue culture plastic. Degenerative NP cells were transduced with lentiviral vectors targeting ZNF865 for upregulation (CRISPRa) using the VPR system. Healthy NP cells were transduced with lentiviral vectors targeting ZNF865 for downregulation (CRISPRi) using the KRAB system. A subset of the healthy NP cell population was subjected to low pH conditions ranging from 7.1 to 6.2 over a period of two weeks. The alkaline comet assay was used to quantify DNA damage before and after treatment in all cell populations. DNA damage was quantified using the OpenComet software.

RESULTS: Nucleus pulposus cells isolated from degenerative disc samples displayed a significantly higher amount of baseline DNA damage relative to healthy cell populations (**1a**). Cells derived from a healthy intervertebral disc subjected to low pH conditions began to display significantly increased DNA damage relative to naïve untreated (pH 7.4) cell populations following 1-14 days of culture in media with decreased pH (7.1, 6.8, 6.5, 6.2) (**1c**). A significant increase in DNA damage was observed at 3 days in pH 6.2 and at 7 days in pH 6.5 culture relative to naive. CRISPRi of ZNF865 in healthy cell populations significantly increased the amount of DNA damage in all patient samples (**1e, 1f**). CRISPRa of ZNF865 in degenerative cell populations significantly decreased the amount of DNA damage, returning the cell populations to an insignificant difference in DNA damage compared to untreated healthy cell populations (**1h, 1i**).

DISCUSSION: In this study, we demonstrate that baseline DNA damage in degenerative NP cell populations is significantly increased relative to healthy cell populations. Using CRISPRi, healthy NP cell populations develop increased DNA damage after ZNF865 downregulation to the same extent as those in the naïve degenerative cell population. Interestingly, CRISPRa of ZNF865 in degenerative cell populations decreases DNA damage, returning the amount of DNA damage to the same as the healthy cell populations. Overall, this research not only elucidates ZNF865's involvement in DNA damage pathways but also suggests that upregulation of ZNF865 could be used as a tool for returning cells to a healthy phenotype. Further, we found that pH decreases, commonly observed in degenerative discs, induce DNA damage in nucleus pulposus cells in vitro and may offer one explanation of the DNA damage observed in painful degenerative human IVDs.

CLINICAL RELEVANCE: As one of the leading causes of pain and disability worldwide, novel treatments for low back pain offer the potential to significantly improve quality of life for millions of people. Current treatments are primarily palliative or require invasive surgical intervention; as such, the primary goal of this work is to continue developing an understanding of ZNF865 for potential future use as a therapeutic for low back pain, as well as understanding the biological effects of this novel gene in the IVD.

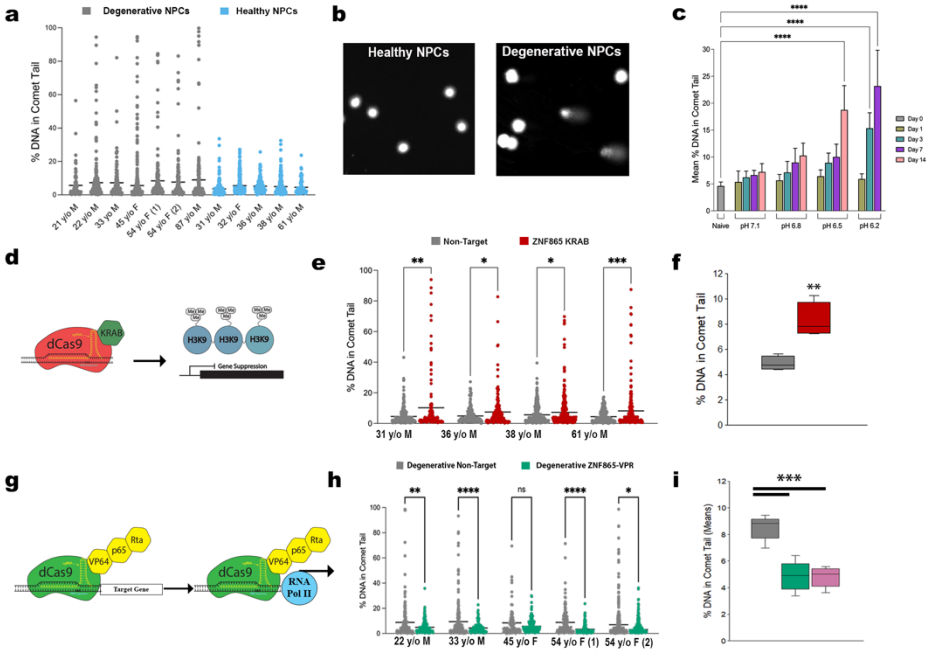

Figure 1. DNA Damage in healthy and degenerative human NP Cells. (a) NP cells derived from degenerative human IVD samples display significantly increased DNA damage relative to NP cells derived from healthy populations. **(b)** Representative alkaline comet assay images of degenerative and healthy NP cells. **(c)** Low pH significantly increases DNA damage in healthy NP cell populations. **(d)** Schematic of the CRISPRi KRAB downregulation system. **(e)** CRISPRi of ZNF865 in healthy NP cells significantly increases DNA damage in all patient samples. **(f)** Mean DNA damage across all samples increases significantly between unmodified NP cells and NP cells subjected to CRISPRi ZNF865 downregulation. **(g)** Schematic of CRISPRa VPR upregulation system. **(h)** CRISPRa of ZNF865 in degenerative NP cells significantly decreases DNA damage. **(i)** Mean DNA damage in ZNF865 CRISPRa degenerative cells returns to the level of DNA damage observed in healthy cells.