CRISPRa Regulation of ZNF865 Decreases SASP Expression and Reprograms Gene expression in Human Degenerative NP cell to a Healthy NP Cell State

Christian Lewis[1], Hunter Levis[1], Brandon Lawrence[2], Robby D. Bowles[1]
[1]The University of Utah Department of Biomedical Engineering, Salt Lake City, UT
[2]University of Utah Orthopedic Center, Salt Lake City, UT

INTRODUCTION: Pathology of the intervertebral disc (IVD) and associated back pain is a major healthcare concern in the US. About 40% of this back pain can be attributed to degeneration of the IVD. Degenerative disc disease (DDD) has been closely associated with several hallmark phenotypes, relating to increased inflammation and senescence in degenerating discs. Current treatments for IVD degeneration are mainly palliative, aimed at reducing pain, providing a critical need for the development of novel treatments for IVD degeneration. Cell engineering has provided the opportunity to tune cell function to address therapeutic needs and discover novel biology. Recently, in a set of CRISPRa genome-wide screens, our lab identified a previously uncharacterized zinc finger protein, ZNF865, also known as BLST, that produces robust cell-engineering phenotypes relating to cell cycle, protein processing, and cellular senescence. Preliminary data from publicly available RNAseq datasets suggests that ZNF865 is differentially regulated within degenerating human IVDs. Here we investigate the molecular phenotypes associated with CRISPRa of ZNF865 within primary human IVD samples and examine differences in inflammatory factor secretion and gene expression between healthy and degenerative samples.

METHODS: Human IVD samples were obtained from surgical waste tissue from deidentified Trauma or IVD replacement patients. Nucleus pulposus (NP) from IVD samples was macroscopically dissected and digested with pronase/collagenase to release cells form native ECM, then plated onto tissue culture plastic. Relative gene expression in naïve degenerative (n=28) and healthy (n=4) NP samples were measured by qRT-PCR after one passage and normalized to the youngest patient. Degenerative NP cells were then transduced with lentiviral vectors targeting ZNF865 for upregulation (CRISPRa) at passage 1-2. Differences in senescence associated secretoy phenotype (SASP) proteins were examined by running a custom Luminex panel for protein on cell culture supernatants (n=4). Differences in gene expression were determined by RNA sequencing. Here, total RNA was extracted from healthy NP cells (hNPCs), degenerative NP cells (dhNPCs) and ZNF865 upregulated degenerative NP cells (ZNF865-VPR dhNPCs) and submitted for sequencing. Reads were normalized and differential analysis was performed using EdgeR. Enriched GO biological and molecular process were determined from significantly regulated genes using Enrichr.

RESULTS: Fold difference in ZNF865 expression showed that decreased ZNF865 expression is correlated with increased aging in hNPCs, but not in dhNPCs (**1a**). Upregulation of ZNF865 using CRISPRa (**1b**) within dhNPCs showed significantly decreased protein levels in 9/10 SASP proteins compared to non-target samples (**1c**) with non-significant differences to hNPCs when combined into a composite SASP score that weights each analyte equally (**1d**). Between dhNPCs and hNPCs 2296 genes were differentially expressed with many pathways relating to senescence and inflammation (**1e**,**1h**). Upon upregulation of ZNF865 in dhNPCs, 1753 genes were differentially expressed with several overlapping pathways with the dhNPCs vs hNPCs (**1f**,**1h**). However, when comparing the ZNF865-VPR dhNPCs to the hNPCs, there is a 95% decrease in the number of differentially expressed genes with 103 genes, and zero significant molecular pathways associated with those genes (**1f**,**1h**).

DISCUSSION: This research examines the ZNF865 gene within degenerative IVD tissue and further investigates the effects of CRISPR regulation of ZNF865 within the IVD *in vitro*. Our results suggest that ZNF865 may be linked to aging and DDD, as we saw that decreased ZNF865 expression was correlated with increased age, with hNPCs show increased expression of ZNF865 compared to dNPCs cells. Additionally, we show that upregulation of ZNF865 can be used as a tool to restore dhNPCs to a healthier state, as we saw that upregulation of ZNF865 in dhNPCs leads to a decrease in SASP phenotype, and decreased the number of differentially expressed genes by 95% when compared to hNPCs.

SIGNIFICANCE: Overall, this work highlights ZNF865 as a potential therapeutic target for treating disc degeneration by mitigating inflammation and SASP within degenerative IVD cells.

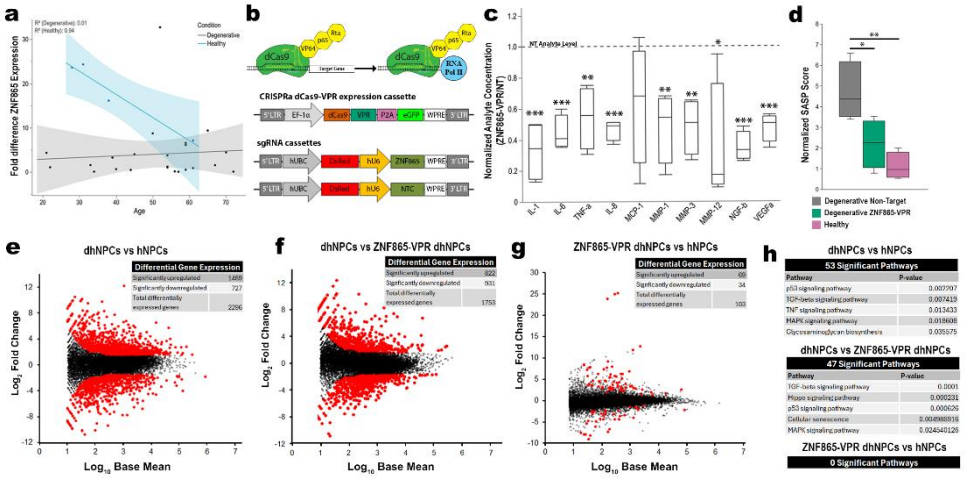

Figure 1: (**a**) Fold difference ZNF865 expression in hNPCs and dNPCs. (**b**) dCas9-VPR CRISPRa system. (**c**) Analyte concentration for SASP related inflammatory cytokines, proteases and proteins present in degenerative ZNF865 VPR human NP cells normalized to non-target samples. **(d)** SASP score normalized to healthy NP cell values. (**e**) MA plots comparing differentially expressed genes in dNPCs vs hNPCs, (**f**) ZNF865-VPR dhNPCs vs dhNPCs and (**g**) ZNF865-VPR dhNPCs vs hNPCs. (**h**) GO biological and molecular process associated with the significantly differentially expressed genes.