CRISPR Regulation of a Novel Gene, ZNF865, to Modulate Cellular Activity and Senescence in Intervertebral Disc Disease

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Introduction: Pathology of the intervertebral disc (IVD) and associated back pain is a major healthcare concern in the US with health care costs exceeding $100 billion annually. About 40% of this back pain can be attributed to degeneration of the IVD. Degeneration of the IVD has been closely associated with IVD cell senescence, with many cells present in degenerative IVD tissue demonstrating a senescent state. 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 Recently, in a set of CRISPRa genome wide screens, our lab identified a novel zinc finger protein previously unpublished on, ZNF865, that produces robust cell-engineering phenotypes relating to cell cycle, protein processing and cellular senescence. Here we investigate ZNF865s phenotype when upregulated (CRISPRa) and downregulated (CRISPRi), perform RNAseq in immortalized cell types, and perform follow-up experiments to understand how ZNF865 is regulating the observed phenotypes in both immortalized cell lines and primary IVD cells to determine the therapeutic potential of ZNF865 within the IVD.

Materials and Methods: Human embryonic kidney (HEK293) and Adipose derived stem cells (ASCs) were transduced with lentiviral vectors targeting ZNF865 for upregulation (CRISPRa) or downregulation (CRISPRi) and non-target control vectors for both systems. Cell proliferation was evaluated for 5 days following successful transduction and RNA was isolated from ZNF865 and NTC cells for qRT-PCR of ZNF865 expression (n=4). Cell cycle analysis was performed using Vibrant Dyecycle Violet staining. RNAseq was performed for both ASC and HEK cells (data not shown). Primary human nucleus pulposus cells (hNPCs) were transduced with CRISPRi-ZNF865, or -NTC expression cassettes and evaluated for proliferation via CCK8 assay. Senescence was evaluated using SA-β-gal staining at 1- and 3-weeks post transduction and p16 and p21 staining at 3 weeks post-transduction (n=4). Degenerative hNPCs were transduced with CRISPRa-ZNF865 expression cassettes and evaluated for proliferation via CCK8 assays. Additionally, ZNF865 was upregulated in Jurkat cells with CRISPRa, and their proliferation measured via CCK8, and cytokine production (IL-2) measured via ELISA to investigate cellular activity and immune response.

Results: Fold change gene expression showed significant activation by VPR and inhibition by KRAB. (**1A**). Downregulation of ZNF865 in HEK 293 cells and ASCs showed a significant decrease in cell viability within 3 days of expression (**1B**). CRISPRa of ZNF865 in HEK 293 cells and ASCs significantly decreased doubling time (**1C**). RNA-seq analysis shows the top molecular processes (Cell Cycle, DNA replication, cellular senescence, and protein processing) affected by ZNF865 upregulation (**1D**). CRISPRi of ZNF865 in hNPCs prevented proliferation compared to NTC (**1E**). CRISPRi in hNPCs showed significant increases in senescence at 1-week and 3-week timepoints via SA-β-gal staining (**1F**) and 3 weeks with p16 and p21 staining (**1G,1H**), indicating ZNF865 may be a key regulator of senescence. CRISPRa of ZNF865 in degenerative hNPCs increased proliferation and significantly decreased doubling time (**1I**). CRISPRa of ZNF865 in Jurkat cells increases proliferation and cytokine production when stimulated (**1J,1K**). One-way (qRT-PCR, ELISA, p16, p21) and two-way ANOVA (proliferation, SA-β-gal) were used to determine significance ($\alpha = 0.05$).

Discussion: This research examines the effects of CRISPR-guided gene modulation of ZNF865 *in vitro*. Overall, our results suggest that ZNF865 regulates key processes in cell activity and senescence across a broad range of human cell types. Upon upregulation of ZNF865, robust increases in cellular proliferation was seen across all cell types. Downregulation of ZNF865 led to cell death or senescence in immortalized and primary cells respectively. These results indicate that ZNF865 is necessary for the normal function of cells in all cell types we tested indicating a previously unknown and significant cell biology discovery. These results indicate that ZNF865 has potential to be used as a tool to boost cell therapies and regulate senescence within the IVD as well as other diseased or aging environments via epigenetic control.

Significance: The apparent regulation of senescence by ZNF865 provides potential for this gene to be used as a cell engineering tool to regulate senescence within diseased or aging environments and boost cell therapy potential across a broad range of cell therapies.

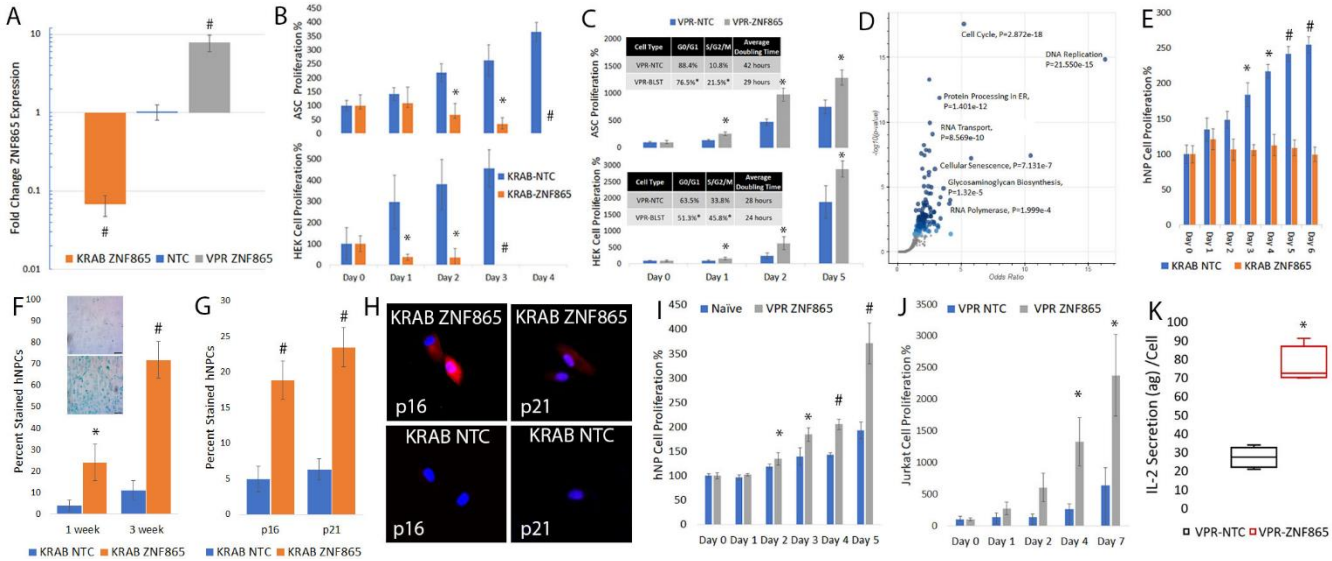

Figure 1: (A) Fold change ZNF865 expression. (B) Cell proliferation for HEK 293 and ASCs transduced with ZNF865 and NTC for CRISPRi and (C) CRIPSRa. (D) RNAseq data from ZNF865 upregulated ASCs. (E) Cell proliferation for ZNF865 downregulated and NTC hNPCs. (F) SA-β-gal staining for ZNF865 downregulated ant NTC hNPCs at 1 week and 3 weeks with representative 3-week images. (G) P16 and p21 staining for KRAB NTC and KRAB ZNF865 hNPCs. (H) Representative images of p16 and p21 staining of hNPCs (Blue=DAPI, Red=p16 or p21) (I) Cell proliferation of naïve and ZNF865 upregulated degenerative hNPCs (J) Jurkat cell proliferation for VPR-ZNF865 and -NTC. (K) IL-2 secretion from stimulation jurkat cells. *=p<0.05 #=p<0.001.