#17

Epigenome Editing for Endogenous Activation of Periodontal and Tendon Transcription Factors

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INTRODUCTION

- Severe periodontitis is the sixth most prevalent medical condition.
- Tendon injuries account for approximately half of the 33 million musculoskeletal injuries in the US.
- Current treatments are limited and fail to restore these tissues back to their native state.
- Regeneration using stem cell programming is an attractive treatment approach for restoring periodontal and tendon tissues.
- However, proper cellular programming and is elusive and will likely require transcriptional gene network regulation.
- Transcription factors Scleraxis (SCX) and Mohawk (MKX) are essential in periodontal ligament and tendon development.
- Simultaneous upregulation of both SCX and MKX may further enhance periodontal ligament and tendon restoration.
- Simultaneous upregulation of genes may enhance periodontal ligament and tendon restoration. We take a multiplexed approach to precisely target and activate both periodontal and tendon master regulators.

HYPOTHESIS

We hypothesize that endogenous activation of key transcriptional factors, SCX and MKX, using the genomic epigenetic editing dCas9-VPR CRISPR activation system, will activate downstream lineage pathways toward periodontal and tenogenic differentiation and repair.

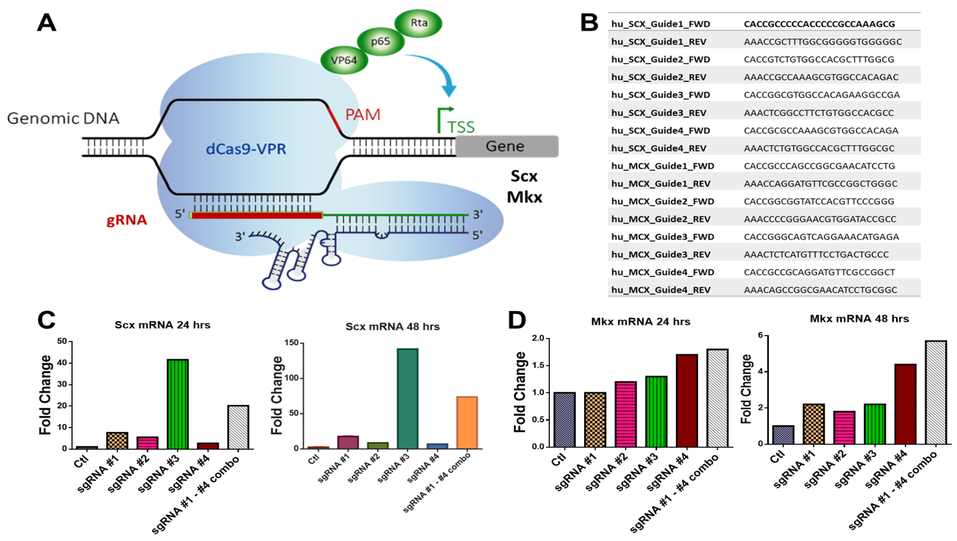
METHODS

CRISPR activation system: Overexpression of SCX and MKX was conducted using a SP-dCas9-VPR plasmid vector and designed gRNAs cloned into pSB700 (A. Chavez-Columbia).

- Four gRNAs per target gene, targeting the upstream promoter region of both MKX and SCX within 250 base pairs of the transcriptional start site, were screened.
- HEK-293T cells were co-transfected using 2 µl Lipofectamine Stem Reagent with 200 ng of dCas9 and 10 - 100 ng of gRNA.

RESULTS

1. CRISPR activation system utilizes designed gRNAs to upregulate tendon transcription factors SCX and MKX



B	
hu_SCX_Guide1_FWD	CACCGCCCCCACCCCCGCCAAAGCG
hu_SCX_Guide1_REV	AAACCGCTTTGGCGGGGGTGGGGGC
hu_SCX_Guide2_FWD	CACCGTCTGTGGCCACGCTTTGGCG
hu_SCX_Guide2_REV	AAACCGCCAAAGCGTGGCCACAGAC
hu_SCX_Guide3_FWD	CACCGGCGTGGCCACAGAAGGCCGA
hu_SCX_Guide3_REV	AAACTCGGCCTTCTGTGGCCACGCC
hu_SCX_Guide4_FWD	CACCGCGCCAAAGCGTGGCCACAGA
hu_SCX_Guide4_REV	AAACTCTGTGGCCACGCTTTGGCGC
hu_MCX_Guide1_FWD	CACCGCCCAGCCGGCGAACATCCTG
hu_MCX_Guide1_REV	AAACCAGGATGTTCGCCGGCTGGGC
hu_MCX_Guide2_FWD	CACCGGCGGTATCCACGTTCCCGGG
hu_MCX_Guide2_REV	AAACCCCGGGAACGTGGATACCGCC
hu_MCX_Guide3_FWD	CACCGGGCAGTCAGGAAACATGAGA
hu_MCX_Guide3_REV	AAACTCTCATGTTTCCTGACTGCCC
hu_MCX_Guide4_FWD	CACCGCCGCAGGATGTTCGCCGGCT
hu_MCX_Guide4_REV	AAACAGCCGGCGAACATCCTGCGGC

Figure 1. CRISPR activation system via dCas9-VPR upregulates specific gene targets guided by designed gRNAs.
(A) Model of CRISPR activation system **(B)** Sequences of gRNAs for SCX and MKX **(C)** Initial screening of gRNA activity of SCX **(D)** and MKX.

2. Modulation of MKX expression using dCas9-VPR system in HEK-293T cells

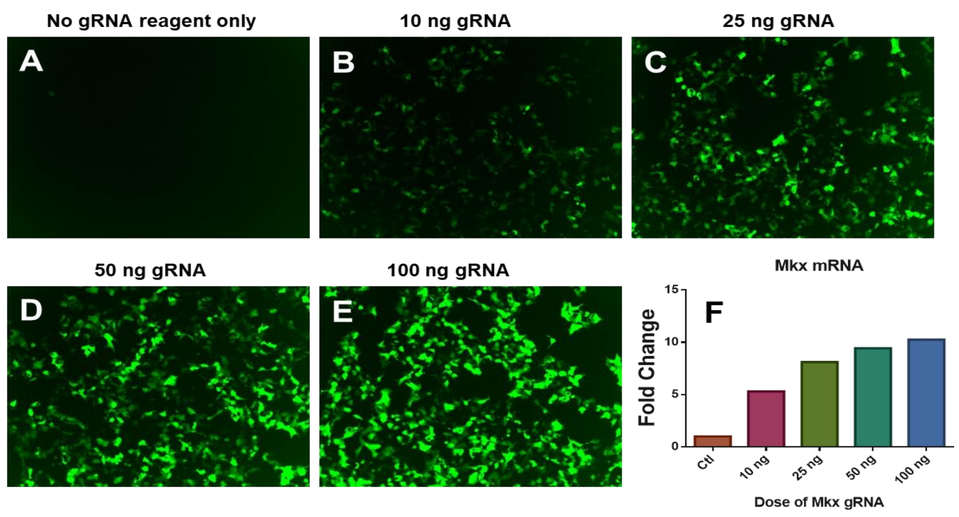


Figure 2. Increasing amounts of MKX gRNA #4 increases transfection efficiency and MKX gene expression in a dose dependent manner.
(A-E) Fluorescence microscopy images of MKX gRNA #4 transfection efficiency in HEK-293T cells **(F)** MKX gene expression may be enhanced up to ~10 fold at highest gRNA dose (100 ng)

3. Multiplex activation of SCX and MKX expression using dCas9-VPR

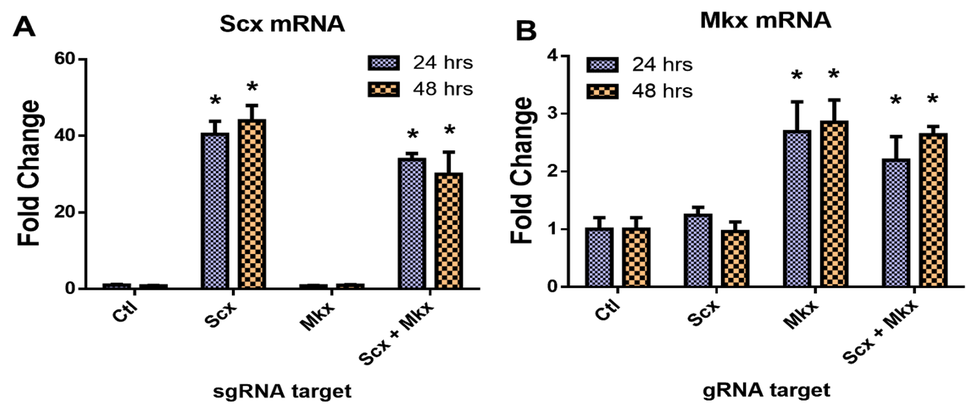


Figure 3. dCas9-VPR transfection with the best performing SCX and MKX gRNAs endogenously and concurrently upregulate gene expression.
(A) Multiplex of best performing gRNAs from SCX and MKX achieves endogenous activation up to ~43 fold **(B)** and ~3 fold respectively (n = 3 per group: *:p<0.0001 compared to control).

4. Neon® Electroporation improves transfection efficiencies in human BM-MSCs and DPSCs

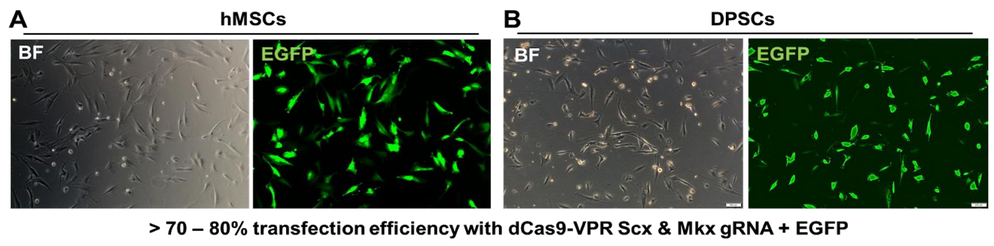


Figure 4. Electroporation using the Neon® system and a GFP reporter plasmid significantly enhances transfection efficiencies in BM-MSCs and DPSCs
(A) Transfection efficiencies of >80% using Neon® Electroporation in BM_MSCs **(B)** and DPSCs

FUTURE DIRECTIONS

5. Design for transcriptomic analysis of periodontal and tendon lineage programming

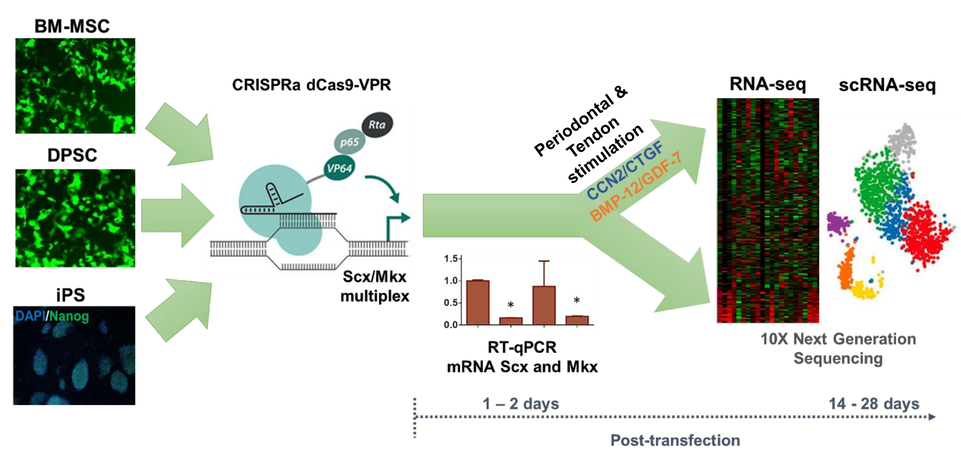


Figure 5. Transcriptomics: Upon confirmation of short-term genetic upregulation of SCX and MKX in biologically relevant cells, we will use single-cell transcriptomics to characterize periodontal and tendon lineage programming, potentially regulated by multiplexed activation of SCX and MKX.

CONCLUSIONS

- We have established a reproducible protocol to epigenetically edit periodontal and tendon regulators using a CRISPRa dCas9-VPR system.
- We identified standout gRNAs for robust activation and efficient transfection conditions for HEK-293T, BM-MSCs, and DPSCs.
- Increasing the concentration of MKX gRNA consistently increased the expression of MKX.
- Activation of SCX was stronger compared to MKX, which could be due to guide RNA design, basal expression or chromatin configuration of the promoter region of each target gene.
- Sufficient activation of these master regulators have the potential to activate necessary downstream targets to further stimulate periodontal ligament and tendon repair.
- To achieve proper lineage programming for periodontal and tendon stem cell replacement, a transcriptional network approach that endogenously regulates multiple genes simultaneously may be necessary. This novel cell fate engineering approach may lead to enhanced cell-based periodontal ligament and tendon regeneration and future therapeutics.

ACKNOWLEDGEMENTS

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